PROTEIN AND AMINO ACID TURNOVER DURING DIFFERENTIATION IN THE SLIME MOLD

I. UTILIZATION OF ENDOGENOUS AMINO ACIDS AND PROTEINS

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SUMMARY

During starvation and multicellular differentiation, the slime mold sequentially utilizes endogenous amino acids, ethanol soluble and ethanol insoluble protein. The amino acid pool drops to approximately 30 % of its initial value, the ethanol soluble protein to 60 % and the ethanol insoluble protein to about 80 % of its initial value during the course of differentiation.

INTRODUCTION

The slime mold, *Dictyostelium discoideum*, multiplies as individual myxamoebae by engulfing bacteria on a complex medium[1,2]. When the myxamoebae have consumed the available bacteria, the cells can be washed and spread on purified agar in order to observe the subsequent stages of their life cycle in the absence of any nutrients. In response to a diffusible hormone, the cells aggregate at various points on the plate to form multicellular pseudoplasmodia[2,3]. Further differentiation of this new "organism" proceeds during starvation of its cells. They begin to respire more actively, and to utilize, with increasing intensity, endogenous proteins as a source of energy and building blocks for the synthesis of carbohydrates[4,5]. Histochemical studies[6,7] as well as *in vitro* analyses[8,9] demonstrate that a number of enzymes change in activity during development. The exact extent of these changes is difficult to ascertain, since enzyme activities in cell-free extracts display different degrees of stability, depending on the stage of differentiation from which they are prepared[10].

In order to describe and analyze further some of the biochemical events accompanying morphological differentiation, studies have been carried out on the manner in which endogenous amino acids and proteins are utilized.

MATERIALS AND METHODS

L-[^{35}S]*methionine* was obtained from Abbott Laboratories. Chromatographic and microbiological analyses demonstrated that this material is 100 % L-methionine.

Growth of D. discoideum

The procedures used for growing the slime mold and for following various biochemical changes of the multicellular unit in the absence of growth have been

previously described[11]. In the present studies, the myxamoebae were always grown on *E. coli* strain ML304d which has an absolute requirement for L-methionine. The *E. coli* mutant was grown in a minimal medium supplemented with an excess of L-[^{35}S] methionine (0.1 μmoles/ml) and then mixed with spore heads of *D. discoideum* on complex media in the usual manner. The myxamoebae which subsequently grew were thus labeled by ingestion of the bacteria as well as by the excess labeled methionine present in the complex media. All the experiments to be described were performed after the myxamoebae were washed and incubated on purified agar with no supplements.

Protein and amino acids

These were measured by the biuret reagent[12] and by quantitative ninhydrin determinations[13] respectively.

Methionine assay

E. coli Strain ML304d was used to determine methionine quantitatively. A total volume of 5.0 ml minimal medium supplemented with various amounts of methionine was shaken overnight at 37° in a 50-ml Erlenmeyer flask and the density of the culture read in the Klett-Summerson colorimeter with a blue filter (420 mμ). Under these conditions the growth response to L-methionine is linear at concentrations from 0.005 μmoles to 0.06 μmoles.

Fractionation of cells

The cells were fractionated into the following general classes of compounds: amino acid pool and other small molecules, nucleic acid and carbohydrates, ethanol soluble protein and residual, ethanol insoluble protein. The method of SCHNEIDER, as modified by ROBERTS *et al.*, was used [14,15]. It was found that each class of compounds was essentially homogeneous and uncontaminated by other fractions.

EXPERIMENTAL RESULTS

[^{35}S]methionine was chosen as a specific label of amino acids and proteins since the extensive carbohydrate synthesis from the carbon skeleton of amino acids occurring in the later stages of differentiation obscured results with [^{14}C]amino acids. Preliminary information was obtained for more than one class of protein by extraction of the proteins precipitated by TCA with ethanol. The fate of ethanol soluble protein was investigated because of its preferential utilization in *E. coli* under starvation conditions[16].

