1978

Analysis of the Flagellar Membrane Proteins of Chlamydomonas moewusii

Cheryl Lynn Jamieson
The College at Brockport

ANALYSIS OF THE FLAGELLAR MEMBRANE PROTEINS OF CHLAMYDOMONAS MOEWUSII

Cheryl Lynn Jamieson, B.S.

State University College at Brockport, 1978

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Biological Sciences

at

State University College at Brockport

1978

APPROVAL PAGE

Master of Science in Biological Sciences Thesis

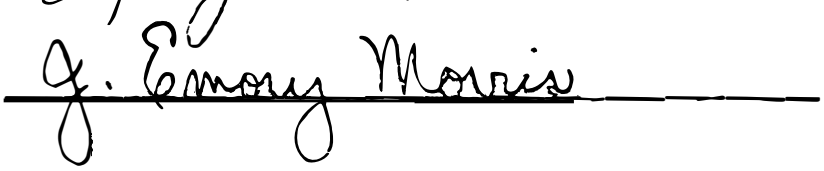
ANALYSIS OF THE FLAGELLAR MEMBRANE PROTEINS OF CHLAMYDOMONAS MOEWUSII

Presented by

Cheryl Lynn Jamieson, B.S., 1976

Major Advisor 

Associate Advisor 

Associate Advisor 

State University College at Brockport

1978

TABLE OF CONTENTS

Acknowledgements iii

List of Illustrations iv

Abstract v

Introduction and Literature Review 1

Materials and Methods 12

Results 22

Discussion 30

Literature Cited 36

ACKNOWLEDGEMENTS

I would like to express my deep appreciation to Dr. Robert J. McLean for his guidance and encouragement during this investigation and in the preparation of the text. I would also like to thank Drs. Larry K. Kline and J. Emory Morris for their helpful suggestions and critical reading of this thesis. My gratitude is extended to my friends and coworkers and those faculty members whose support gave me incentive to finish this project.

LIST OF ILLUSTRATIONS

Figure		Page
1	Effect of CsCl gradient centrifugation on the purity of (+) gametic gamone	44
2	Electron micrographs of mastigonemes, whole flagella, and cell wall material negatively stained with 2% PTA in 0.4% sucrose	45
3	Electron micrographs of (+) and (-) vegetative and gametic gamone negatively stained with 2% PTA in 0.4% sucrose	46
4	SDS-Polyacrylamide gel electrophoresis of (+) gamone, cell wall material, and mastigonemes	47
5	SDS-Polyacrylamide gel electrophoresis of (-) gamone and whole flagella	48
6	Radioactive elution profile of $Na^{125}I$ obtained from a Con A affinity chromatography column	49
7	Elution profiles from Con A affinity chromatography of (+) gametic gamone	50
8	Elution profiles from Con A affinity chromatography of (-) gametic gamone	51
9	SDS-Polyacrylamide gel electrophoresis of fractions from an SDS Con A affinity chromatography column	52

ABSTRACT

This investigation was concerned with the analysis of the proteins isolated from the gamone (flagellar membrane vesicles isolated from the medium) of Chlamydomonas moewusii.

SDS-polyacrylamide gel electrophoresis of gamone isolated from (+) and (-) cell types indicated possible differences between vegetative and gametic gamone within mating types and a degree of similarity within vegetative and gametic gamone of both mating types. Electrophoretic analysis of several molecular weight standards indicated that the major proteins from all gamone types are glycoproteins of relatively high molecular weight (100-150,000 D.)

Con A affinity chromatography of membranes solubilized in 1% DOC in 10 mM Tris, pH 8.2, showed that 4.2% of the proteins isolated from the (-) gamone and 7.35%-16.4% of the proteins isolated from the (+) gamone bound to Con A. These proteins could subsequently be eluted with 2% α-methylmannoside. Attempts to recover the proteins from the Con A affinity chromatography column were unsuccessful.

INTRODUCTION

AND

LITERATURE REVIEW

The plasmalemma is the outer barrier between the external environment and the cell matrix. It is responsible for many aspects of cell function and behavior such as cellular recognition, adhesiveness, adsorption, transport, immunologic behavior and electrical characteristics. Cellular recognition and adhesive properties of cell surfaces seem to determine many morphogenetic processes including differentiation, organogenesis, contact inhibition, and metastasis. The biochemical composition of cell surfaces may reflect the mechanisms by which cells recognize each other and adhere. If these mechanisms are known, a better understanding of cellular dedifferentiation and uncontrolled growth (cancer and metastasis), among others, will be forthcoming.

Glycoproteins as adhesive factors

A variety of systems have been used to study specific cell adhesion. They include sponge cell reaggregation (1, 2), neural retina cell aggregation (3), teratoma cell adhesion (4, 5), the mating reaction in Chlamydomonas (6-9), conjugation of Blepharisma (10, 11, 12), egg-sperm adhesion (13), and others. The adhesive properties of cell surfaces have been attributed in some cases to the presence of large glycoproteins (1, 2, 14, 16-18). In mammalian cells, the evidence implicating glycoproteins in adhesion has been fairly recent, since in the past, few workers analyzed

for carbohydrate. In non-mammalian cells, evidence implicating glycoproteins in cell adhesion arose much earlier. Forster et al. (16) isolated an adhesive factor (gamone) from the medium of Chlamydomonas eugametos which was identified as glycoprotein by paper chromatographic analysis. In several species of marine sponges, aggregation factors responsible for species-specific adhesion were identified as glycoproteins (1, 19). Henkart et al. (20) have characterized the aggregation factor from the marine sponge Microciona panthena. Chemical analysis indicated that it was comprised of 47% amino acids and 49% sugars with a M. W. between 20-40, 000. Electron microscope examination showed the molecular complex to be fibrous in a sunburst configuration with an inner circle and radiating arms (1). In a more recent investigation, the gamones of the protozoan Blepharisma intermedium have been characterized (11, 12). The gamone of mating type I is a glycoprotein of M. W. 20, 000. The gamone produced by mating type II, however, is not a glycoprotein. McClay and Moscona have isolated a glycoprotein from the medium of chick embryo neural retinal cells of M. W. 50, 000 (14) which causes adhesion when added to cultured cells in vitro.

In mammalian cells, Lloyd and Cook (15) have isolated a glycoprotein fraction from the membranes of rat fibroblasts which caused increased adhesion when added to fibroblasts cultured in vitro. In an investigation of transformed cells, large, external, transformation sensitive proteins (LETS) or cell surface proteins (CSP) have been discovered on the cell surfaces of normal cells which are absent or

diminished on transformed cells (21-27). Periodic acid-Schiff staining (a stain specific for carbohydrates) of membrane components from cultured chick fibroblasts indicated that the LETS protein was a glycoprotein (25). Further analysis of this LETS protein indicated that it contained 5-6% carbohydrate consisting of N-acetylglucosamine, mannose, galactose, sialic acid, and glucose. There did not appear to be a preponderance of any one class of amino acid (28). Gamberg et al. present further evidence that the LETS protein is a glycoprotein by isolating a galactoprotein of M.W. 200,000 from NIH cells which was absent in NIH cells transformed by polyoma virus. Yamada et al. have recently quantitated the LETS protein on various fibroblastic cell lines from several species, indicating that it comprises 1-3% of the total cell protein of early passage cells and is greatly diminished after transformation (29). LETS glycoproteins have been shown to have adhesive properties (30, 32). Using formalinized sheep erythrocytes, Yamada et al. (25), demonstrated that the LETS protein isolated from chick embryo fibroblasts would cause haemagglutination. In addition to binding to erythrocytes, LETS can be readily adsorbed back onto chick fibroblasts (30). In various cell lines transformed by oncogenic viruses, LETS proteins were detected only in cell-cell contact areas whereas untransformed cells had the LETS protein distributed over the entire cell surface (31). When the LETS protein isolated from chick embryo fibroblasts was added to several transformed lines, the cells showed an increased adhesion to the substratum as well as increased cell-cell adhesion. Thus, the

LETS protein promoted a reversion to a more normal phenotype. This LETS protein does not appear to regulate growth rate or nutrient transport (26, 30, 32, 33).

Mechanisms of cell adhesion

A variety of theories have been proposed to explain the mechanism of specific cell adhesion. These range from purely chemical criteria (ionic or physical cell surface properties) to biochemical and enzymatic reactions.

The Weiss-Tyler hypothesis (34, 35) explains specific cell adhesion in terms of an antigen/antibody-like reaction. Since cell surfaces have been shown to be covered with antigenic compounds such as blood group and transplantation antigens, the only other requirement by the cell would be to have antibodies or similar substances to form adhesive bonds. This hypothesis seems quite adequate except for one major consideration. In embryonic differentiation adhering cells often dissociate, migrate to other locations in the embryo, and readhere to each other or different cells. Dissociation would require changing or destroying the antigen, antibody, or antigen/antibody complex and reinstatement in the precise location for adhesion to again occur (36). Although this theory has been somewhat disregarded, Crandall and Brock (37) present some evidence in its favor. Using the yeast Hansenula wingii they studied the sexual fusion in which agglutination is the initial step when opposite mating types are mixed. They isolated a

glycoprotein agglutination factor from strain 5 which is neutralized by a cell surface non-agglutinin from strain 21 thus producing a reaction analagous to an antigen/antibody reaction.

Another mechanism involving complex carbohydrates is based on the formation of hydrogen bonds between glycose units on adjacent surfaces. To form intercellular adhesions, however, a large number of hydrogen bonds would be necessary. The major drawback in this hypothesis arises from the fact that homologous cells adhere strongly and heterologous cells weakly or not at all (36). It has been suggested (36) that this mechanism would require a high order of architectural specificity on each cell surface. Due to the complexity of tertiary structure and isolation methods which may alter this structure, associating the tertiary structure of glycoproteins to cell adhesiveness would be difficult.

A different approach to cell adhesion concerned with surface energies rather than physiochemical aspects of cell adhesion was proposed by Steinberg (38). The differential adhesion hypothesis is a thermodynamic one stating that the final association of cells will be the one in which the adhesive strength is greatest, corresponding to a minimized free energy. It seems, however, that surface energies cannot be measured with any degree of confidence (39) thereby making it difficult to assess in experimental terms.

Another model for intercellular adhesion is a simple and more flexible model proposed by Roseman (36). Glycosyltransferases and

complex carbohydrates located on neighboring cell surfaces interact by forming enzyme-substrate complexes resulting in adhesion. The advantage of this mechanism is that adhesions are not static since adhesiveness is lost or diminished on completion of the enzymatic reaction unless the acceptor provides a substrate for further glycosylation. Glycosyltransferases have been reported on the cell surfaces of several different cell types (40-44).

Lectins

Lectins, most of which appear to be glycoproteins, have the property of binding specific carbohydrates. Some lectins, in addition to being agglutinins, have mitogenic activity (45) while others are cytotoxic (46, 47). Although lectins are usually associated with plants, similar proteins also exist in numerous other organisms such as fungi (48), the horseshoe crab, Limulus polyphemus (49), sea snail, Helix dolabella (50), sponge (2), and others. Many of these lectins have been purified and have become useful reagents for detecting glycoproteins histochemically on cell surfaces (46, 47, 51, 55).

