Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tcar20

Sulphated Polysaccharides and the Differentiation of the Cellular Slime Mould Dictyostelium Discoideum

Vincenzo P. Chiarugi[a], Mario Del Rosso[a], Renzo Cappelletti[a], Simonetta Vannucchi[a], Cristina Cella[a], Gabriella Fibbi[a] & Pasquale Urbano[a]

[a] Istituti di Patologia Generale e di Microbiologia, Università degli Studi di Firenze, Firenze, Italy

Published online: 30 Jan 2014.

To cite this article: Vincenzo P. Chiarugi, Mario Del Rosso, Renzo Cappelletti, Simonetta Vannucchi, Cristina Cella, Gabriella Fibbi & Pasquale Urbano (1978) Sulphated Polysaccharides and the Differentiation of the Cellular Slime Mould Dictyostelium Discoideum, Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 31:2, 183-189, DOI: 10.1080/00087114.1978.10796742

To link to this article: http://dx.doi.org/10.1080/00087114.1978.10796742

SULPHATED POLYSACCHARIDES AND THE DIFFERENTIATION OF THE CELLULAR SLIME MOULD *DICTYOSTELIUM DISCOIDEUM* *

VINCENZO P. CHIARUGI, MARIO DEL ROSSO, RENZO CAPPELLETTI, SIMONETTA VANNUCCHI, CRISTINA CELLA, GABRIELLA FIBBI and PASQUALE URBANO

Istituti di Patologia Generale e di Microbiologia,
Università degli Studi di Firenze, Firenze, Italy

Received: 28th June 1977

INTRODUCTION

Evidences have been accumulated in our laboratories that glycosaminoglycans (GAGs) play a basic role in the regulation of eukariotic cells: the relative amounts of sulphated and non sulphated polysaccharides of the cell surface vary widely following the transformation of fibroblasts with oncogenic viruses (CHIARUGI *et al* 1974; VANNUCCHI and CHIARUGI 1977) the differentiation of neuroblastoma cultures *in vitro* (AUGUSTI-TOCCO and CHIARUGI 1976) and the removal of the density dependent inhibition of growth in 3T3 cells (CHIARUGI and VANNUCCHI 1976). The results of our experiments point to cell-surface GAGs as regulative elements of growth and modulators of cellular adhesiveness and differentiation (CHIARUGI *et al.* 1975; CHIARUGI 1977). Our interest is attracted at present on the role of GAGs as surface elements controlling cellular recognition and tissue assembly in metazoa: we found that the exposure of the latter molecules is specific for any given organ thus suggesting that the specific recognition of cell surfaces in order to form a stable tissue aggregate could be mediated by GAGs.

We have been interested to extend our studies from fibroblasts to *Dictyostelium discoideum* cultures as the latter model appeared a suitable

* Supported by C.N.R., Rome, Italy.

one in order to study surface phenomena accompanying specific cellular recognition, adhesion and differentiation.

MATERIALS AND METHODS

Maintenance of cultures.

Dictyostelium discoideum (AX2 strain) was a gift of Dr. Piero Cappuccinelli, Sassari, Italy; it was maintained in axenic cultures on HL-5 medium. Vegetative cells were grown in large roller bottles at 22°, and harvested in log growing phase.

Differentiation procedure.

Washed vegetative cells were spread on agar dishes (Agar Noble Difco) to obtain a sparse monolayer, and excess water was drained. Incubation continued until the majority of cells were streaming before slug formation.

Cell labelling.

The cells were labelled with ^{35}S-Inorganic sulphate and ^{3}H-glucosamine (Amersham, Busks, U.K.) at a concentration of the label of 20 μC/ml and 1 μC/ml respectively for 24 hours during log growth phase. An aliquot of growing cells were directely processed for trypsinization and an aliquot was put on agar plates in order to allow the differentiation. After 15/20 hours when the cells reached the streaming condition and aggregation centers were evident the same were treated with trypsin as following.

Trypsin treatment of growing and differentiated cells.

The cells were washed with unlabelled medium and then suspended in a trypsin solution (Difco Trypsin 1/250) at 37° for 10 Min with shaking. The cells were then spun down by certifugation (5000 x g x 15 min) and the supernatants (indicated as trypsinates) and the sedimented cells were processed for biochemical analyses.

Isolation of polysaccharides from the cells and from the trypsinates.

