INCORPORATION OF *N*-ACETYLGLUCOSAMINE INTO THE SLIME SHEATH OF THE CELLULAR SLIME MOULD *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

There are two distinct phases in the life cycle of *Dictyostelium discoideum.* During the growth phase, the slime mould myxamoebae remain solitary and feed on bacteria but, when the food source is depleted, the myxamoebae stop dividing and enter a phase of differentiation [1]. Aggregates of about 10^5 cells are formed and these pass through several morphological stages before finally forming fruiting bodies in which only two cell types (spore and stalk) have been identified. Several theories [2–5] have been proposed recently to explain what sort of signal indicates to a given cell within an aggregate whether to differentiate into a spore or stalk cell. Two of these theories [2,3] involve the slime sheath. The slime sheath contains a polysaccharide consisting of *N*-acetylglucosamine, fucose and mannose [3,6] and is secreted when the aggregate is formed. When development reaches the pseudoplasmodium stage, the sheath forms a tube through which the slug-like pseudoplasmodium migrates towards heat and light. Hence, as the pseudoplasmodium moves forward through the stationary slime sheath, the posterior cells must pass through slime secreted by the anterior cells.

Loomis [3] incubated pseudoplasmodia in *N*-acetyl-[^{14}C]-glucosamine to study slime sheath synthesis and showed by autoradiography that the *N*-acetyl-[^{14}C] glucosamine was incorporated into surface material all over the pseudoplasmodia. He therefore claimed that all surface cells synthesise slime. The present work shows that this incorporation is not indicative of slime synthesis and instead demonstrates activity of an extracellular enzyme in the slime that hydrolyses terminal *N*-acetylglucosamine residues off the slime sheath polysaccharide.

2. Materials and methods

After growth in dual culture with *Aerobacter aerogenes,* myxamoebae of *Dictyostelium discoideum* NC-4 were harvested, washed with water and resuspended at 2×10^8 myxamoebae/ml [7]. A sample containing 2×10^7 myxamoebae was placed on 2% agar along the edge (7 cm) of a piece of Oxoid cellulose acetate elctrophoresis strip. During incubation at 22°C in a light gradient, pseudoplasmodia formed and migrated towards the light and onto the strip. Small pieces of strip bearing pseudoplasmodia were cut out and, for each experiment, five pseudoplasmodia were placed on a 13 mm Millipore Solvinert filter (0.25 μ porosity) resting on a glass (at 20°C) or metal (at 0°C) sinter. 2 mM *N*-acetyl-l-[^{3}H]-acetylglucosamine (10 μCi/μmol) was added to form a droplet on the filter over the aggregates. After incubation the [^{3}H]-acetylglucosamine was removed by suction through the sinter and the pseudoplasmodia were rinsed with 5 ml 2 mM *N*-acetylglucosamine at 0°C. The filters were prepared for scintillation counting by digestion with 30% (v/v) H_2O_2 and 60% (v/v) $HClO_4$ [8].

3. Results and discussion

When pseudoplasmodia were incubated in [^{3}H] acetylglucosamine for 30 min at 20°C, [^{3}H] acetylglucosamine was incorporated into water insoluble material as was shown previously by Loomis [3]. It was not possible to obtain a detailed time course for incorporation but little [^{3}H]-acetylglucosamine was incorporated after 15 min incubation and the incorporations after 45 and 60 min were similar to

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that at 30 min. At 0°C, incorporation was greatly reduced (table 1) suggesting that incorporation at 20°C is enzyme mediated. The lag in incorporation at 20°C may indicate that it takes some time for the [^{3}H]-acetylglucosamine to diffuse through the slime to the enzyme which must be located between the cell mass and the slime sheath.

[^{3}H] acetylglucosamine incorporated during 30 min of incubation was released after 30 min further incubation with [^{3}H] acetylglucosamine to which Pronase and 2,3 dimercaptopropan-1-ol had been added although there was no release of radioactivity during 60 min incubations with [^{3}H] acetylglucosamine alone. This demonstrates that incorporation was into the slime sheath and not into the cells since Pronase in the presence of a sulphydryl compound digests the slime sheath to release disaggregated but undamaged, viable cells [8].