Concanavalin A (Con A), a lectin isolated from the jack bean, has been extensively studied (52, 56-59). Crystalline Con A has been shown to be a tetramer. In solution the basic subunit of 25,000 D. associates to form dimers below pH 5 and tetramers above pH 7 (56, 57). Each subunit binds one Mn^{++}, one Ca^{++}, and one saccharide. The metals are required for saccharide binding. Con A specifically reacts with α-D-

mannopyranosyl and α-D-glucopyranosyl residues (58, 59). Reeke et al. (60) have determined the protein composition and tertiary arrangement of amino acids and metal ions in the Con A molecule.

Con A has had widespread applicability to numerous investigations. In an extensive study of the lysosomal enzymes of brain, Con A was used to 1) demonstrate the glycoprotein nature of these enzymes, 2) purify lysosomal enzymes, and 3) immobolize various glycoproteins such as arylsulphatase A, after precipitation with Con A (61). Con A, as well as other lectins, has been used to study cell surfaces of transformed cells (21, 53-55). Certain lectins have been shown to agglutinate transformed cells more readily than normal cells (53-55).

Affinity Chromatography

Affinity chromatography utilizes an immobilized support to which an adsorbant having specific biological activity has been attached. Because of this biospecificity, affinity chromatography has become an important tool used for the isolation of molecules such as enzymes, antigens, and membrane glycoproteins. Membrane glycoproteins isolated by this technique include brain glycoproteins (62), glycoproteins from lymphocyte plasma membranes (63), glycoproteins from Dictyostelium discoideum (64, 65) and glycoproteins from normal and transformed cells of BHK (baby hamster kidney cells) (66). Several lectins have been utilized as adsorbants in these investigations. Those most commonly used are the lectins isolated from Lens culinaris, Ricinus communus, and the

jack bean (concanavalin A).

In several investigations of the slime mold D. discoideum it has been demonstrated by using Con.A affinity chromatography that the plasma membrane of this organism has at least 15 proteins which bind Con A. One appears to be under developmental regulation (64, 65). An analysis of enzyme activity of those proteins specifically bound to Con A when D. discoideum was in its vegetative state showed that 90% of the activity for alkaline phosphatase, 5' nucleotidase, and cAMP phosphodiesterase are bound to the column. As cells acquired aggregation competence, the percentage of total activity bound for each enzyme decreased (67).

In this investigation, Con A is of particular interest as it has been demonstrated that Con A will cause flagellar tip isoagglutination of Chlamydomonas gametes (see below). By utilizing an immobilized Con A-agarose column, it may be possible to isolate the glycoproteins found in the flagellar membranes which bind Con A.

Description of organism

Chlamydomonas, a unicellular, heterothallic, biflagellated, alga, has a specific mating reaction which has been studied in great detail (6-9, 42, 68-71). Gametogenesis is induced by a nitrogen deficient medium and is followed by a flagellar agglutination or clumping of cells when opposite mating types are mixed. From the above investigations, it has been well established that the agglutination phenomenon is dependent on glycoproteins located on the flagellar tips. Investigations of

C. reinhardtii (70) have demonstrated that both sexes are sensitive to the proteases trypsin and pronase thus destroying agglutinability. In C. eugametos and C. moewusii, however, only the (-) sex was sensitive to the proteases where the (+) are not (17, 18). The susceptibility of the agglutination reaction to proteases suggests that proteins contribute to the stickiness. Furthermore, the (+) sexes are sensitive to α-mannosidase, blocking agglutination, whereas the (-) sexes are resistant (17, 70). Wiese et al. (17) demonstrated that 0.01% Con A will cause isoagglutination (agglutination of a single sex) of all gametes of C. moewusii and C. eugametos. A 0.001% solution of Con A will not cause isoagglutination of gametes. If, however, the (+) mating type is treated with 0.001% Con A prior to mixing with untreated (-) gametes agglutination did not occur. Agglutination was not affected if the (-) gametes were treated with 0.001% Con A prior to mixing with untreated (+) gametes. From this sensitivity to various enzymes, Wiese and coworkers concluded that the active sites of the (+) gamete are composed of a carbohydrate while the active sites of the (-) gamete are proteinaceous (17, 18). Wiese and Hayward (18) have suggested that the mating site and Con A binding sites are the same. By using monovalent Con A and competitive inhibitors of Con A, McLean et al. (71) demonstrated that the receptors for Con A could blocked, eliminating the Con A agglutinability without altering mating ability. This indicated that the Con A binding site(s) and mating agglutinin are not the same.

Isolated flagella and flagellar membranes from gametic cells cause

isoagglutination when added to the opposite mating type thus mimicking the mating agglutination. Vegetative cells exhibit no such isoagglutinating activity (7). This system thus provides a means for comparison of flagellar membrane surfaces before and after gametogenesis. This may give a clue to the mechanism by which gametogenesis is accompanied by increased cellular adhesiveness. Morphological studies of C. reinhardtii, however, show no differences in the fuzzy surface coats and associated mastigonemes (fine hairlike projections on flagella) in all cell types (6). Electrophoretic analysis of protein components also revealed few, if any differences between cell types (6, 8). The only differences accompanying gametogenesis appear to be an alteration in the distribution of intramembranous particles (6) and the flagellar length (72). It has not been demonstrated that either of these differences is related to membrane agglutinability. Thus, the difference between vegetative and gametic cell types as reflected in the adhesive properties of these cells, must be very subtle.

Forster et al. (16) isolated an adhesive factor from the medium of gametic cells which causes isoagglutination when added to the opposite mating type. They named this factor gamone and defined it as a particulate material of high molecular weight. Ultrastructural studies, however, show that gamone is really flagellar membrane vesicles (69). These membrane vesicles are also present in vegetative cells, but demonstrate no isoagglutinating activity (8). Biochemical studies on gamone from C. reinhardtii show that many of the isolated proteins

were identical to those found on the flagellar surface (8). Forster et al. (16) determined that the (+) mating type gamone is composed of 21% protein while the (-) mating type gamone is 36% protein. A comparison of vegetative and gametic gamone by SDS polyacrylamide gel electrophoresis showed (+) gametic gamone to contain an additional protein not present in vegetative gamone or in the flagellar membranes of all cell types. Since this protein was not present in the flagellar membranes, it was disregarded as being related to adhesiveness (8).

This investigation is directed toward a study of the flagellar membrane proteins from the gamone of both vegetative and gametic cells of C. moewusii.

MATERIALS AND METHODS

EXPERIMENTAL ORGANISMS

The organism used in this investigation was Chlamydomonas moewusii Syngen I obtained from the Culture Collection of Algae at the University of Texas. No. 96 = (+); No. 97 = (-).

CULTURING PROCEDURES

The cells were grown under continuous illumination of 200-300 ft-c for two weeks in liquid growth medium containing the following nutrients:

distilled water	1000	ml
trace elements	1	ml
sodium citrate	0.5	g
$CaCl_2 \cdot 2H_2O$	0.1	g
$MgSO_4 \cdot 7H_2O$	0.6	g
NH_4Cl	0.4	g
K_2HPO_4	2.1	g
KH_2PO_4	1.1	g
$FeCl_3$	0.001	g
sodium acetate	0.2	g
yeast extract	0.4	g

The trace element solution was composed of:

distilled water	1000	ml
H_3BO_4	0.618	g
$MnCl_2$	0.880	g
$ZnCl_2$	0.109	g
$CoCl_2 \cdot 6H_2O$	0.044	g
$Na_2MoO_4 \cdot H_2O$	0.024	g
$CuCl_2$	0.03	mg
Na_2EDTA	7.44	g

After two weeks growth, 5 ml of the cultures were transferred to

150 ml new growth medium and subjected to 200-300 ft-c of light for a period of 2 weeks. The remaining cells were transferred to agar plates containing growth medium plus 1.5% agar. These plates were grown under similar conditions as liquid cultures and used when they were between 10-14 days old.

HARVESTING CELLS

Approximately 100-200 of 10-14 day old plates were harvested by flooding the plates with induction medium (described below) and scraping the cells off using a sterile glass microscope slide.

GAMETIC INDUCTION

After harvesting, the cells were directly resuspended in induction medium (IM) composed of the following:

distilled water	1000	ml
sodium citrate	0.05	g
$CaCl_2 \cdot 2H_2O$	0.01	g
$MgSO_4 \cdot 7H_2O$	0.06	g
K_2HPO_4	0.717	g
KH_2PO_4	0.363	g
$FeCl_3$	0.001	g
trace elements	0.1	ml

pH of the solution was adjusted to 7.6 with NaOH

The (+) mating type was placed in the dark for a period of 12-14 hr in which time they became gametic and were subjected to light of 200-300 ft-c for 20 min. The (-) mating type was placed in dim light for a period of 8-10 hr.

VEGETATIVE CELLS

Vegetative cells were prepared as above except the cells were directly resuspended in vegetative growth medium (regular growth medium plus 1g/l additional NH_4Cl).

ISOLATION OF GAMONE

Gametic gamone was obtained from cells induced in IM and vegetative gamone was obtained from cells resuspended in vegetative growth medium. Gamone was obtained by centrifuging the cell suspension 1000 rpm (164 X g) for 20 min in a Sorvall Model RC2B centrifuge with GSA rotor. This allowed the cell pellet to be used for flagellar detachment as the flagella would remain attached to the cell bodies at this speed. The supernate was removed and centrifuged 2500 rpm (1020 X g) for 20 min using the GSA rotor. This supernate was removed and centrifuged 8000 rpm (10, 400 X g) for 20 min as above. The supernate was removed and filtered through a 0.45 filter to remove cell wall material and other debris. The filtrate was poured into 50 ml polycarbonate tubes and centrifuged 15, 500 rpm (29, 000 X g) for 1 hr. The rough gamone pellet was resuspended in 10 mM Tris, pH 7.2, layered over an equal volume of 40% sucrose in 10 mM Tris, pH 7.2, and centrifuged 10, 000 rpm (16, 300 X g) for 1 hr using the HB-4 swinging bucket rotor. The interface and upper layer was removed and centrifuged at 15, 500 rpm for 1 hr to pellet gamone. If further purification was required, the

pellet was resuspended in 2.8 M CsCl in 10 mM Tris and centrifuged 40,000 rpm (148,000 X g) for 22 hr in a Beckman Model L3-50 with SW 50.1 rotor. Gamone usually banded 1.1-1.7 cm from the top of the tube. The band was removed by pipette, rinsed in 10 mM Tris and centrifuged 15,500 rpm for 1 hr to pellet the gamone.

ISOLATION OF MASTIGONEMES

The cell free gamone supernate was centrifuged 19,500 rpm (30,000 X g, SS-34 rotor) for 1 hr. This supernate was centrifuged again for 45 min at 50,000 rpm (166,500 X g, Spinco Ti 50 rotor) and the resulting mastigoneme pellet was prepared for polyacrylamide gel electrophoresis as described below.

ISOLATION OF CELL WALL

Cell wall material was in the pellet obtained after layering gamone over 40% sucrose. This pellet was resuspended directly in 2.8 M CsCl and centrifuged 40,000 rpm (148,000 X g, SW 50.1 rotor) for 22 hr. The cell wall material banded as a distinct zone 0.9 cm from the bottom of the tube.

FLAGELLAR DETACHMENT

The cell suspension was poured into 250 ml plastic bottles and centrifuged for 20 min at 1,000 rpm (164 X g) in the Sorvall RC2B centrifuge with GSA rotor.