The cells and the trypsinates were digested extensively with pronase (Calbiochem 'Grade II') and then centrifuged at 10.000 x g. The supernatant was made 15% respect to TCA and the precipitate was discarded; to the clear supernatant 2.5 volumes of ethanol were added and the precipitate allowed to develop overnight at —30°. The precipitated polysaccharides were collected, solubilized in distilled water, centrifuged at 10.000 x g and lyophilized.

Chemical analyses of the polysaccharides.

Polysaccharides were subjected to anion exchange chromatography on AGIX2 resin (Bio-Rad), to cellulose acetate electrophoresis (CAE) with various buffers which work in the separation of the polysaccharides by different physico-chemical

principles. Degradation with nitrous acid was carried out as well as sensitivity to different polysaccharidases. The presence of aminosugars in the hydrolysate was revealed with a Jeol Aminoacid Analyser.

Calcium distribution assays.

The cells were labelled with 1 μC/ml of $^{45}Ca\ Cl_2$ (Amersham, Bucks, U.K.) added to the culture media. The cells were allowed to equilibrate and to distribute their calcium for 12 hours, then they were washed once with saline and treated with trypsin as indicated above. The radioactivity of the cell trypsinate and of the trypsinized cells was then measured.

RESULTS

Table I shows that the ectocellular to endocellular ratio of sulphated polysaccharides varies widely during the differentiation, with a shift of the label to an external location. The same event takes place after addition of cAMP to the cultures and it is paralleled by the behaviour of the cellular localization of calcium ions (Table II). The endocellular calcium decreases and the ectocellular calcium increases either after cAMP administration or differentiation of the cultures on agar plates for 15 hours as indicated in Table III.

TABLE I

Distribution of ^{35}S-labelled polysaccharides in the cell trypsinates and in the trypsinized cells of growing and differentiated Dictyostelium discoideum.

	Growing	Differentiated
Exp. 1	1.98	10.62
Exp. 2	2.49	9.25

* The values indicate the ratios between the radioactivities of the trypsinates and of the trypsinized cells expressed as cetyl-pyridinium-chloride (CPC) precipitable C.P.M. after pronase digestion.

TABLE II

Distribution of calcium ions (^{45}Ca) and sulphated polysaccharides (CPC precipitable ^{35}S) in the cell trypsinates and in the trypsinized cells in the early aggregation of Dictyostelium discoideum produced by exogenous cAMP addition.

	Hours after 0	5mM 2	cAMP added 4
^{45}Ca	0.88	1.04	1.74
^{35}S	7.55	9.92	15.82

* Log growing cells prelabelled with ^{45}Ca and ^{35}S were incubated in centrifuge tubes in a roller apparatus at 22°. cAMP was added and cells were sampled after the indicated time intervals. The values indicate the same ratios as in Table I.

TABLE III

Distribution of Ca^{45} in the cell trypsinates and in the trypsinized cells of growing and differentiated Dictyostelium discoideum.

	Growing	Differentiated
Exp. 1	1.41	4.89
Exp. 2	1.54	4.33
Exp. 3	1.32	4.02

* The values indicate the ratios of the radioactivities of the trypsinates and of the trypsinized cells expressed as CPM/mg protein.

