METHOD USED TO ISOLATE SECRETORY COMPONENT FROM SECRETORY IMMUNOGLOBULIN A

by

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A thesis submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Master of Science

College of Nursing

University of Utah

June 1974

UNIVERSITY OF UTAH GRADUATE SCHOOL

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ACKNOWLEDGMENTS

I express my gratitude to those who have assisted me in the completion of this study: Camilla S. Wood, R.N., Ph.D.; Maeona K. Jacobs, R.N., Ph.D.; and William B. Wiley, Ph.D.

Appreciation is extended to Lester A. Allen, Ph.D., Dean of the College of Biological and Agricultural Sciences, for his support and assistance in the laboratory.

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ABSTRACT

An attempt was made to isolate secretory component from the molecule secretory immunoglobulin A. Two methods were used, each with colostrum as the source of secretory component. In the first method, colostrum was centrifuged, and the supernatant was chromatographed on a Sephadex G-200 column. Half of the eluates were concentrated by negative pressure dialysis, and half of the eluates were chromatographed on a carboxymethyl-50 cation exchange column. All eluates collected were subjected to immunoelectrophoresis. In the second method the colostrum was clarified, and the proteins were precipitated by the addition of ammonium sulfate. The eluates were chromatographed on a DEAE cellulose column using a stepwise elution of three sodium phosphate buffers. Further chromatography included a CM-50 cation exchange column and identification of the proteins by the method of Ouchterlony using rabbit antiserum against secretory IgA.

Free or unattached secretory component was found in the second elution peaks from the Sephadex G-200 gel filtration column and from the first fraction of colostral whey eluted using 0.01M sodium phosphate buffer, at pH 7.6.

Secretory component was highly contaminated with lactoferrin and was not obtained in a pure state.

Failure to obtain spurring between second peak eluates and anti S-IgA could be the result of differences in quarternary structure between the tetrameric free form and the IgA bound form.

INTRODUCTION

Immunoglobulin A is the predominant immunoglobulin in the secretions of the exocrine glands. IgA present in external secretions such as colostrum and secretions of the urinary tract differs structurally from that present in serum. Not only does exocrine IgA or secretory IgA (S-Iga) have a higher sedimentation constant (11S as compared to 7S in serum IgA), but it has a greater molecular weight and contains additional antigenic determinants not present in the serum IgA. The extra antigenic determinants are due to an additional glycoprotein moeity called secretory component (SC). This glycoprotein can either exist as an unbound moeity called "free" SC, or as an integral part of the IgA molecule (1,2,3,4,5,6,7,8,9).

There has been much research on the production sites of SC and IgA. Evidence supporting different production sites for IgA and SC have been reported. First, SC is found in, and thought to be synthesized by, epithelial cells in various parts of the body (10,11). Second, agammaglobulinemic persons have been reported as producing free secretory component, and third, an excess of SC above IgA levels has been shown. Thus, the IgA is believed to be produced in local plasma cells, whereas the SC glycoprotein

molecule is believed formed in the epithelial cells of the exocrine glands (12,13).

The exact function of SC has not been determined. Tomasi has postulated that the component serves as a "transport piece" from the site of production in the lamina propria, through the epithelial cells of the surface of the mucous membrane (14). The biological function of SC binding to IgA molecules is thought to be facilitation of the transport of S-IgA across the mucous membranes into secretions (3), and/or protection of the IgA from proteolytic degradation in the digestive tract (2,15).

Tomasi has reported that since S-IgA bathes the mucous membranes of the body it serves as the first line of defense (14). Thus, a study of the effectiveness of the S-IgA portion of the secretory immune system against urinary tract infections may offer some insight into its etiology and pathogenesis.

In order to quantify the levels of S-IgA in urine it is necessary to have monospecific antisera. Monospecific antisera is required since antisera for S-IgA reacts in an identical manner with both the serum IgA, S-IgA, and free or fragmented alpha chains in the urine. The purpose of this project is to perfect the isolation of SC to produce the antisera for this glycoprotein. This antisera will then be used in conjunction with antisera to S-Iga to identify the whole molecule.

METHODS AND MATERIALS

Method One

Colostrum was collected during the antepartum period. The whole colostrum was refrigerated after collection and processed in the following manner. The lipids and cell fragments were removed by centrifugation at 26,000 Xg for 30 minutes. Lipids and debris were discarded, and the supernatant was divided into 2 ml aliquots and frozen.