Flagella were detached using two methods described by Witman (68). 1) Ca Steep Method: the packed cells were resuspended in an equal volume of cold 10 mM Tris, pH 7.2 and cooled to 10°C. Four to five times the volume of the cell suspension of cold (2°C) Gibbon's solution or "steep" (0.15 M sucrose, 15 mM Tris, 2.5 mM disodium EDTA, 11% EtOH, 30 mM KCl) was added and the solution vigourously stirred. Enough 1 M CaCl was added to give a final concentration of Ca^{++} of 15 mM. Flagella detached in approximately 30 sec. This suspension was then layered over 15 ml of 25% sucrose in 10 mM Tris, pH 7.2, and centrifuged 4,300 rpm 10 min in the Sorvall RC2B with HB-4 rotor. The upper layer and interface were removed, layered again over 15 ml of 25% sucrose in 10 mM Tris and centrifuged as above to remove any remaining cell bodies. The top layer and interface were removed, diluted with 10 mM Tris, pH 7.2 and centrifuged 15,500 rpm (29,000 X g) for 1 hr to obtain flagella. 2) pH shock method: the cells obtained after harvesting were resuspended in 4-5 times their volume in 5% sucrose in 10 mM Tris, pH 7.2 and cooled on ice. As the solution was vigourously stirred, the pH was lowered to 4.5 by the addition of 0.5 M acetic acid. After the flagella were detached (approx. 30 sec.) the pH was raised to 7.0 by the addition of 0.5 M KOH. The flagella were isolated as described in the Ca Steep procedure.

DETERMINATION OF PROTEIN

Protein concentration was determined by the method of Lowry (73)

using the Bio-Rad gamma-globulin as standard.

NEGATIVE STAINING

Negative staining of membrane preparations was done to determine purity of preparations. One drop of flagellar suspension was put on a copper grid mesh 300 coated with formvar. A drop of 2% phosphotungstic acid (PTA) containing 0.4% sucrose was placed on top of the membranes for 30 sec. The liquid was drawn off with filter paper and the grid allowed to dry 15 min.

SDS POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

SDS polyacrylamide gel electrophoresis was carried out using a modification of the procedure of Weber and Osborne (74). Gel buffer contained 7.8 g NaH_2PO_4, 38.6 g Na_2HPO_4 and 2 g SDS in 1000 ml of distilled water. The acrylamide stock solution contained 10 g acrylamide and 0.175 g N, N' methylenebisacrylamide in 100 ml of distilled water. Final gels were 5% acrylamide, 0.087% N, N' methylenebisacrylamide, 0.16% N, N, N', N'-tetramethylethylenediamine (TEMED), and 0.25% ammonium persulfate. The electrophoresis buffer was a 1:1 dilution of gel buffer. Samples were run at 5 ma/tube for 10 min and then increased to 10 ma/tube for 5.5 hr.

PREPARATION OF SAMPLE FOR SDS PAGE

Samples were prepared for PAGE by resuspending the pellet in a

solution containing 2% SDS and 0.5% beta-mercaptoethanol to a final concentration of 1 mg/ml and heated in a boiling water bath for 5 min. Insoluble material was pelleted by centrifugation at 40,000 rpm for 1 hr in a Beckman Model L3-50 ultracentrifuge with Ti 50 rotor. The supernate was removed and a few crystals of sucrose were added. Bromophenol blue was used as a tracking dye. Approximately 50-100 μg of protein were layered on each gel.

GEL STAINS

Duplicate gels were stained for either protein (Coomassie Brilliant Blue-CBB) or carbohydrate (Periodic acid-Schiff-PAS).

1) Protein: gels were stained in a solution of 25% isopropyl alcohol, 10% acetic acid, and 0.025-0.05% CBB 8-12 hr. Destaining was in 10% acetic acid for several hr. 2) Carbohydrate: gels were stained for carbohydrate as described by Fairbanks et al. (75). Gels to be stained for carbohydrate were fixed in cold 10% TCA 15 min before staining. All rinses were done at room temperature with vigourous stirring. The staining procedure was as follows:

A. 0.5% periodic acid for 2 hr.
B. 0.5% sodium arsenite, 5% acetic acid 30-60 min.
C. 0.1% sodium arsenite, 5% acetic acid 20 min, twice.
D. acetic acid 10-20 min.
E. 10 ml Schiff reagent overnight
F. Rinse with 0.1% sodium metabisulfate, 0.01 N HCl several hr. Gels rinsed several times.

LACTOPEROXIDASE LABELLING

Lactoperoxidase iodination was carried out using a modification of

a method described by Hynes (23). The pellet of membranes was resuspended in IM containing 5 mM glucose and 78 μCi of carrier-free $Na^{125}I$ (ICN Chemical). To initiate the reaction, a final concentration of 25 U/ml of lactoperoxidase (E.C. 1.11.1.7. Sigma Chemical) and 3 U/ml glucose oxidase (E.C. 1.1.3.4 Sigma Chemical) were added. After incubating the suspension 10 min, the reaction was stopped by the addition of 200 volumes of IM containing 1% NaI. This suspension was then centrifuged 15, 500 rpm (29, 000 X g) in an International Refrigerated Centrifuge with 870 rotor for 1 hr. The labelled material was resuspended in IM and dialyzed against 1000 volumes of IM in the cold overnight.

AFFINITY CHROMATOGRAPHY

Column Preparation

Con A agarose (Sigma Chemical) was resuspended in 100 volumes of IM for several hr. An 8 mm X 5 cm column was packed to 1 ml with the Con A agarose. The packed column was then rinsed with 200 volumes of 10 mM Tris, pH 7.2, 10 volumes of 2% α-methylmannoside (aMM) in 1% sodium deoxycholate, 10 mM Tris, pH 8.2, and 200 volumes of 1% sodium deoxycholate in 10 mM Tris, pH 8.2 (DOC buffer), respectively. The flow rate was adjusted to 0.1 ml/min.

Binding Capacity

The binding capacity of the Con A agarose column was tested using ovalbumin (4 mg/ml) suspended in DOC buffer. A column was packed

with 1 ml of Con A agarose as previously mentioned. An elution profile of protein (optical density at 280 nm) indicated that approximately 22% or 1 mg/ml of the total sample was bound and subsequently eluted with 2% aMM.

Sample Preparation

Membranes were dissolved in 500 µl of DOC buffer and placed in a 37 °C water bath for 1 hr. Insoluble material was pelleted by centrifuging the suspension 40,000 rpm (148,000 X g) for 1 hr in a Beckman Model L3-50 with Ti 50 rotor. The supernate was removed, optical density at 280 nm recorded, and the sample layered onto the Con A agarose column. After the sample entered the column, the column was washed with DOC buffer until the radioactivity or optical density at 280 nm reached background levels. Bound proteins were then eluted with 2% aMM in DOC buffer. When the aMM entered the column, the flow was stopped for 1 hr to let the inhibitor react. Elution with aMM was continued for the remainder of the experiment.

Elution Profile

Fractions collected were 1 ml. A radioactive elution profile was obtained by taking 100 µl aliquots from each fraction and counting in a Packard Model 3320 Tri-carb scintillation counter. The counting solution contained 10 g Omnifluor, 1 liter triton X, and 2 liters toluene.

A protein elution profile was obtained by monitoring the optical density of each fraction at 280 nm.

Recovery of Protein

Unbound fractions were pooled and fractions eluted with aMM were pooled. Two volumes of absolute ethanol at -20 ^{o}C were added and the sample was left overnight at -20 ^{o}C. The tubes were brought to room temperature 30 min before centrifuging at 16,000 rpm (30,000 X g) for 30 min. The pellet was washed twice with ethanol and traces of ethanol were removed with a light air stream. Samples were resuspended in 1% SDS and subjected to PAGE as previously described.

SDS Affinity Chromatography

A Con A agarose column was prepared as described above except 0.07% SDS was substituted for 1% DOC. The sample was solubilized in 0.07% SDS. Fractions were pooled as described above, lyophilyzed, and applied to gels.

RESULTS

Isolation of gamone

A characteristic difference noticed during gamone preparations was that the medium isolated from (+) gametes was deep gold and that of (-) gametes was faintly yellow. Correspondingly, gamone isolated from (+) gametes was brown, whereas gamone isolated from (-) gametes was white.

Negative stains of gamone preparations purified by layering over 40% sucrose showed them to be generally free of cell wall material. Gamone purified further with CsCl, however, had fewer bands when subjected to PAGE than those preparations isolated by layering over 40% sucrose (Fig. 1). When the gamone was subjected to the CsCl purification procedure, it always banded as a broad zone between 1.1-1.7 cm from the top of the tube. Cell wall contamination would be found banding as a distinct zone 0.9 cm from the bottom. No cell wall bands were observed in CsCl gradients when the preparations were first layered over 40% sucrose.

Throughout this investigation, isolation of (-) gamone or flagella was always more difficult than (+). With equal quantities of starting material, the quantity of end product was usually far less for (-) than that obtained for the (+) mating type.

Negative stains of gamone preparations after CsCl, showed the preparations to be free of cell wall material, mastigonemes, or whole

flagella. Negative stains of these contaminants can be seen in Fig. 2, A-C. Negative stains of the (+) and (-) gametic and vegetative gamone can be seen in Fig. 3. It appeared from these pictures that (+) membrane vesicles may be somewhat larger than those isolated from the (-). In all negative stain preparations, a small globular-shaped material was always present. Since it appeared in all negative stains (cell wall, mastigonemes, and gamone), it is probably an effect of the sucrose -- PTA staining solution (see arrows Fig. 3)

Isolation of whole flagella

The flagellar detachment procedures described by Witman et al. (68) (pH shock and Ca Steep) worked adequately for C. moewusii. The pH shock procedure often had to be repeated twice within an experiment because many flagella remained attached after the first shock. This may have been a result of a too high cell density. Light microscope examination of the suspension after flagellar detachment showed a high percentage of cell lysis. When isolated flagella were examined as above, the flagella appeared shorter than those isolated by Ca Steep. This suggested that the pH shock method was breaking up flagella as well as cells. Witman and coworkers suggest that the Ca Steep procedure produces more intact flagella (68). Negative stains of Ca Steep flagella isolated in this investigation are in agreement with Witman (Fig. 2B). The Ca Steep method was more efficient in detaching flagella in a smaller volume than pH shock. Because intact flagella and smaller

volumes were more desirable, the Ca Steep method was utilized rather than the pH shock method.

SDS PAGE

The optimal running time for gels was determined to be $5\frac{1}{2}$ hr. In this time frame the molecular weight standards of trypsin (23,000 D.), Con A (100,000 D.), and gamma-globulin (150,000 D.), as well as samples, could be separated and resolved in the same gel system. (Fig. 4, A-C) (76). Trypsin, however, was the only standard in which one band was observed in the gel. Con A and gamma-globulin produced multiple bands. Only a molecular weight range was, therefore, obtained for samples.

Gels of whole flagella, cell wall material, and mastigonemes were run simultaneously with gamone to provide a complete set of protein profiles and a basis for comparison.

Cell wall material (Fig. 4H, F) - gels of cell wall material stained with CBB showed four bands in the upper 25% of the gel (Fig. 4H). The fastest migrating band was always the most intensely stained. The next fastest band did not stain as intensely, but was very prominent. The other two bands were faintly stained. When gels were stained with PAS, two intensely stained bands were observed corresponding to those intense bands observed with CBB (Fig. 4I).