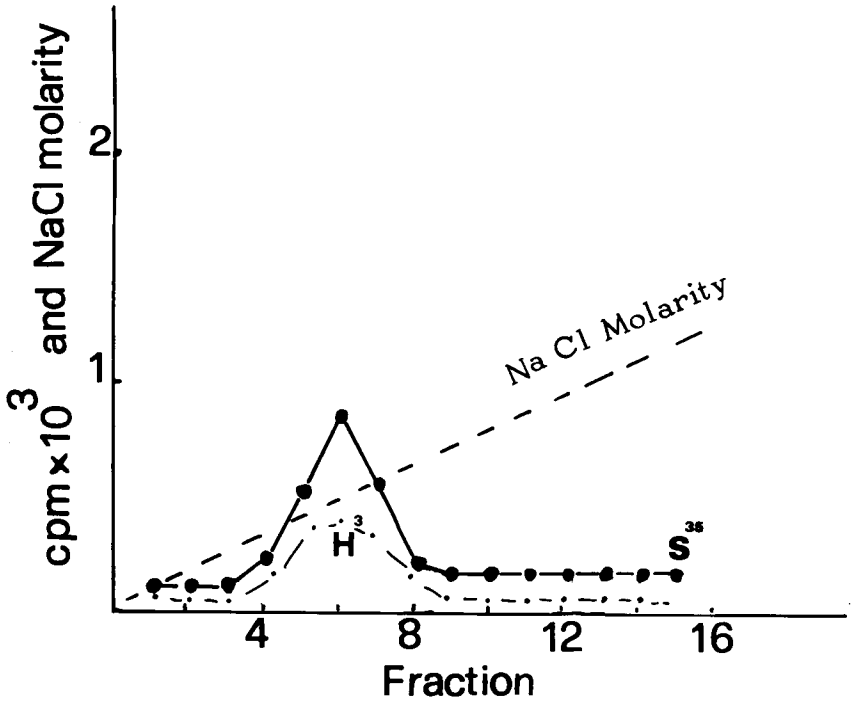

Fig. 1. — Elution of cell surface polysaccharides from *Dictyostelium discoideum* during AG 1 x 2 ion exchange chromatography. The material was labelled with S^{35}-inorganic sulphate and 3H-glucosamine.

The analyses of the GAGs obtained from the ameboe are reported in Fig. 1 which shows that the polysaccharides elute as a single peak from AGIX2 chromatography at a salt concentration ranging from 0.5 to 0.8 M. No differences in the elution molarity of differentiated and

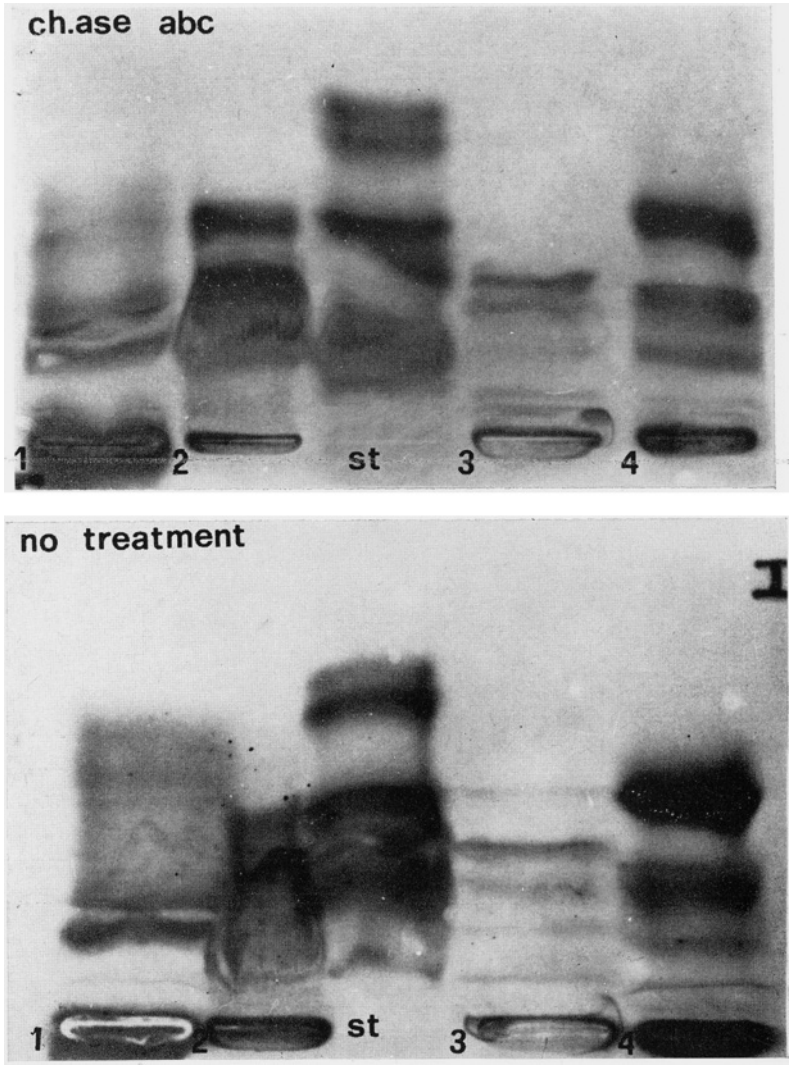

Fig. 2. — Cellulose acetate electrophoresis of unlabelled polysaccharides from *Dictyostelium discoideum* stained with Alcian bleu. 1 and 2). Cell trypsinates from indifferentiated and differentiated cells respectively; st: standards mixture (from the start: heparin, heparan sulphate, hyaluronic acid, dermatan sulphate and chondroitin sulphate A/C); 3 and 4) indifferentiated and differentiated whole cells. Untreated and chodroitinase ABC treated polysaccharides are compared. The standards mixture is untreated in both cases.

undifferentiated cells polysaccharides has been revealed. On the contrary when sulphated polysaccharides obtained from the surface of indifferentiated cells and differentiated cells have been compared with cellulose acetate electrophoresis striking differences have been revealed (Fig. 2). Also the comparison of trypsinized cells in the two conditions revealed strong variations. Some bands present in indifferentiated cells disappear following the differentiation and vice versa. The treatment of the polysaccharides with enzymes that are well known to destroy animal compounds (as chodroitinases ABC and AC and either testicular of Streptomices hyaluronidases) was uneffective. Only chondroitinase ABC destroied a minor faster band of the undifferentiated cells.