The incorporated [^{3}H] acetylglucosamine was also lost when the incubation was continued with unlabelled acetylglucosamine, virtually all the radioactivity being released within 15 min. This could have occurred had the slime sheath begun to dissolve in the incubation medium during the second 30 min of incubation (i.e. with unlabelled acetylglucosamine), but, since there was no loss of radioactivity during 60 min incubations with [^{3}H] acetylglucosamine, it seems improbable that the slime sheath was dissolving. Instead the experiment would seem to indicate that the enzyme catalysing incorporation will also catalyse exchange between the [^{3}H] acetylglucosamine incorporated into the slime and unlabelled acetylglucosamine in the incubation medium. Such behaviour would be expected of an enzyme normally catalysing the hydrolysis of terminal acetylglucosamine residues off polysaccharides. The enzyme would catalyse exchange only in non-physiological conditions where the reaction was made favourable by incubating the pseudoplasmodia in a high acetylglucosamine concentration. [^{3}H] acetylglucosamine incorporation by such exchange would be limited by the availability of terminal acetylglucosamine residues in the slime polysaccharide as was found in experiment 5.

The presence of the hydrolytic enzyme was demonstrated in the final experiment where the incorporated [^{3}H] acetylglucosamine was lost from the slime sheath when incubation was continued in the absence of acetylglucosamine. Thus neither these experiments nor those of Loomis [3] demonstrated net synthesis of the slime polysaccharide. This was to be expected since nucleotide sugars, not simple sugars, are the

Table 1
Incorporation of [^{3}H] acetylglucosamine by pseudoplasmodia

Experiment	Conditions	[^{3}H] acetylglucosamine incorporated
1	30 min incubation - no pseudoplasmodia	4 ± 0.7
2	30 min incubation	100
3	30 min incubation at 0°C	19 ± 1.1
4	30 min incubation, then 30 min with Pronase + BAL + [^{3}H] acetylglucosamine	4 ± 1.9
5	30 min incubation, then 30 min with fresh [^{3}H] acetylglucosamine	97 ± 7
6	30 min incubation, then 30 min with unlabelled acetylglucosamine	4 ± 1.4
7	30 min incubation, then 30 min without	3 ± 0

5 pseudoplasmodia were incubated with [^{3}H] acetylglucosamine in each experiment except for experiment 1 where five pieces of electrophoresis strip holding no pseudoplasmodia were incubated. In experiment 4, the 2 mM [^{3}H] acetylglucosamine also contained Pronase (1 mg/ml) and 2 mM 2,3 dimercaptopropan-1-ol (BAL). Three determinations were made for each experiment and results are given as a percentage ± SEM. of the mean incorporation in experiment 2 which varied from day to day between 8000 and 10 000 cpm.

precursors for polysaccharide synthesis. Instead these experiments show that there is an extracellular enzyme that can hydrolyse terminal acetylglucosamine residues off the slime sheath polysaccharide.

Loomis [3] showed by autoradiography that the [^{3}H] acetylglucosamine is incorporated into surface material all over a pseudoplasmodium and therefore concluded that all the surface cells synthesise the slime sheath polysaccharide. In consequence the slime sheath should, during migration of the pseudoplasmodium, become thicker at the posterior and present a greater barrier to outward diffusion of metabolites than at the anterior giving rise to concentration gradients along the pseudoplasmodium. Such gradients could induce cells to differentiate into spores at the posterior but stalk cells at the anterior. However, our experiments show that Loomis was not studying slime synthesis and there is, therefore, no experimental evidence to support his theory about the role of the slime sheath.

The hydrolytic enzyme studied was probably the *N*-acetylglucosaminidase which, during development, is secreted by the slime mould cells [10] and increases more than tenfold in specific activity [11]. Since it has now been shown that it may catalyse modification of the slime sheath polysaccharide, it is possible that this is its role in development. Such activity is compatible with Ashworth's proposal [2] that the slime sheath could act as a positional signal to the cells in the pseudoplasmodium if it were structurally modified as it moved backwards along the pseudoplasmodium. If the acetylglucosaminidase does remove terminal *N*-acetylglucosamine residues from the slime sheath polysaccharide, the slime would contain fewer terminal *N*-acetylglucosamine residues at the posterior than at the anterior because of longer contact with the enzyme. Posterior cells would then differentiate into spores rather than into stalk cells as a response to the modified slime.

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