Mastigonemes (Fig. 4J) - the gel of mastigonemes showed eight bands

distributed throughout the upper 75% of the gel when stained with CBB. The two fastest migrating bands (lower half of the gel) and two bands in the middle section of the gel were the most prominent, staining with equal intensity. Four slow migrating bands were observed in the upper quarter of the gel. These were narrow bands, but stained with equal intensity to the others.

Whole flagella (Fig. 5D, E) - approximately eleven bands were observed in the upper half of the gel stained with CBB (Fig. 5D). Nine bands were visible in gels stained with PAS (Fig. 5E). Bands 8-10 in CBB stained gels were not present in gels stained with PAS. An additional band in PAS stained gels was a faintly staining band located in the lower 10% of the gel. Whole flagella from (+) and (-) types were similar.

Gamone (Figs. 4D-G, 5F-I) - both (+) and (-) vegetative and gametic gamone were subjected to PAGE. CBB and PAS stained gels of (+) gametic gamone were identical indicating three glycoprotein bands (Fig. 4F, G). Two were in the upper quarter of the gel and the third was in the lower half. The slowest migrating band stained faintly with CBB and PAS. The second band stained intensely with CBB, but stained only faintly with PAS. Gels of the (+) vegetative gamone indicated four bands when stained with CBB (4D, E), but only the two slowest migrating bands stained with PAS. It appears that the second band in the (+) vegetative and gametic gels may be common (Fig. 4D-G). All the bands in the (+) gamone migrated slower than trypsin (Fig. 4A). The two upper bands

migrated slower than the slowest of the gamma-globulin (Fig. 4C) and Con A (Fig. 4B) bands. This suggests that all the glycoproteins have a M.W. of at least 23,000 D. and that the largest is probably between 100-150,000 D.

Because of the difficulty in isolation of (-) membranes, most gels were run on material only purified over 40% sucrose (Fig. 5F-I). When gels of (-) gametic gamone were stained with CBB, eight bands were visible. Six bands were in the upper quarter, one was in the middle, and one was in the lower quarter (Fig. 5F). Seven bands stained with PAS (Fig. 5G). Five were in the upper quarter and the other two were broad, very faint bands at the bottom of the gel. Gels of (-) vegetative gamone which had been purified over 40% sucrose, had 10 bands (Fig. 5H). Only three of these, however, stained PAS positive (Fig. 5F). The appearance of so many protein bands suggests that there may be some contaminants in the preparation. A comparison of (-) gamone with molecular weight standards indicate that the majority of proteins have a M.W. of at least 23,000 D. and the largest probably between 100-150,000 D. (Fig. 5A-I).

Lactoperoxidase Labelling

A control experiment was performed to test whether the radioactivity associated with the membranes was actually due to labelling of proteins by the iodination procedure or a nonspecific association of ^{125}I with the proteins. A gamone preparation isolated from (+) gametes was divided

in half (approx. 250 μg protein--O. D. of 1. 5). The labelling reactions were done simultaneously with identical conditions except that lactoperoxidase was eliminated from one tube. The proteins subjected to ^{125}I and lactoperoxidase bound 30 times more radioactivity than that without lactoperoxidase.

Affinity Chromatography

To determine the effects of stopping the column and to test for nonspecific binding of ^{125}I to agarose, an identical column to those used for samples was run with just $Na^{125}I$. Before the addition of aMM, the column was stopped for 1 hr. Several fractions were collected with no significant rise in radioactivity. A peak was observed after the addition of 2% aMM. This peak, however, corresponded to only 0. 3% of the total radioactivity added to the column (Fig. 6) as compared to 4-16% for samples. This amount of nonspecific binding does not significantly alter values obtained for samples. An experiment substantiating these results was a set of identical columns run with the sample containing all components required for iodination and the sample without lactoperoxidase. Both protein and radioactive elution profiles were determined (Fig. 7). The protein elution profiles from both columns were identical. The radioactive elution profiles, however, were not. The elution profile from the sample lacking lactoperoxidase showed the majority of radioactivity eluted in the first two fractions. From that point on, there was no increase in radioactivity above background, even after the addi-

tion of aMM. The increase in radioactivity after the addition of aMM corresponded to an increase in optical density at 280 nm.

A slight difference was observed between radioactive elution profiles obtained from (+) and (-) mating types (Figs. 7, 8). The percentage of bound radioactive material eluted with aMM compared to the total radioactivity put on initially for (-) gamone was 4.2%. This value was consistant between experiments. The percentages obtained for (+) gamone ranged from 7.35%-16.4%. This set of experiments shows conclusively that both the (+) and (-) gametic gamones contained proteins specifically labelled by lactoperoxidase iodination and that a certain percentage of these proteins were specifically bound to Con A agarose and eluted with 2% aMM.

In the affinity chromatography with 0.07% SDS several problems were encountered: 1) the protein elution profile could not be monitored as the SDS absorbed too high at 280 nm. At the time this experiment was done, $Na^{125}I$ radio-iodination was not being employed in this investigation, so a radioactive elution profile was not possible. 2) the binding capacity of the Con A agarose in SDS was about half that obtained with Con A agarose in DOC. Pooled fractions of protein not bound to the Con A agarose column and pooled fractions of the protein eluted with aMM were lyophylized and the residues subjected to PAGE. Staining patterns can be seen in Fig. 9. Con A and aMM controls were run simultaneously with the sample. The Con A banding pattern can be seen in Fig. 9A. The aMM did not migrate into the gel. Comparing the

Con A gel with gels of the protein eluates suggests that a substantial amount of Con A was leaching out from this column.

DISCUSSION

The mating reaction in the various species of Chlamydomonas has been extensively studied. The most extensive investigations, however, involve the species of C. reinhardtii (6-9, 68). This investigation has been concerned with the mating reaction of C. moewusii. By analysis of flagellar membrane proteins, through the isolation of the membrane vesicles isolated from the medium (gamone), an insight to the mechanism of cell adhesion for this organism may evolve.

Wiese and Shoemaker (17) demonstrated that 0.01% Con A caused flagellar tip isoagglutination of (+) and (-) cells in C. moewusii. Wiese (70) demonstrated that 0.125 M aMM prevents the Con A induced agglutination and also prevents the adsorption of all gamete types to Con A-coated Sephadex beads. In this study, Con A affinity chromatography of radio-iodinated membrane proteins indicated that the majority of labelled membrane proteins did not bind to Con A agarose. Only 4.2% of the (-) and 7-16% of the (+) type was bound by Con A. This percentage of bound radioactivity of C. moewusii flagellar membrane proteins appeared to be slightly greater than that found in membrane preparations in other investigations. Studies of D. discoideum employing Con A affinity chromatography indicated that 0.25% of the input counts were released with 0.1 M aMM with a residual 1.75% of the material remaining bound to the column (64). Pearlstein indicated in his study of normal and transformed cells that 3-5% of the total plasma protein was speci-

fically eluted from an affinity column depending on the lectin used (66).

The (+) mating type appeared to have a slightly greater affinity for the Con A column that the (-) type as demonstrated by the greater percentage of radioactivity bound to the column. Although Wiese demonstrated that both mating types were agglutinable by 0.01% Con A, only the (+) mating type was affected by 0.001% Con A, blocking agglutination when added to the (-) gamete. This may indicate that the (+) mating type has a greater amount of Con A receptors or that the steric configuration of these receptors is such that increased binding to an insoluble Con A support is possible. Solubilization of the membranes to release the proteins may also affect the steric configuration in favor of exposing more receptor sites.

The protein elution profile (O.D. at 280 nm) indicated that a higher percentage of protein had bound than that measured by radioactive labelling. This may be explained by the occurrence of Con A leaching which has been demonstrated in other investigations employing affinity chromatography even after extensive washing. Although Con A would lead to an increased absorbance at 280 nm, it would not lead to an increase in radioactivity. Quantitation of bound membrane proteins is feasible using radio-iodination, where just monitoring the optical density at 280 nm may give erroneous results.

Recovery of proteins eluted from the Con A agarose column proved to be very difficult. Removal of DOC from the eluted fractions by several methods reported successful by other investigators were not

successful in this investigation (63, 64). The addition of absolute ethanol at -20 ^{o}C should have precipitated the proteins leaving the DOC in solution. When absolute ethanol was added to the combined fractions a precipitate did form. The precipitate, however, was gummy, impossible to resolubilize, and in far too great a quantity to be entirely protein. Affinity chromatography with SDS was tried because lyophylization of the combined fractions was all that was necessary to prepare the sample for PAGE analysis. Due to the substantial amounts of Con A obtained in the combined fractions (Con A leaching), bands corresponding to membrane proteins could not be identified in polyacrylamide gels of these fractions. Gombos (76) described a method for affinity chromatography which employs SDS and substantially reduces the amount of Con A leaching, if not completely eliminating it. Recovery of these proteins for PAGE analysis could provide some valuble information for the role of these proteins in flagellar membrane activity. This would also provide yet another tool for comparison of vegetative and gametic flagellar proteins. For example, an analysis of membrane proteins from vegetative cells of D. discoideum demonstrated that 90% of the activity for alkaline phosphatase, 5' nucleotidase, and cAMP phosphodiesterase bound to a Con A Sepharose column. As cells acquired aggregation competence, the percentage of total activity bound for each enzyme decreased (67). In another investigation, Yoshimoto et al. (77) found that 90% of the biologically active placental human chorionic gonadotropin (HCG) bound to Con A Sepharose, but 90% of liver or colon

HCG (not biologically active) did not bind; they felt that additional carbohydrate moieties (indicated by the greater binding) might account for the biologic activity. A mechanism similar to one of those just described could be involved with the differentiation of Chlamydomonas from the vegetative to gametic state. In investigations of C. reinhardtii, PAGE reveals no differences between vegetative and gametic cells. However, PAGE may not reveal a quantitative change in the addition of a carbohydrate moiety to an already existing glycoprotein.

Polyacrylamide gel electrophoresis was performed on various components of (+) and (-) cell types in an attempt to finger print the banding patterns obtained under the conditions used in this investigation. The importance of this was twofold: 1) to try to establish any obvious differences between mating types or between vegetative and gametic cells within the same mating type. 2) to establish a basis of comparison for PAGE analysis of unbound and eluted fractions from Con A affinity chromatography.

It has been previously demonstrated in C. reinhardtii by electrophoretic analysis that proteins isolated from flagellar membranes and from gamone are identical (68). In investigations of vegetative and gametic gamone of C. reinhardtii (8) electrophoretic analysis indicates a similarity between vegetative and gametic gamones of both mating types. The only difference was a unique protein band detected in the gametic gamone of the (+) mating type that was not present in any other preparations. Since the protein was not present in flagellar membranes,

Snell (8) suggests that it is not related to the mating reaction. The electrophoretic banding pattern obtained by Snell of all gamone types revealed a major band of relatively high molecular weight corresponding to membrane proteins, and two bands corresponding to mastigonemes and tubulin, respectively. Similar results were obtained by Bergman et al. (6).

In this investigation, electrophoretic analysis of several molecular weight standards indicated that the major proteins from all gamone types of C. moewusii are glycoproteins of relatively high molecular weight. In contrast to the gamone isolated from C. reinhardtii where the presence of mastigonemes was clearly indicated, it appears that none of the protein bands obtained from the gamone of C. moewusii correspond to mastigonemes. This was concluded from the following: 1) the electrophoretic banding pattern obtained for mastigonemes showed no similarities to that of gamone; 2) negetative stains of gamone isolated from C. moewusii showed no mastigonemes whereas negative stains of the gamone from C. reinhardtii clearly showed the presence of mastigonemes; and 3) McLean et al. (69) indicated that mastigonemes were easily lost during the gamone preparations.

