*THE ACRASIN ACTIVITY OF ADENOSINE-3′,5′-CYCLIC PHOSPHATE**

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The life cycle of the cellular slime mold is marked by a phase of vegetative reproduction and, after the exhaustion of the food supply, a phase of morphogenetic activity. The first visible event to occur after the onset of starvation is the aggregation of the myxamoebae into a mound of cells which subsequently differentiates into a migrating slug and ultimately into a fruiting body of varying complexity. It has been recognized for some time that this first event, the aggregation of cells, is mediated by a chemical messenger which attracts the cells over a distance, presumably by establishing a diffusion gradient against which these cells may orient.[1] The chemical itself has been named acrasin.[1]

Attempts to identify this substance have been reported by Shaffer,[2] Sussman,[3] and Wright,[4] all using the Shaffer assay.[5]

Konijn[6] reported that *E. coli* is able to attract myxamoebae from a distance, and Bonner[7] has suggested that this material may be chemically identical to acrasin. This may indeed be so since amoebae are most sensitive to this attraction just prior to aggregation.[8]

We wish to report that purification of this material, using a different assay,[9, 10] has now progressed sufficiently far to be able to make significant comments upon its chemical structure. We suggest that the attracting compound may very likely be adenosine-3′,5′-cyclic phosphate (3′,5′-cyclic AMP), and that pure, crystalline material obtained from a commercial source shows extremely high activity in the bioassay.

Materials and Methods.—Bioassay: Details of purification and bioassay will be published subsequently, though some information on the assay is already available.[9, 10] Droplets of a suspension of myxamoebae were deposited on hydrophobic agar of defined rigidity such that all cells remained within the margin of the drop. The test solution (0.1 μl) was placed nearby (100–500 μ), and the assay was scored positive if cells moved outside the boundaries of the droplet.

Purification: Purification involved repeated gel-filtration on Sephadex G-10, followed by descending paper chromatography using a solvent system consisting of butanol, acetic acid, water (4:1:5). Finally, the active fraction was run on paper electrophoresis with pyridine and acetate buffer at pH 3.9.

Spectra: Ultraviolet spectra of our purest fraction were obtained on a Cary, model 14 recording spectrophotometer.

Chemicals: 3′,5′-Cyclic AMP and 5′-AMP were obtained from Calbiochem, and Mann Research Biochemicals, respectively. They were of the highest available purity. Sephadex G-10 was obtained from Pharmacia Fine Chemicals.

Strains: E. coli B/r and *D. discoideum* NC-4(H) were used in these experiments.

Results.—Attracting activity was isolated from several strains of *E. coli* and other bacteria. Activity was also found in human urine from both male and female.

TABLE 1

DATA FOR ACTIVE FRACTION AT PRESENT PURITY

Property	Result
Molecular weight by gel-filtration	200–400
Net molecular charge in electrophoresis at pH 3.9	Minus
ϵ_{260} at pH 10.5	270
λ_{max}	259 mμ
Heat stability	High

To date, the following information is available concerning the active component of the *E. coli* extract. Gel-filtration on Sephadex G-10 indicates a molecular weight of between 200 and 400. It is heat stable. Migration of the active band in paper electrophoresis indicates a negative charge for the molecule, even at low pH. Ultraviolet absorption spectroscopy shows a prominent, symmetrical peak at 259 mμ at pH 7. A shift in the shape of the curve is seen at pH 2, although the λ_{max} remains the same. The spectrum at pH 12 is identical to that at pH 7. These data suggest that the absorbing material might be an adenine derivative. The extinction coefficient at 260 mμ (ϵ_{260}) is 270. This indicates that about 2 per cent of our purest material absorbs ultraviolet if it has an ϵ_{260} of 14,200.[11]

Even at this low purity, however, we felt that the data were sufficiently indicative to warrant investigation of several known adenine nucleotides. 5′-AMP gives no activity. 3′,5′-Cyclic AMP has shown activity in our assay at amounts as low as 0.01 mμg. Amounts as low as 2.50 mμg of our purest fraction give similar activity. An estimation of purity from the bioassay is therefore in the same order of magnitude as that from the spectrophotometric data.

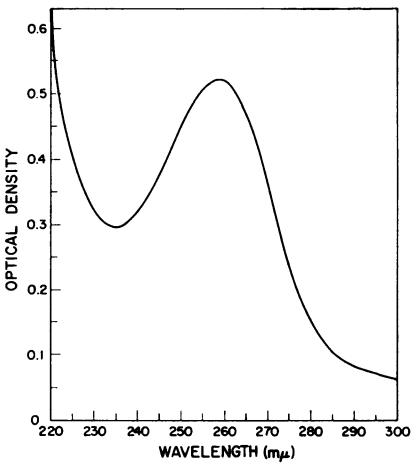

FIG. 1.—Ultraviolet absorption spectrum of purest fraction derived from *E. coli* extract.

Discussion.—It seems to us likely that 3′,5′-cyclic AMP is responsible for the activity from *E. coli*. It is known that *E. coli* produces this material in large quantities.[12] It would be isolated by our procedure, and our assay would detect it. We do not, of course, know whether there are other compounds with similar properties.

It is also interesting to note that we have obtained activity from human urine, and that preliminary data show it to migrate similarly to *E. coli* activity on Sephadex G-10. Human urine contains significant quantities of 3′,5′-cyclic AMP.[12]

We recognize that the attraction of myxamoebae towards an extract of *E. coli* does not identify 3′,5′-cyclic AMP as the chemical messenger which mediates the normal morphogenesis of cellular slime molds, although we consider this likely. We are especially intrigued with the fact that sensitivity to the *E. coli* extract is highest just before aggregation of the myxamoebae. Specificity of aggregation in mixed populations of cells may reside in a mechanism such as differential adhesion of cell membranes, rather than in the messenger itself.

Experiments designed to identify the active fraction of the *E. coli* extract, and the active material in normal slime mold morphogenesis are underway.

Summary.—3′,5′-Cyclic AMP has been found to attract sensitive slime mold myxamoebae. It is active in amounts of 0.01 mμg. It is suggested that this is probably the orientation factor in *E. coli* responsible for the attraction of sensitive myxamoebae. The implications of this finding with reference to slime mold morphogenesis are discussed briefly.

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