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An Immunological Assessment of Lysosomal Enzymes and Other Macromolecules Sulfated During Vegetative Growth of *Dictyostelium discoideum*

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Western blotting and immunoprecipitation data indicated that lysosomal enzymes represent a subset of the sulfated macromolecules present in vegetative *Dictyostelium discoideum* amoebae and account for less than 2.5% of the total sulfate incorporated during vegetative growth. These data suggest that the majority of the highly sulfated macromolecules of vegetative *D. discoideum* amoebae are not related to the lysosomal enzymes.

Key words: **sulfated macromolecules, slime moulds, *N*-acetylglucosaminidase, alpha-mannosidase, beta-glucosidase**

The biphasic life cycle of *Dictyostelium discoideum* is facilitating the biochemical analysis of both cell growth and development.

The vegetative growth of bacterially grown, wild-type *D. discoideum* amoebae is arrested when sulfation is inhibited [1]. At least ten macromolecules are sulfated during vegetative growth, and this sulfation declines rapidly during early development [2]. These macromolecules [2] share several of the properties of the lysosomal enzymes of axenic amoebae, which have been extensively characterized [3–5]: the macromolecules are highly sulfated, have acidic isoelectric points and relatively low subunit molecular weights, and are secreted by amoebae during early development. It was of interest, therefore, to determine the relationship between these molecules and lysosomal enzymes in the vegetative growth of wild-type *D. discoideum* amoebae.

D. discoideum, wild-type strain NP73, was grown and was labelled with [^{35}S]sulfate as described previously [1,2]. *N*-acetylglucosaminidase, alpha-mannosidase, and beta-glucosidase were assayed by the use of chromogenic substrates (Sigma Chemical Co., St. Louis, MO) as described by Loomis [6] for determining alpha-mannosidase activity.

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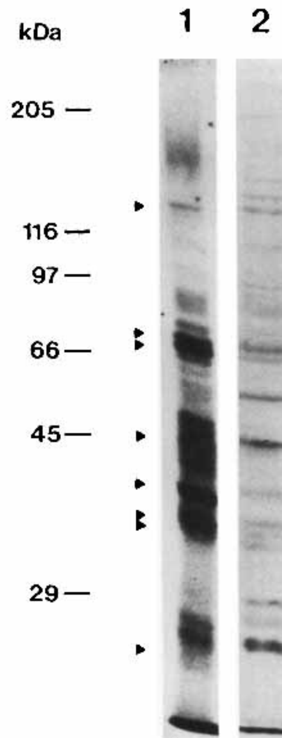

Fig. 1. Western blot analysis of the sulfated macromolecules. After electrophoresis on 12% polyacrylamide gels in the presence of sodium dodecyl sulfate, [^{35}S]sulfate-labelled macromolecules from vegetative *D. discoideum* strain NP73 amoebae were visualized by fluorography (**lane 1**). The same macromolcules separated in another lane of the same gel were transferred to nitrocellulose for an incubation with mLE1 antibody. Macromolecules recognized by the antibody were visualized with a second antibody conjugated to alkaline phosphatase (**lane 2**).

Autoradiographic analysis of macromolecules separated by gel electrophoresis and Western blotting with monoclonal antibody mLE1, generously supplied by Dr. David Knecht (University of California, San Diego), were performed as described [2,5]. The glycosidase activities and [^{35}S]sulfate-labelled macromolecules were precipitated according to the method of Knecht and Dimond [7].

The monoclonal antibody (mLE1) used in this study was first described by Knecht et al. [5] and was obtained by immunising mice with a crude mixture of *D. discoideum* lysosomal enzymes. The antibody has been extensively characterized; in competition studies mLE1 recognizes an antigenic determinant consisting of carbohydrate residues with immunogenicity absolutely dependent on the presence of sulfate [7]. Moreover, this determinant is present on all examined lysosomal enzymes of vegetative amoebae [5,8,9]. However, two-dimensional gel-electrophoretic analyses indicate that the antibody also recognizes a number of other minor sulfated cellular proteins [5]. Therefore, the diagnostic use of the antibody may over estimate the proportion of sulfated macromolecules that are lysosomal enzymes.