Unlike the similarity observed between vegetative and gametic gamone of C. reinhardtii, the results of this investigation indicated differences between vegetative and gametic gamone within mating types of C. moewusii and a degree of similarity within vegetative and gametic cells of both mating types. Although one expects to find differences

in protein composition between C. reinhardtii and C. moewusii, the fact that no differences are observed between vegetative and gametic cells in one species and several differences observed in the other species leaves many questions. The sensitivity of the electrophoretic analysis used in this investigation may need to be increased, perhaps by employing gradient slab electrophoresis. Less sample is required and better resolution is generally obtained. It seems unusual that membrane preperations in other cell systems contain numerous proteins (62, 64, 65), yet investigations in Chlamydomonas to date reveal very few (6, 8).

A more sensitive gel system combined with affinity chromatography and analysis for enzyme activities could provide some interesting clues as to the mechanism of recognition and adhesion in Chlamydomonas.

In conclusion, the results of this investigation have indicated that there may be differences in the protein composition of gamone between the vegetative and gametic cells of C. moewusii. It has also been demonstrated that a percentage of the proteins isolated from gametic gamone of both mating types are specifically bound to a Con A agarose column. A difference between mating types with regard to the quantity or physical state of Con A receptors may be indicated by the percentages of bound radioactivity obtained for each mating type.

REFERENCES

1. Cauldwell, C.B., Henkart, P. and Humphreys, T. (1973). "Characterization of sponge aggregation factor. A Unique Proteoglycan Complex." BIOCHEM. 12: 3051-3055.

2. Bretting, H., Kabat, E.A., Liao, J. and Pereira, M.E.A. (1976). "Purification and Characterization of the Agglutins from the Sponge Aadaptos Papillata and a Study of their Combining Sites." BIOCHEM. 15: 5029-5038.

3. Hausman, R.E., and Moscona, A.A. (1976). "Isolation of Retina-Specific Cell-Aggregating Factor From Membranes of Embryonic Neural Retina Tissue." PROC. NATL. ACAD. SCI. USA 73: 3594-3598.

4. Oppenheimer, S.B. and Humphreys, T. (1971). "Isolation of Specific Macromolecules Required for Adhesion of Mouse Tumour Cells." NATURE 232: 125-127.

5. Meyer, J.T. and Oppenheimer, S.B. (1976). "The Multicomponent Nature of Teratoma Cell Adhesion Factor." EXP. CELL RES. 102: 359-364.

6. Bergman, K., Goodenough, U., Goodenough, D.A., Jawitz, J., and Marhn, H. (1975). "Gametic Differentiation in Chlamydomonas reinhardtii. II. Flagellar Membranes and the Agglutination Reaction." J. CELL BIO. 67: 606-622.

7. Wiese, L. (1965). "On sexual agglutination and mating-type substances (gamones) in isogamous heterothallic Chlamydomonads. I. Evidence of the identity of the gamones with the surface components responsible for sexual flagellar contact." J. PHYCOL. 1: 46-54.

8. Snell, W.J. (1976). "Mating in Chlamydomonas. A system for the Study of Specific Cell Adhesion. I. Ultrastructural and Electrophoretic Analysis of Flagellar Surface Components Involved in Adhesion." J. CELL BIO. 68: 48-69.

9. Snell, W.J. (1976). "Mating in Chlamydomonas: A system for the Study of Specific Cell Adhesion. II. A Radioactive Flagella-Binding Assay for Quantitation of Adhesion." J. CE.. BIO. 68: 70-79.

10. Honda, H. and Miyake, A. (1976). "Cell to cell Contact by Locally Differentiated Surfaces in Conjugation of Blepharisma." DEV. BIO. 52: 221-230.

11. Miyake, A. and Beyer, J. (1974). "Blepharmone. A Conjugation-inducing Glycoprotein in the Ciliate Blepharisma." SCIENCE 185: 621-623.

12. Braun, V. and Miyake, A. (1975). "Composition of Blepharmone, a Conjugation-inducing Glycoprotein of the Ciliate Blepharisma." FEBS LETT. 53: 131-134.

13. Vacquier, V. D. and Moy, G. W. (1977). "Isolation of Bindin: the Protein Responsible for Adhesion of Sperm to Sea Urchin Eggs." PROC. NATL. ACAD. SCI. USA 74: 2456-2460.

14. McClay, D. R. and Moscona, A. A. (1974). "Purification of the Specific Cell-Aggregating Factor from Embryonic Neural Retina Cells." EXPTL. CELL RES. 87: 438-443.

15. Lloyd, C. D. and Cook, G. M. W. (1975). "A Membrane Glycoprotein-containing Fraction which promotes Cell Aggregation." BIOCHEM. & BIOPHYS. RES. COMM. 67: 696-700.

16. Forster, H., Wiese, L., and Braunitzer, G. (1956). "The Gynogamone of Chlamydomonas eugametos." A NATURFORSCH B11, 315-317.

17. Wiese, L. and Shoemaker, D. W. (1970). "On sexual agglutination and mating-type substances (gamones) in isogamous heterothallic Chlamydomonads. II. The effect of Concanavalin A upon the mating-type reaction." BIOL. BULL 138: 88-95.

18. Wiese, L. and Hayward, P. C. (1972). "On sexual agglutination and mating-type substances (gamones) in diocedious Chlamydomonads. III. The sensitivity of sex cell contact to various enzymes." AMER. J. BOT. 59: 530-536.

19. Muller, W. E. G. and Zahn, R. K. (1973). "Purification and Characterization of a Species-specific aggregation Factor in Sponges." EXPTL. CELL RES. 80: 95-104.

20. Henkart, P., Humphreys, S., Humphreys, T. (1973). "Characterization of Sponge Aggregation Factor. A Unique Proteoglycan Complex." BIOCHEM. 12: 3045-3050.

21. Burridge, K. (1976). "Changes in Cellular Glycoproteins After Transformation: Identification of Specific Glycoproteins and Antigens in Sodium Dodecyl Sulfate Gels." PROC. NATL. ACAD. SCI. USA 73: 4457-4461.

22. Gahmberg, C.G. and Hakamori, S. (1974). "Altered Growth Behavior of Malignant Cells Associated with Changes in Externally Labelled Glycoprotein and Glycolipid." PROC. NATL. ACAD. SCI. USA 70: 3329-3333.

23. Hynes, R.O. (1973). "Alteration of Cell-Surface Proteins by Viral Transformation and by Proteolysis." PROC. NATL. ACAD. SCI. USA 70: 3170-3174.

24. Hogg, N.M (1974). "A Comparison of Membrane Proteins of Normal and Transformed Cells by Lactoperoxidase Labelling." PROC. NATL. ACAD. SCI. USA 71: 489-492.

25. Yamada, K.M. and Weston, J.A. (1974). "Isolation of a Major Cell Surface Glycoprotein from Fibroblasts." PROC. NATL. ACAD. SCI. USA 71: 3492-3496.

26. Yamada, K.M., Yamada, S.S., and Pastan, I. (1976). "Cell Surface Protein Partially Restores Morphology, Adhesiveness, and Contact Inhibition of Movement to Transformed Fibroblasts." PROC. NATL. ACAD. SCI. USA 73: 1217-1221.

27. Bussell, H.R. and Robinson, W. S. (1973). "Membrane Proteins of Uninfected and Rous Sarcoma Virus--Transformed Avian Cells." J. VIROLOGY 12: 320-327.

28. Yamada, K.M., Schlesinger, D.H., Kennedy, D.W., and Pastan, I. (1977). "Characterization of a Major Fibroblast Cell Surface Glycoprotein." BIOCHEM. 16: 5552-5559.

29. Yamada, K.M., Yamada, S.S., and Pastan, I. (1977). "Quantitation of a transformation-sensitive, adhesive cell surface glycoprotein." J. CELL BIO. 74: 649-654.

30. Yamada, K.M. and Weston, J.A. (1975). "The Synthesis, Turnover, and Artificial Restoration of a Major Cell Surface Glycoprotein." CELL 5: 75-81.

31. Chen, L.B., Gallimore, P.M., and McDougall, J. (1976). "Correlation Between Tumor Induction and the Large, External, Transformation-Sensitive Protein on the Cell Surface." PROC. NATL. ACAD. SCI. USA 73: 3570-3574.

32. Yamada, K.M., Yamada, S.S., and Pastan, I. (1975). "The Major Cell-Surface Glycoprotein of Chick Embryo Fibroblasts is an Agglutinin." PROC. NATL. ACAD. SCI. USA 72: 3158-3162.

33. Yamada, K.M., Ohanian, S.H., and Pastan, I. (1976). "Cell Surface Protein Decreases Microvilli and Ruffles on Transformed Mouse and Chick Cells." CELL 9: 241-245.

34. Weiss, P. (1947). "The Problem of Specificity in Growth and Development." YALE J. BIOL. MED. 19: 235.

35. Tyler, A. (1947). "The Problem of Specificity in Growth and Development." GROWTH (Sup) 10: 7.

36. Roseman, S. (1970). "The Synthesis of Complex Carbohydrates by Multiglycosyltransferase Systems and Their Potential Function in Intercellular Adhesion." CHEM. PHYS. LIPIDS 5: 270-297.

37. Crandall, M.A. and Brock, T.D. (1968). "Molecular Aspects of Specific Cell Contact." SCIENCE 161: 473-475.

38. Steinberg, M.S. "The Problem of Adhesive Selectivity in Cellular Interactions." in CELLULAR MEMBRANES IN DEVELOPMENT. M. Locke, ed. Academic Press, New York (1964). pp. 321-366.

39. Steinberg, M.S. (1970). "Does Differential Adhesion Govern Self-Assembly Processes in Histogenesis? Equilibrium Configuration and the Emergence of a Hierarchy Among Populations of Embryonic Cells." J. EXP. ZOOL. 173: 395.

40. Patt, L.M. and Grimes, W.J. (1975). "Ectoglycosyltransferase Acitivity in Suspensions and Monolayers of Cultured Fibroblasts." BIOCHEM. & BIOPHYS. RES. COMMUN. 67: 483-490.

41. Patt, L.M. and Grimes, W.J. (1973). "Cell Surface Glycolipid and Glycolipid Glycosyltransferase of Normal and Transformed Cells." J. BIOL. CHEM. 249: 4157-4165.

42. McLean, R.J. and Bosmann, H.B. (1975). "Cell-cell Interactions: Enhancement of Glycosyl Transferase Ectoenzyme Systems During Chlamydomonas Gametic Contact." PROC. NATL. ACAD. SCI. USA 72: 310-313.

43. Roth, S., McGuire, E.T., and Roseman, S. (1971). "Evidence for Cell-Surface Glycosyltransferases." J. CELL BIO. 51: 536-547.

44. Porter, C.W. and Bernacki, R.J. (1975). "Ultrastructural Evidence for Ectoglycosyltransferase Systems." NATURE 256: 648-650.

45. Allen L. W., Svenson, R. H., and Yachnin, S. (1969). "Purification of Mitogenic Proteins Derived from Phaseolus vulgaris: Isolation of Potent and Weak Phytohemagglutinins Possessing Mitogen Activity." PROC. NATL. ACAD. SCI. USA 63: 334-341.