Colostral proteins were subjected to Sephadex G-200 gel filtration, carried out in a 40 x 2.5 column, using upward flow elution. The flow rate was maintained between 16-18 ml/hr using the elution buffer, 0.01M Tris pH 8 with 0.14M sodium chloride. The temperature was maintained at 4C. Approximately half of the second peak eluates were concentrated by negative pressure dialysis for immunoelectrophoretic studies. The second peak eluates not concentrated from the Sephadex G-200 column were subjected to a carboxymethyl-50 Sephadex cation exchange column. CM Sephadex cationic exchange was carried out in a 40 x 2.5 cm column, using upward flow elution at a rate of 20-22 ml/hr. The elution buffer used was 0.5M potassium monobasic with 0.05M sodium phosphate dibasic pH 6 and 7, at 4C. Eluates were concentrated by negative pressure dialysis.

The optical density of the eluates was measured at 280nm in a Beckman DB-G grading spectrophotometer and the data plotted as optical density versus fraction number.

Immunoelectrophoresis using special Difco noble agar at a concentration of 1.5% was carried out according to Wood's procedure (16).

Method Two

Colostrum was prepared as described in the first method except the casein was extracted by a pH adjustment to 4.0 with the addition of concentrated hydrochloric acid followed by centrifugation at 30,000 Xg for 30 minutes. The supernatant, now termed clarified colostral whey, was saved and the pH readjusted to 7.4 with 0.5N sodium hydroxide. The proteins were then precipitated with a solution of ammonium sulfate at 50% saturation, at pH 6.5. The precipitate was then dialyzed against 0.01M sodium phosphate at pH 7.4, which was the starting buffer for DEAE column chromatography.

The prepared colostral whey was chromatographed on a DEAE cellulose column using a stepwise elution of three sodium phosphate buffers: (1) 0.01M, pH 7.6; (2) 0.1M, pH 6.4; and (3) 0.3M, pH 4.8. The fraction eluted from 0.1M sodium phosphate buffer, pH 6.4 was further chromatographed on a CM-cellulose column (2 x 2.5 cm) using a

linear gradient between 0.005M and 0.5M sodium acetate buffer, pH 5.0.

The fraction containing IgA was identified through double diffusion, using the method of Ouchterlony, with a rabbit antiserum against S-IgA.

RESULTS

Method One

Sephadex G-200 gel filtration chromatography of clarified human milk produced an elution pattern marked by three or four peaks (Figure 1). The first elution peak of human milk contained S-IgA and at least one component with a larger molecular weight than S-IgA (16). The second elution peak contained free SC, lactoferrin, and several serum proteins including albumin (12). The remaining two peaks contained protein material; however, the identification of these proteins was not done for the purposes of this study.

In Figure 2 the upper wells contained clarified colostral whey, and the lower wells contained the eluates of the second peak. The troughs contained anti S-IgA. The eyebrow arcs seen in the slides exhibited the same electrophoretic mobility as the secretory component identified by Tomasi and Bienenstock (2).

In Figure 3 the upper wells contained first peak eluates, the lower right wells contained anti S-IgA, and the lower left wells contained second peak eluates. After 72 hours the slide showed a precipitation line between the anti S-IgA and first peak eluates but no spurring with