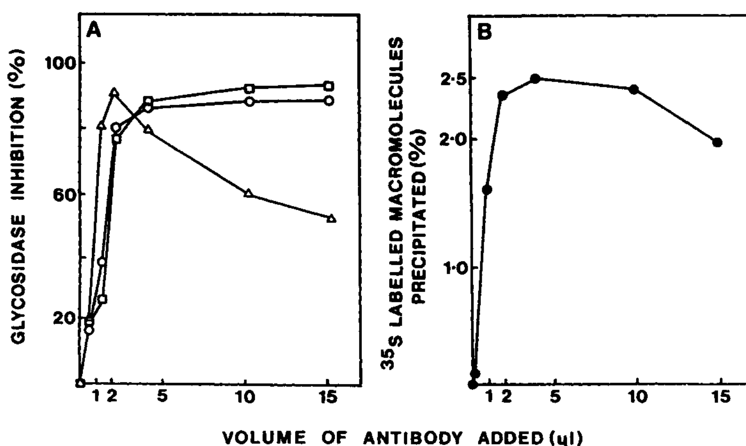

Fig. 2. Immunoprecipitation of lysosomal enzyme activities and [^{35}S]sulfate-labelled macromolecules. Triton X-100 extracts of vegetative *D. discoideum* strain NP73 amoebae exposed to [$^{35}SO_4^-$] were incubated with various quantities of monoclonal antibody mLE1. Antigen-antibody complexes were precipitated with fixed *S. aureus* and loss of activity resulting from immunoprecipitation of *N*-acetylglucosaminidase (○), alpha-mannosidase (△), and beta-glucosidase (□) activities was determined (**A**). The precipitation of [$^{35}SO_4^-$]-labelled macromolecules (●) by antibody was examined by determining the percentage of radioactivity found in the pellet (**B**).

Activities of the three best characterized glycosidases of strain NP73 amoebae during the vegetative growth are two- to tenfold higher than those described for another wild-type strain, NC4 (data not shown [3]). These elevated activities are correlated with the high levels of sulfation observed during vegetative growth of strain NP73 amoebae.

Immunological experiments indicated that sulfate incorporation into the lysosomal enzymes was not a major contributor to high levels of sulfation. First, a Western blot analysis of these [^{35}S]sulfate-labelled macromolecules from vegetative amoebae indicated that although some of the sulfated macromolecules have molecular weights similar to those of proteins recognized by mLE1 (marked with arrows in Fig. 1), the majority of the highly sulfated macromolecules have molecular weights different from proteins recognized by the antibody (Fig. 1). Second, at antibody concentrations sufficient to precipitate approximately 90% of the three lysosomal enzyme activities (Fig. 2A), mLE1 precipitated no more that 2.5% of [^{35}S]sulfate-labelled macromolecules present in vegetative amoebae solubilized with Triton X-100 (Fig. 2B). The percentage of the sulfated macromolecules precipitated by mLE1 after solubilization in boiling sodium dodecyl sulfate solution was the same (2.5%; data not shown), indicating that the antigenic determinant was not masked in undenatured protein.

These data indicate that the majority of highly sulfated macromolecules present in vegetative wild-type *D. discoideum* amoebae are not related to lysosomal enzymes. This is consistent with the observation by Knecht et al. [5] that proteins present in axenic amoebae during vegetative growth and very early development that are recognized by antibody mLE1 constitute a subset of the sulfated proteins present during these phases. It is likely that the larger group of sulfated proteins described by these authors is similar if not identical to the group of highly sulfated macromolecules present in wild-type amoebae [2].

The function(s) of these highly sulfated macromolecules and the relevance of sulfation to function(s) remain to be determined. During early development of *D. discoideum* the synthesis and presumably also the sulfation of lysosomal enzymes increases dramatically [3,10], in contrast to declining sulfation noted for other macromolecules sulfated during vegetative growth. Moreover, the oligosaccharides present on the highly sulfated macromolecules are extremely heterogeneous [11].

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