46. Tomita, M., Osawa, T., Sakurai, Y., and Ukita, T. (1970). "On the Surface Structure of Murine Ascites Tumours. I. Interactions with Various Phytohaemagglutinins." INT. J. CNACER 6: 283-289.

47. Tomita, M., Kurokawa, T., Onozaki, K., Osawa, T., Sakurai, Y., and Ukita, T. (1972). "The Surface Structure of Murine Ascites Tumours. II. Difference in Cytotoxicity of Various Phytoagglutinins Towards Youshida Sarcoma Cells in vitro." INT. J. CANCER 10: 283-289.

48. Presant, C. A. and Kornfield, S. (1972). "Characterization of the Cell Surface Receptor for the Agaricus biophorus hemagglutinin." J. BIOL. CHEM. 247: 6937-6945.

49. Marchalonis, J. J. and Edelman, J. M. (1968). "Isolation and Characterization of a Hemagglutinin from Limulus polyphemus." J. MOL.BIO. 32: 453-465.

50. Ishiyama, I., Takatsu, A., and Gielen, N. (1972). "An Agglutinin from the Sea Snail Dolabella Reacting with a Neuraminic Acid-Containing Structure." HAEMATOLOGIE (Budapest) 6: 109-112.

51. Nicholson, G. L. and Yanagimachi, R. (1972). "Terminal Saccharides on Sperm Plasma Membranes: Identification by Specific Lectins." SCIENCE 177: 276-279.

52. Chowdhury, T. K. "Concanavalin A: An Introduction." in CONCANAVALIN A. T. K. Chowdhury and A. K. Weiss (eds.) Advances in Exp. Medicine and Biology, Vol. 55. Plenum Press, N. Y. 1975. pp. 1-7.

53. Burger, M. M. and Boldberg, A. R. (1967). "Identification of a Tumour Specific Determinant on Neoplastic Cell Surfaces." PROC. NATL. ACAD. SCI. USA 57: 359-366.

54. Burger, M. M. and Martin, G. L. (1972). "Agglutination of Cells Transformed by Rous Sarcoma Virus by Wheat Germ Agglutinin and Concanavalin A." NATURE 237: 9-12.

55. Borek, C. (1973). "Neoplastic Transformation in vitro of a Clone of Adult Liver Epithelial Cells into Differentiated Hepatoma-like Cells Under Conditions of Nutritional Stress." PROC. NATL. ACAD. SCI. USA 69: 956-959.

56. Kalb, A. J. and Lustig, A. (1968). "The Molecular Weight of Concanavalin A." BIOCHEM. BIOPHYS. ACTA 168: 366.

57. McCubbin, W. D. and Kay, C. M. (1971). "Molecular Weight Studies on Concanavalin A." BIOCHEM. BIOPHYS. RES. COMMUN. 44: 101.

58. Goldstein, I. J., Hollerman, C. E., and Merrick, J. M. (1965). "Protein-carbohydrate-interactions. I. The interaction of polysaccharides with Concanavalin A." BIOCHEM. BIOPHYS. ACTA 97: 68.

59. Agrawal, B. B. L. and Goldstein, I. J. (1968). "Protein-carbohydrate-interaction. VII. Physical and Chemical studies on Concanavalin A, the hemagglutinin of Jack Bean." ARCH. BIOCHEM. & BIOPHYS. 124: 218.

60. Reeke, G. N., Jr., Becker, J. W., Cunningham, B. A., Wang, J. L., Yahara, I. and Edelman, G. M. "Structure and Function of Concanavalin A." in CONCANAVALIN A. T. K. Chowdhury and A. K. Weiss (eds.). Advances in Exp. Medicine and Bio., Vol. 55. Plenum Press, N. Y. 1975. pp. 13-29.

61. Surolia, A., Bishayee, S., Ahmad, A., Balasubramanian, K. A. Thambi-Dorai, D., Podder, S. K., and Bachhawat, B. K. "Studies on the Interaction of Concanavalin A with Glycoproteins." in CONCANAVALIN A. T. K. Chowdhury and A. K. Weiss (eds.). Advances in Exp. Medicine and Bio., Vol 55. Plenum Press, N. Y. 1975. pp. 95-111.

62. Zanetta, J. P., Morgan, I. G., and Gombos, G. (1975). "Synaptosomal Plasma Membrane Glycoproteins: Fractionation by Affinity Chromatography on Con A." BRAIN RES. 83: 337-348.

63. Allan, D. and Crumpton, M. J. "Application of Concanavalin A-'Sepharose' to the Purification of Glycoproteins from the Lymphocyte Plasma Membrane." CONANAVALIN A AS A TOOL. Bittiger, H., and Schnebli, H. D., (eds.). John Wiley and Sons, N. Y. 1976. pp. 399-420.

64. Geltosky, J. E., Siu, C. H., and Lerner, R. A. (1976). "Glycoproteins of the Plasma Membrane of Dictyostelium Discoideum during development." CELL 8: 391-396.

65. West, C. W. and McMahan, D. (1977). "Identification of Concanavalin A receptors and Galactose-binding Proteins in Purified Plasma Membranes of Dictyostelium Discoideum." J. CELL BIO. 74: 391-396.

66. Pearlstein, E. (1977). "Isolation and Partial Characterization of Plasma Membrane Glycoproteins from Normal and Transformed Mammalian Cells Employing Plant Lectin Affinity Chromatography." EXP. CELL RES. 109: 95-103

67. Crean, E. V. and Rossomando, E. F. (1977). "Developmental Changes in Membrane Bound Enzymes of Dictyostelium Discoideum Detected by Concanavalin A Sepharose Affinity Chromatography." BIOCHEM. & BIOPHYS. RES. COMM. 75: 488-495.

68. Witman, G. B., Carlson, K., Berliner, J., and J. L. Rosenbaum. (1972). "Chlamydomonas Flagella. I. Isolation and Electrophoretic Analysis of Microtubules, Matrix, Membranes, and Mastigonemes." J. CELL BIO. 54: 507-539.

69. McLean, R. J., Laurendi, C. J., and Brown, R., Jr. (1974). "The Relationship of Gamone to the Mating Reaction in Chlamydomonas moewusii." PROC. NATL. ACAD. SCI. USA 71: 2610-2613.

70. Wiese, L. (1974). "Nature of Sex Specific Glycoprotein Agglutinins in Chlamydomonas." ANN. N.Y. ACAD. SCI. 234: 383-394.

71. McLean, R. J. and Brown, R. M. (1974). "Cell Surface Differentiation of Chlamydomonas During Gametogenesis. I. Mating and Concanavalin A Agglutinability." DEV. BIOL. 36: 279-285.

72. Randall, J., Cavalier-Smith, T., McVittie, A., Warr, J. R., and Hopkins, J. M. (1968). "Developmental and Control Processes in the Basal Bodies of Flagella of C. reinhardtii." SYMP. SOC. DEV. BIO. 26: 43.

73. Lowry, O. H., Rosebrough, N. H., Farr, A. L., and Randall, R. J. (1951). "Protein Measurement with the Folin Phenol Reagent." J. BIOL. CHEM. 193: 265-275.

74. Weber, K. and Osborne, M. (1969). "The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis." J. BIOL. CHEM. 244: 4406-4412.

75. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971). "Electrophoretic Analysis of the Major Polypeptides of the Human Erythrocyte Membrane." BIOCHEM. 10: 2606-2617.

76. Sedita, Nancy (1978). "Electrophoretic Analysis of the Flagellar Components of Chlamydomonas moewusii." Independant Study Project, S. U. C. Brockport.

77. Gombos, G. "Solubilization of Brain Membranes for Affinity Chromatography." in CONCANAVALIN A AS A TOOL. H. Bittiger and H. P. Schnebli (eds.). John Wiley and Sons, New York 1976. pp. 379-385.

78. Yoshimoto, Y., Wolfsen, A. R., and Odell, W. D. (1977). "Human Chorionic Gonadotropin-like Substance in Nonendocrine Tissues of Normal Subjects." SCIENCE 197: 457-577.

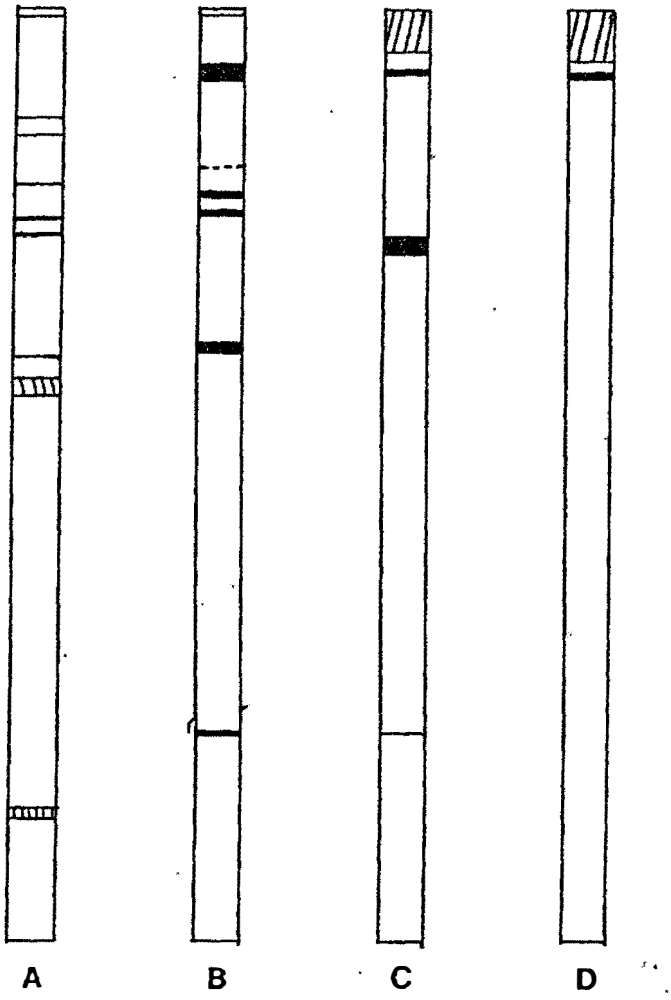


FIG. 1 Effect of CsCl gradient centrifugation on the purity of (+) gametic gamone.

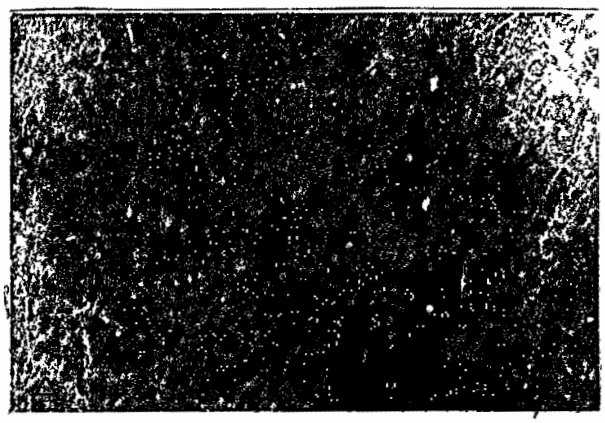
The sample on gels A and B was purified only by layering over 40% sucrose. Gel A was stained with Coomassie Brilliant Blue (CBB); gel B was stained with periodic acid-Schiff (PAS). The sample on gels C and D was further purified by CsCl gradient centrifugation. Gel C was stained with CBB; gel D was stained with PAS.