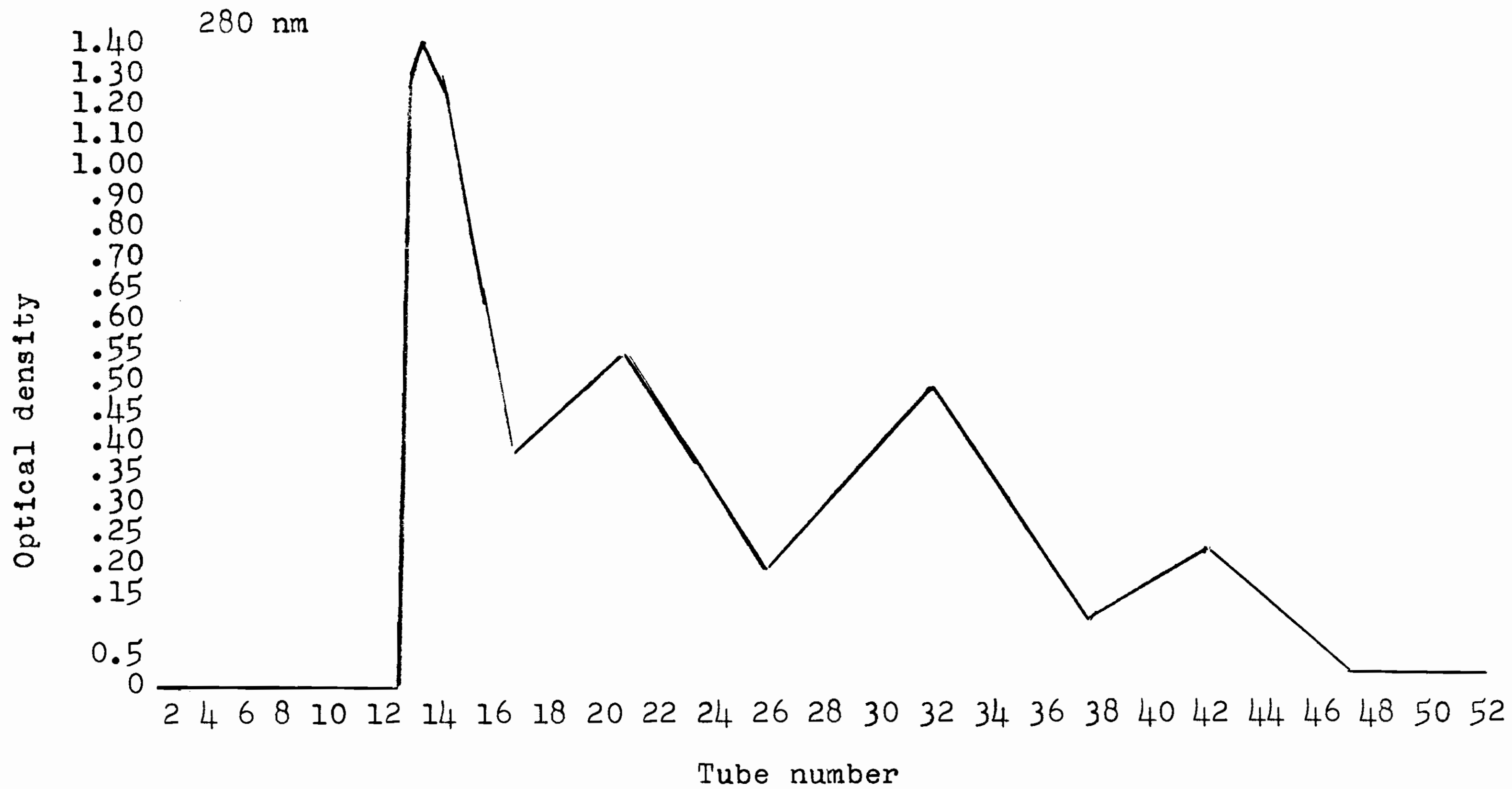

Figure 1 Gel filtration chromatography. The first elution peak contains S-IgA. The second elution peak contains free SC, lactoferrin, and several serum proteins. The remaining two peaks were not identified.

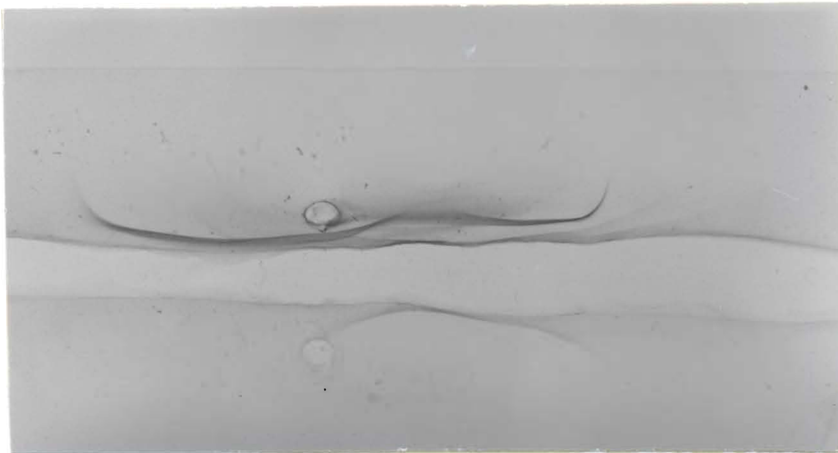

Fig. 2.--Precipitation reaction of clarified colostral whey and second peak eluates. The upper wells contained clarified colostral whey, and the lower wells contained the eluates of the second peak. The troughs contained anti S-IgA.

