Short Communications

Adenosine 3':5'-Cyclic Monophosphate-Binding Protein from the Cellular Slime Mould *Dictyostelium discoideum*

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During the differentiation of myxamoebae of *Dictyostelium discoideum* strain Ax-2 grown in axenic medium there is a seven- to ten-fold increase in the specific activity of cyclic AMP-binding protein(s). Evidence is presented for the belief that cyclic AMP phosphodiesterase and cyclic AMP-binding protein are distinct molecular species.

When starved of food and placed on a solid substratum myxamoebae of the cellular slime mould *Dictyostelium discoideum* aggregate (Bonner, 1967). Cyclic AMP secreted by the myxamoebae has been identified as the substance that acts as a chemotactic attractant causing this aggregation (Konijn *et al.*, 1968; Bonner *et al.*, 1969), and an extracellular phosphodiesterase appears to be required for modulating the cyclic AMP concentration along the chemotactic gradient (Bonner *et al.*, 1969; Goidl *et al.*, 1972). Both the concentration of cyclic AMP (Bonner *et al.*, 1969; Malkinson & Ashworth, 1973) and the specific activity of a particulate phosphodiesterase (Malchow *et al.*, 1972; Malkinson & Ashworth, 1973) increase just before aggregation.

Aggregating amoebae are more sensitive to the cyclic AMP signal than are vegetative amoebae (Bonner *et al.*, 1969). This would seem to indicate an increase in the amount and/or activity of a cyclic AMP receptor within the myxamoebae. Malchow *et al.* (1972) have postulated that a membrane-bound phosphodiesterase, which increases in specific activity in buffered shaken suspensions of myxamoebae and also just before aggregation (Malkinson & Ashworth, 1973), could be the receptor responsible for this increased responsiveness to cyclic AMP. However, proteins that can bind cyclic AMP but that lack phosphodiesterase activity have been found in a variety of biological systems (see, e.g., Gill & Garren, 1971). In the present communication we describe developmental changes in such a cyclic AMP-binding protein and discuss its possible roles during development.

The growth, conditions for obtained developmental samples and the preparation of cell extracts have been described previously (Malkinson & Ashworth, 1973). Crude extracts were dialysed extensively against

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5 mM-Tris-chloride buffer, pH 7.5, and centrifuged at 15000g for 20 min, and the resulting supernatant was further centrifuged at 100000g for 60 min.

Assay of this final supernatant for cyclic AMP-binding activity was carried out by a modification of the procedure of Gilman (1970) for assaying cyclic AMP. Reaction mixtures (230 μ l) contained 24 pmol of cyclic [3 H]AMP (50000 c.p.m.) (The Radiochemical Centre, Amersham, Bucks., U.K.), 20 μ mol of potassium phosphate buffer, pH 6.4, 1 μ mol of MgSO₄, 60 nmol of dithiothreitol and the supernatant preparation. These were incubated in ice for 90 min and filtered on Sartorius membranes (Sartorius Membranfilter G.m.b.H., Göttingen, W. Germany), which were then placed in 8 ml of liquid scintillant and their bound radioactivities determined. The protein concentration of this supernatant fraction was also determined, by the method of Lowry *et al.* (1951), with dry crystalline bovine serum albumin as a standard.

The assay was linear with respect to protein concentration in the range 0–40 μ g/incubation mixture. The K_a for binding cyclic AMP was 3×10^{-8} – 14×10^{-8} M, which is in the same range as values for similar proteins found in other eukaryotic systems (Cheung, 1972). Cyclic GMP competitively inhibited the binding of cyclic AMP.

Fig. 1 shows the binding activity in pmol of cyclic AMP bound/mg of protein as a function of time of development. The specific activity rises slowly from zero time, reaching a peak at 18 h, and then falls sharply. The peak activity at 18 h in this and other similar experiments is seven- to ten-fold higher than the activity at zero time.

Table 1 compares the properties of the phosphodiesterase and the cyclic AMP-binding protein. There seems no doubt that the cyclic AMP-binding protein that we are assaying is different from the cyclic AMP phosphodiesterase. However, it is known that the assay of one of these activities can be affected by the

Table 1. *Properties of cyclic AMP phosphodiesterase and cyclic AMP-binding protein from D. discoideum*

	Phosphodiesterase (from Malkinson & Ashworth, 1973)	Cyclic AMP-binding protein
Location	Mainly particulate	Soluble
Effect of dithiothreitol	Inhibits activity	Stimulates activity
K_m or K_a for cyclic AMP	10^{-5} M	3×10^{-8} – 14×10^{-8} M
Time of maximum specific activity (h)	9 (aggregation)	18 (culmination)
Effect of cyclic GMP	None	Competitive inhibitor of cyclic AMP binding

Fig. 1. *Variation in specific activity of the cyclic AMP-binding protein during development of myxamoebae of D. discoideum*

Experimental details are indicated in the text. Aggregation occurs at 8–12h and fruiting-body contraction is finished by 25h.

presence in the assay mixture of the other (see, e.g., O'Dea *et al.*, 1971), and thus it is quite conceivable, for example, that the apparent effect of dithiothreitol on cyclic AMP-binding protein is a secondary consequence of its effect on the phosphodiesterase activity. Further fractionation will be necessary before the properties of the cyclic AMP-binding protein(s) can be determined. The timing of the maximum specific activity and the location of the phosphodiesterase in a membrane fraction (Malkinson & Ashworth, 1973) favour its identity with the receptor for extracellular cyclic AMP during aggregation, as suggested by Malchow *et al.* (1972). The increased sensitivity of aggregating cells to cyclic AMP is indicated by a more rapid movement of cells towards the aggregation centre (Bonner *et al.*, 1969), and it is possible that even the twofold increase in specific activity of cyclic AMP-binding protein during aggregation over that at zero time (Fig. 1), coupled with its very high affinity for cyclic AMP, may be sufficient to account

for the changes in cell movement and orientation. Thus both phosphodiesterase and cyclic AMP-binding protein may serve as receptors for cyclic AMP during aggregation.

The peak of cyclic AMP-binding activity at 18h, however, suggests a further role of cyclic AMP in slime-mould development. Bonner (1970) has shown that high concentrations of exogenous cyclic AMP convert single myxamoebae into stalk cells, thereby by-passing the normal morphogenetic sequence and eliminating any differentiation to spore cells. We have found (Malkinson & Ashworth, 1973) a second peak of extracellular cyclic AMP that occurs 19–22h after the start of development. This may reflect extrusion of cyclic AMP from a particular portion of the culminating slime mould, leaving the remaining cyclic AMP to interact with the cyclic AMP-binding protein to effect the differentiation to stalk (and perhaps spore) cells. In this context the increased extracellular concentration of cyclic AMP on the onset of stationary phase during growth (Malkinson & Ashworth, 1972) may help to explain the finding that, when stationary-phase cells and exponential-phase cells are mixed and allowed to aggregate together, the differentiated fate of the cells is dependent on their growth stage (Leach *et al.*, 1973).

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