FIG. 2

A. Mastigonemes negatively stained with 2% PTA in 0.4% sucrose for 30 seconds. X 78,000

B. Whole flagella negatively stained with 2% PTA in 0.4% sucrose for 30 seconds. X 13,000

C. Cell wall material negatively stained with 2% PTA in 0.4% sucrose for 30 seconds. X 5,700



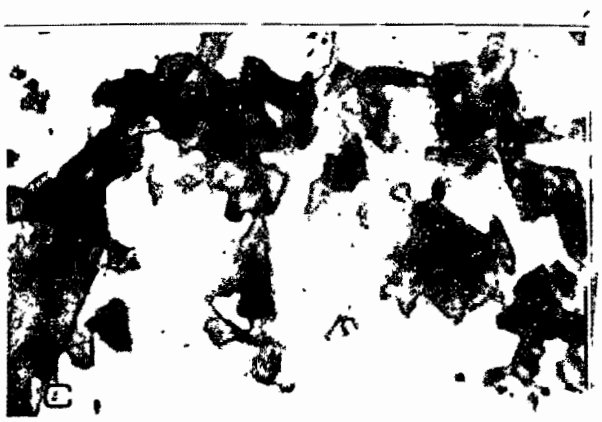


FIG. 2

FIG. 3

A. Negative stain of (+) gametic gamone, stained for 30 seconds with 2% PTA in 0.4% sucrose. X 58,000

B. Negative stain of (+) vegetative gamone, stained for 30 seconds with 2% PTA in 0.4% sucrose. X 58,000

C. Negative stain of (-) gametic gamone, stained for 30 seconds with 2% PTA in 0.4% sucrose. X 58,000

D. Negative stain of (-) vegetative gamone, stained for 30 seconds with 2% PTA in 0.4% sucrose. X 58,000

Arrows indicate the globular material present in all preparations.

FIG. 3

FIG. 4 SDS-polyacrylamide gel electrophoresis of (+) gamone, cell wall material, and mastigonemes

A, B, C-molecular weight standards trypsin, Con A, and gamma-globulin, respectively, stained with Coomassie Brilliant Blue (CBB).

D-vegetative gamone stained with CBB.

E-vegetative gamone stained with periodic acid-Schiff (PAS).

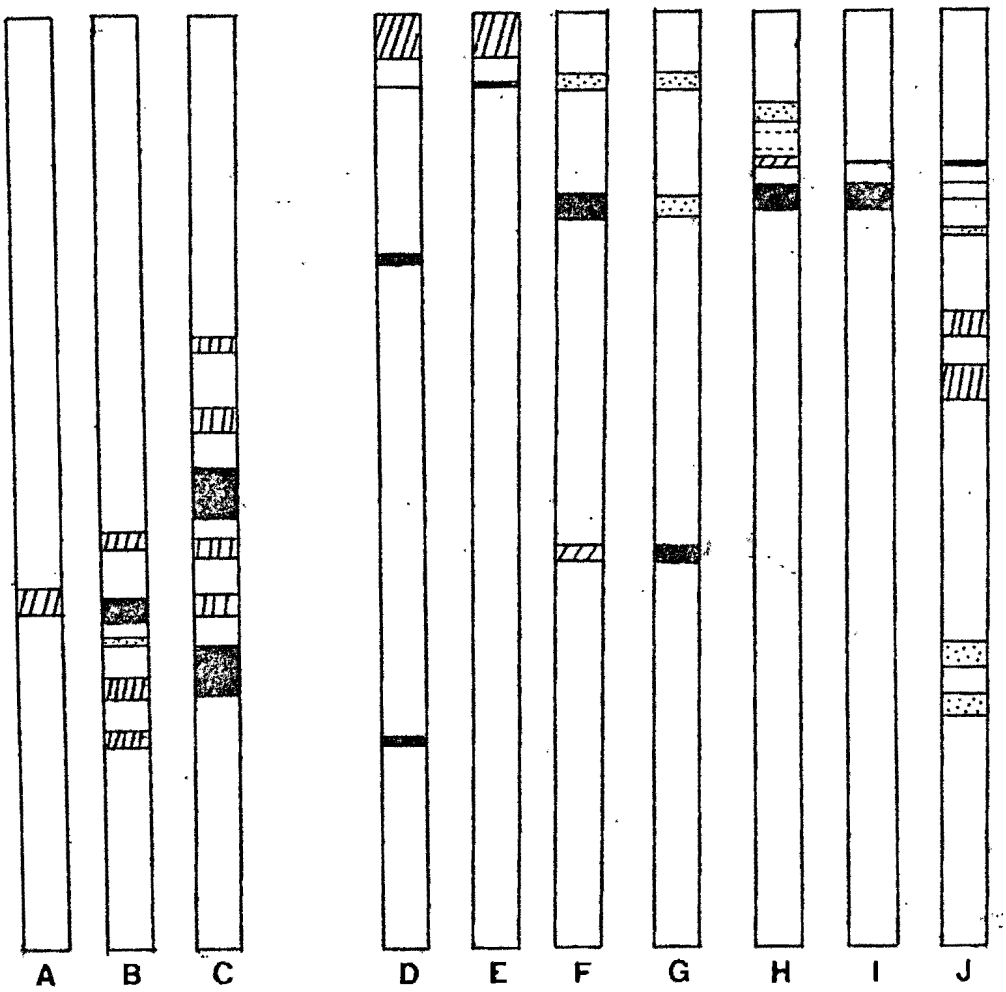
F-gametic gamone stained with CBB.

G-gametic gamone stained with PAS.

H-cell wall material stained with CBB.

I-cell wall material stained with PAS.

J-mastigonemes stained with CBB.



Dark

Light

FIG. 4

FIG. 5 SDS-polyacrylamide gel electrophoresis of (-) gamone and whole flagella

A, B, C-molecular weight standards trypsin, Con A, and gamma-globulin, respectively, stained with Coomassie Brilliant Blue (CBB).

D-whole flagella stained with CBB.

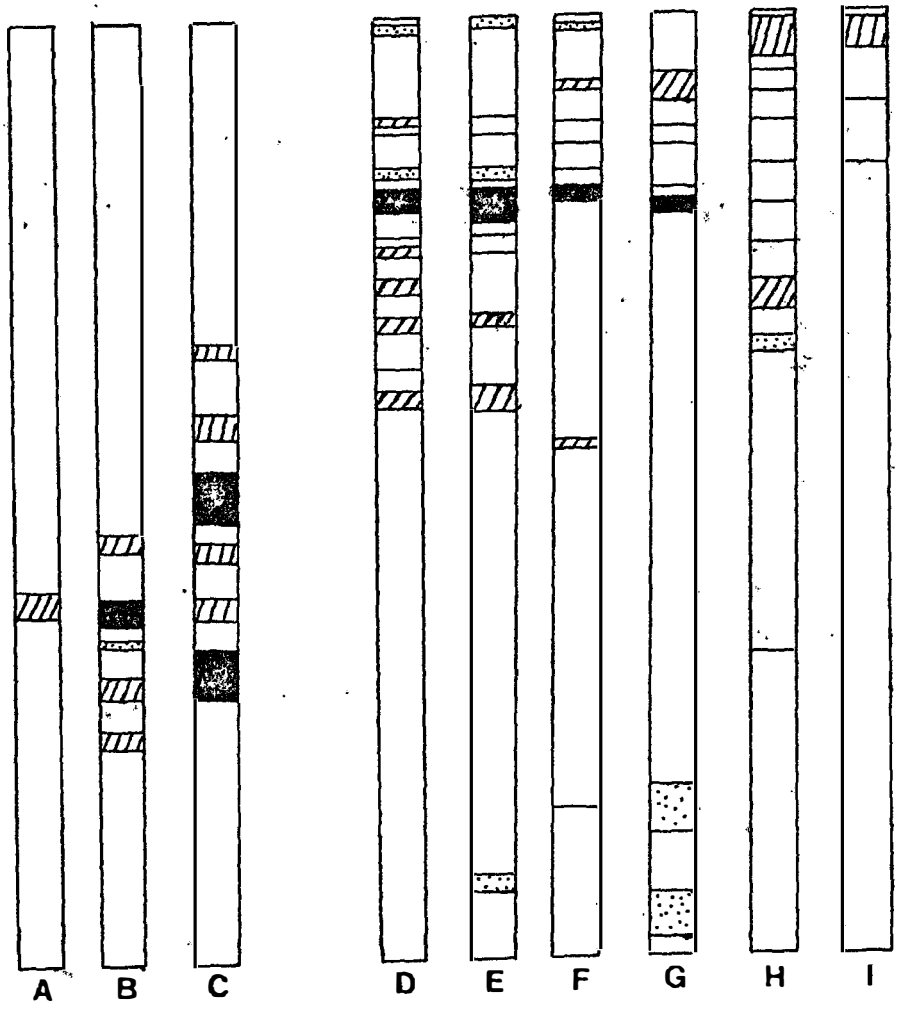
E-whole flagella stained with periodic acid-Schiff (PAS).

F-gametic gamone stained with CBB

G-gametic gamone stained with PAS

H-vegetative gamone stained with CBB

I-vegetative gamone stained with PAS



Dark

Light

FIG. 5

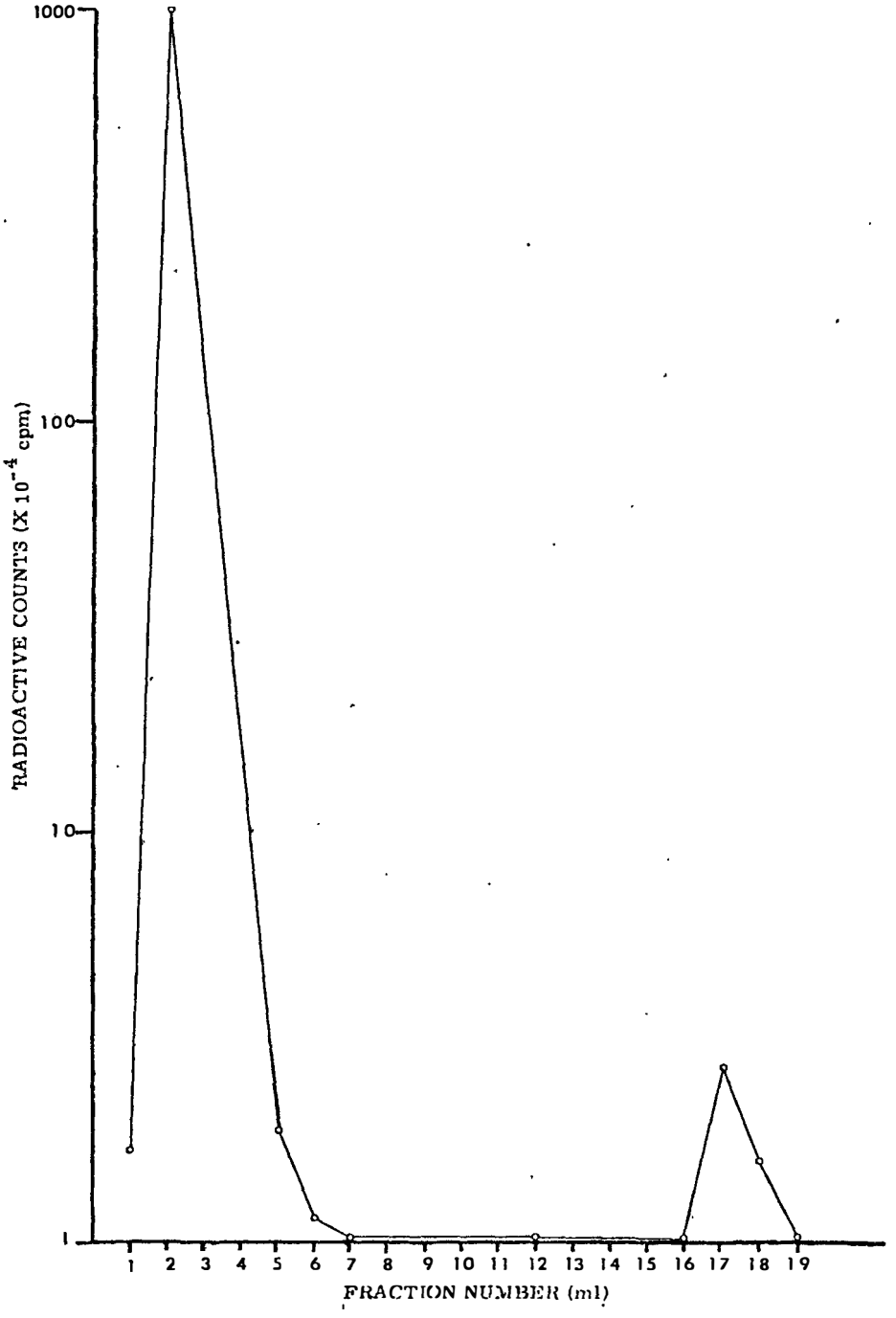


FIG. 6 Radioactive elution profile of $Na^{125}I$ on a Con A affinity chromatography column done under identical conditions as membrane preparations. α-methylmannoside (aMM) was added at fraction number 16.