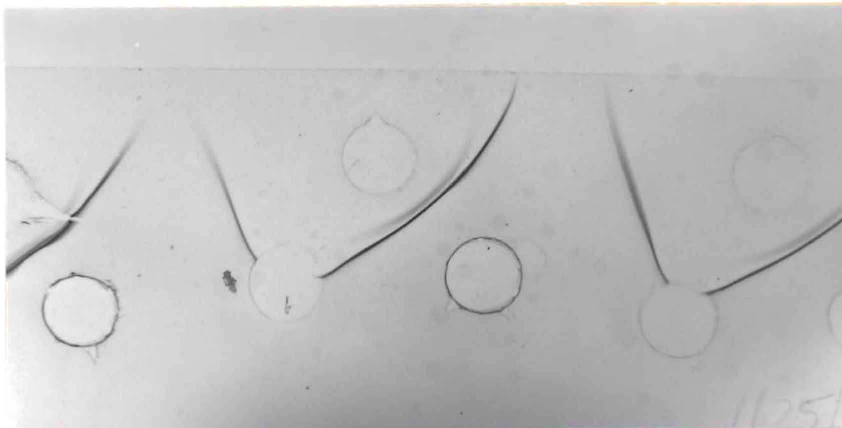

Fig. 3.--Ouchterlony method of double diffusion. The upper wells contained first peak eluates, the lower right wells contained anti S-IgA, and the lower left contained second peak eluates.

this precipitation line and second peak. It is possible that antibodies to the secretory component may have been present but in too small a concentration to show a precipitation line.

Pooled elutions of second peak of human milk, containing free SC and lactoferrin concentrations were used as starting material for the chromatography.

Since there are small differences in elution volume between free SC and lactoferrin, it was difficult to separate these components sufficiently by chromatography on Sephadex G-200. This was also true for the eluates that were subjected to the CM-50 cationic exchange column. Only very small concentrations of protein were obtained.

Method Two

The first fraction of prepared colostral whey eluted from 0.01M sodium phosphate buffer; pH 7.6 from the DEAE cellulose column contained the majority of SC. The second fraction eluted from 0.1M sodium phosphate, pH 6.4, yielded small quantities of S-IgA. The third fraction eluted from 0.3M sodium phosphate, pH 4.8, eluted small amounts of lactoferrin.

The first fraction was further fractionated by the addition of ammonium sulfate to make a 50% saturated solution (17). After discarding the precipitate obtained

through centrifugation at 30,000 Xg for 30 minutes, the supernatant was dialyzed against 0.005M sodium acetate buffer, pH 5.0, and applied on a CM-50 cellulose column against the same buffer. A linear gradient elution between 0.005M and 0.05M sodium acetate buffer, pH 5.0 was used. However, no fractions of protein were collected, possibly due to the small concentrations initially used to begin the linear gradient.

DISCUSSION AND RECOMMENDATIONS

This project was the beginning of a long-term study dealing with the local immune response of the S-IgA molecule and SC against urinary tract infections. The purpose was to isolate SC from S-IgA in order to produce a monospecific antisera for this glycoprotein. Contamination with lactoferrin presented difficulties with the isolation of the SC, and the low immunogenicity of bound SC hampered the production of good antisera to this immunoglobulin component.

Several attempts have been made to release SC from S-IgA in order to make a specific antisera for both the SC and S-IgA, but have not provided conclusive information about the degradation process (1,3,4,5,6,18,13).

In the studies by Munster (1) and Brandzaeg (19) an attempt was made to provide pertinent and important information regarding the degradation process.

Munster reported the antigenic differences between free SC and IgA bound SC to be the result of differences in quarternary structure between the tetrameric free form and the IgA-bound form. Brandzaeg went even further and described the forces stabilizing the quarternary structure of human S-IgA and established conditions for maximum

release of SC with intact antigenicity.

Brandzaeg reported that there is at least one antigenic determinant of free SC which is inaccessible in the bound form and, therefore, may not elicit an antibody response (19). This is termed the I determinant and is apparently the most immunogenic part of the SC molecule (19).

The antigenicity of the accessible part of bound SC does not depend on a single determinant (20). In addition to a specific configurational characteristic, bound SC was found to have two antigenic groups in common with the free component (A_1 and A_2). The conventional antisera, the type I used, whereby reactions are exhibited to secretory components other than SC, for example lactoferrin, show better activity to A_1 than A_2. Since there is better activity to A_1 it is possible that A_2 is partly masked in the bound SC, and it may be visualized as the central region of the molecule (20).

This inaccessible (I) determinant, as well as one of its accessible (A_1) antigenic groups, is extremely susceptible to reduction and alkylation. Brandzaeg obtained secretory component free of lactoferrin from purified colostral IgA after reduction with 0.1M B mercaptoethanol followed by alklation (20).

Failure to obtain spurring between anti S-IgA and

second peak eluates could be a result of the conformational characteristics of SC free and bound to IgA. Brandzaeg's method of reduction and alklation of colostral IgA could be one method to explore.

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