DISCUSSION

The results obtained in our approach to *Dictyostelium* could be summarized as follows:

1) Molecules are present either in the cell body or in their trypsinates which are labelled by inorganic sulphate and glucosamine. Being these molecules protease resistant, highly soluble in water, precipitable with cetyl-pyridinium chloride, and stainable with polycationic dyes, we conclude that they are sulphated polysaccharides.

2) We are not familiar enough with these molecules so far to asses them a definite chemical nature. An assay for aminosugars with the Amino-acid Analyser revealed that they contain glucosamine and so possibly they could be included in the glycosaminoglycans family. They ahe however insensitive to animal polysaccharidases.

3) It appeared that during the differentiation an externalization of these molecules takes place and that this event is paralleled by a concomitant externalization of calcium ions.

These findings are in agreement with our previous results obtained on vertebrate cells. Sulphated polysaccharides could play their negative role on growth and their positive role on cellular differentiation via their high ligand properties of Ca-ions, thus influencing the trapping ability of the cell surface towards divalent cations and modulating the intracellular influx of Ca-ions. Probably growing cells need a higher intracellular concentration of calcium ions for mitotic purposes and a lower ectocellular exposure of the same ions to avoid adhesiveness and to maintain the ameboid condition. When the differentiation begins and the cAMP is raised the endocellular concentration of calcium is lowered to allow the assembly of microtubules which fix the cytoscheleton for the differentiation from an ameboid to a

'metazoal' condition. Accordingly a particular adhesiveness of the cell surface is needed for the assemblage of differentiated tissues of the slug. The sulphated polysaccharides this study is concerned with are good candidates to mediate these phenomena. In fact, because of their strong trapping activity, sulphated polysaccharides are thought to play a not secundary role in the regulation of Ca-ions distribution and therefore on cellular control processes. Further studies are obviously needed to clarify the picture emerging from these preliminary results and in particular to asses a definite chemical nature to the sulphated polysaccharides. *Physarum polycephalum* is reported to secrete a slime coat of sulphated poly-galactose of the carragenans family during differentiation (KONIJN and RAPER 1961). Our information on the nature of the polysaccharides which are involved in the differentiation of *Dictyostelium discoideum* is too preliminary but, the possibility that also this mould exposes on the cell surface a polysaccharide of the carragenans family can not be ruled out.

REFERENCES

AUGUSTI-TOCCO G. and CHIARUGI V. P., 1976. — Cell Differentiation, 5: 161-170.
CHIARUGI V. P., VANNUCCHI S. and URBANO P., 1974. — Biochim. Biophys. Acta, 345: 283-296.
CHIARUGI V. P., VANNUCCHI S., DEL ROSSO M. and URBANO P., 1975. — XI International Cancer. Congress Proceedings, 101, 3. Oct.
CHIARUGI V. P. and VANNUCCHI S., 1976. — J. Theor. Biol., 61: 459-475.
CHIARUGI V. P., 1976. — Exptl. Cell Biology, 44: 251-259.
KONIJN T. M. and RAPER K. B., 1961. — Developmental Biology, 3: 725-730.
VANNUCCHI S. and CHIARUGI V. P., 1977. — J. Cellular Physiology, 90: 503-510.

SUMMARY

Cell surface and endocellular polysaccharides of growing and differentiated *Dictyostelium discoideum* have been isolated and characterized with electrophoretic and chromatographyc procedures.

The mould exhibit a very eterogeneous family of sulphated polysaccharides which are externalized during the differentiation.

The possible role of cell surface polysaccharides in the differentiation process is discussed.

RIASSUNTO

I polisaccaridi di superficie del fungo *Dictyostelium discoideum* sono stati isolati e caratterizzati con varie tecniche durante il processo di differenziazione.

È stato osservato che questo microorganismo ha una popolazione molto complessa di polisaccaridi solforati i quali tendono ad essere esposti maggiormente alla superficie cellulare durante la differenziazione.

Il possibile ruolo di questa categoria di molecole nel processo di differenziazione è brevemente discusso.