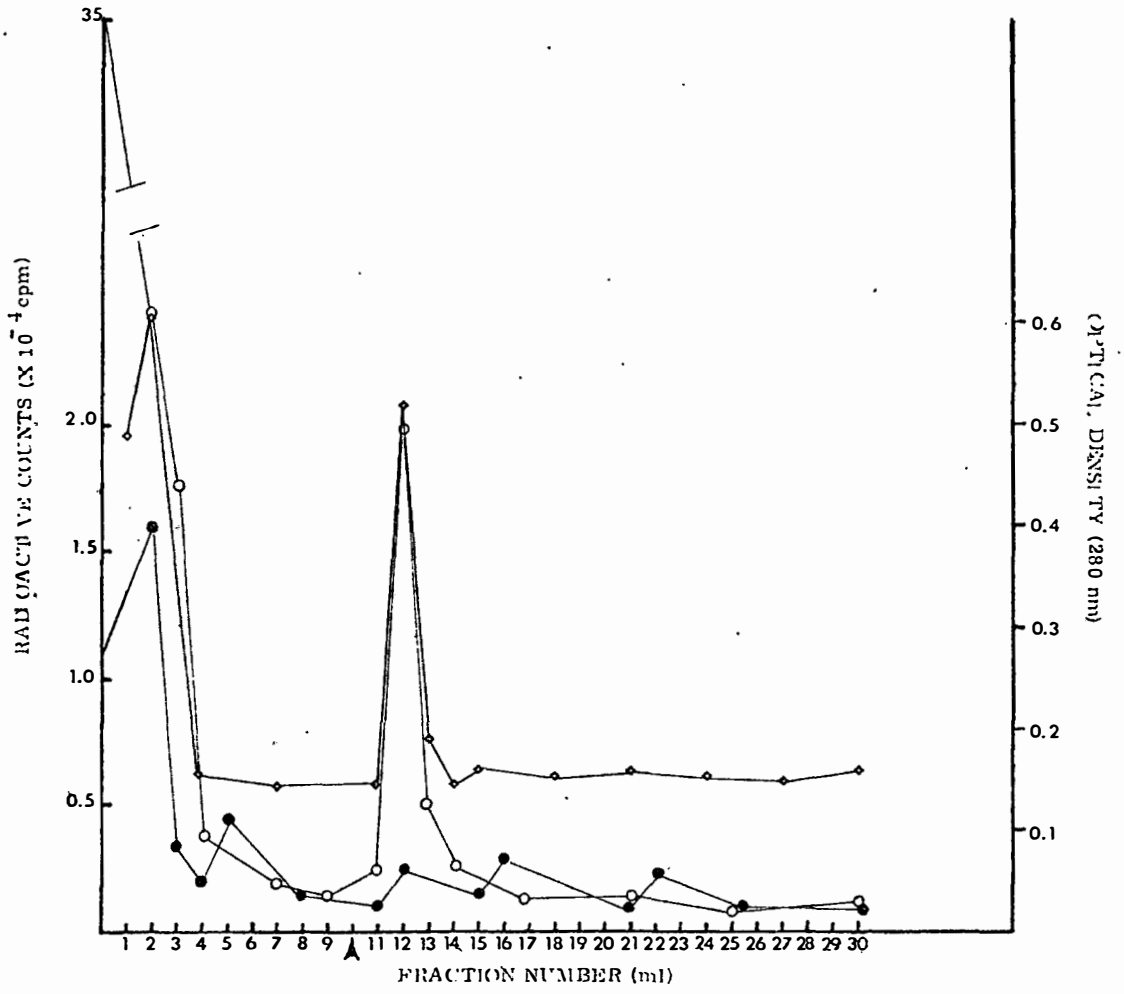


FIG. 7 Elution profiles from Con A affinity chromatography using (+) gametic gamone. o—o is the radioactive elution profile from the sample containing all components required for lactoperoxidase radio-iodination. •—• is the radioactive elution profile for the identical sample lacking lactoperoxidase. ◇—◇ is the protein elution profile corresponding to the sample containing all components for lactoperoxidase iodination. The arrow indicates the last fraction collected before the addition of 2% aMM.

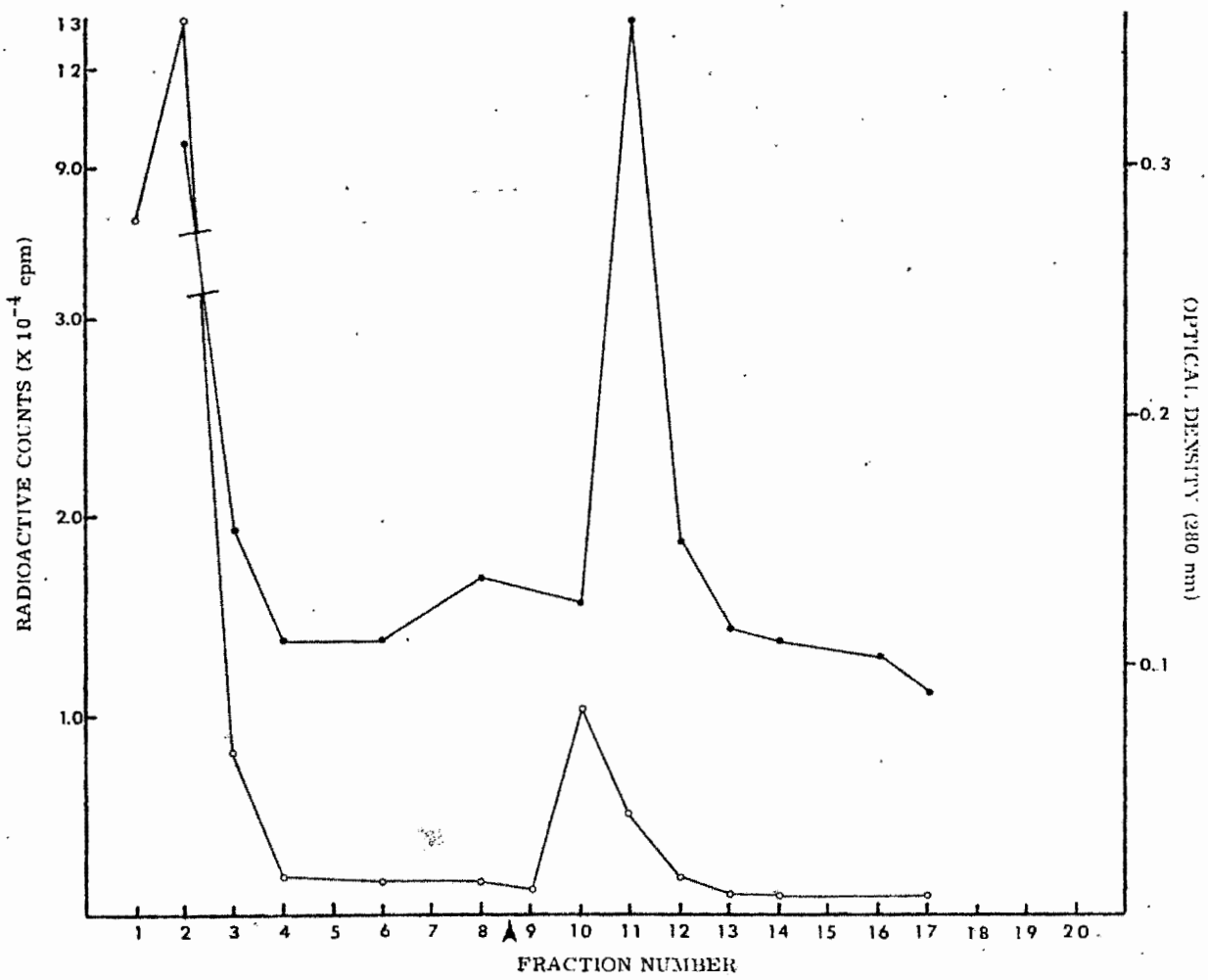


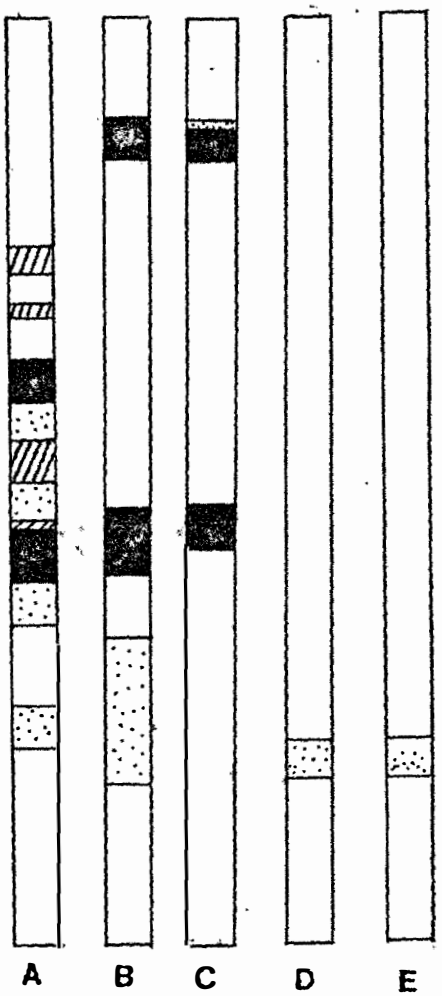
FIG. 8 Elution profile from affinity chromatography of (-) gametic gamone. •—• is the protein elution profile. —○ is the radioactive elution profile. The arrow indicates the addition of 2% aMM.

FIG. 9 SDS-polyacrylamide gel electrophoresis of fractions from an SDS Con A agarose affinity chromatography column.

A- Con A control

B, C- fractions not bound to the Con A

D, E- fractions eluted with 2% α-methylmannoside



Dark

Light

FIG. 9