Overall loss of ^{35}S during development

During differentiation of [^{35}S]methionine-labeled cells on washed agar, there is a gradual loss of total counts with time per aliquot of harvested slime mold. In order to account for this loss in ^{35}S activity, petri dishes with labeled amoebae were inverted over water, to trap any volatile material. After fruiting had occurred, the counts in the slime mold, the agar and the water were compared to the counts of an original control culture which had been harvested immediately after preparation. The fruits had retained 63 % of the original counts, the agar 36 % and the water trap 2 %. The ^{35}S material in the agar has not yet been identified.

Distribution of [^{35}S]methionine in amoebae

Table I gives the percentage distribution of total counts in the various chemical fractions obtained from amoebae labeled with [^{35}S]methionine (see METHODS). The last experiment was done with an aggregateless mutant obtained from Dr. M. SUSSMAN[17]. Qualitatively similar count distributions have been observed for *E. coli* cells labeled with ^{35}S during exponential growth[15]. In *E. coli*, however, the amino acid pool contains only 2 % of the total counts since methionine is present mainly in the proteins of these cells. The size of the amino acid pool in the slime mold is particularly dependent upon how quickly the cells are harvested after being plated on washed agar, since of the four classes of compounds studied, "free" amino acids begin to be used very early during development. In these experiments, the amoebae were harvested for analysis between one and 3 h after plating.

TABLE I

% DISTRIBUTION OF [^{35}S]METHIONINE IN AMOEBAE

Expt.	*Strain*	*Amino acid pool*	*Et. sol prot.*	*Et. insol. prot.*	*nucleic acid**	*% recovered*
I	Wild	9.0	12.8	67.8	2.7	89
II	Wild	17.0	10.0	56.0	3.3	86
III	Wild	12.3	15.5	71.0	2.1	100
IV	Agg 204	8.7	18.0	80.0	2.6	100

* The radioactivity in this fraction may be attributable to thiomethyl adenosine[11,17].

Specific radioactivity determinations

In order to interpret experiments to be described, it was necessary to determine whether or not methionine was present as a constant fraction of the amino acid pool and of protein at all stages of development. ^{35}S-labeled amoebae were plated on purified agar. At the amoeba, slug and fruit stages a number of plates were harvested, and the cells frozen and homogenized to obtain a cell-free supernatant as well as a precipitate fraction containing the cell debris. Protein which could be obtained from the cell debris fractions gave values similar to those found for the companion supernatant fraction. Table II gives specific radioactivity data for the amino acid pool, the ethanol soluble and ethanol insoluble protein. The data demonstrate that methionine is present as a reasonably constant fraction of the amino acids in the pool and in the two classes of proteins at the slug compared to the amoeba stage, but that significant differences may exist when comparing the slug and fruit stages with respect to the amino acid pool and the ethanol insoluble protein. Chromatographic analysis has

TABLE II

SPECIFIC RADIOACTIVITIES OF PROTEINS AND AMINO ACID POOL

Stage	*Counts/min/mg protein*		*Counts/min/μmole amino acid*
	Et. insol.	*Et. sol.*	*Amino acid pool*
Amoebae	3,630	1,540	955
Slug	3,480	1,270	909
Fruit	4,700	1,230	630

shown that about 80–90 % of the radioactivity in the amino acid pool is methionine, and in the hydrolyzed protein is methionine or its chemical breakdown products. KRIVANEK AND KRIVANEK[18] have shown by paper chromatography that the free amino acid composition (including methionine) of the slime mold is essentially similar at the various stages of development.

In vivo *utilization of amino acids and proteins*

Experiments were carried out to obtain information concerning the relative behavior of the amino acid pool and the two types of protein during starvation and differentiation of the slime mold on washed agar. A typical experiment is shown in Fig. 1. The values are expressed as percent of the initial total radioactivity found for each class of compounds. The stage of development is indicated by the figures on the abscissa. By thus following the radioactivity in the various fractions obtained from cells harvested at progressive stages of development, it appears that the "free" amino acids are utilized first, then the ethanol soluble and finally the ethanol insoluble protein. Considering both classes of protein, these data indicate that the most active proteolysis occurs between the early slug and culmination stages.

The data of Table II indicate that corrections should perhaps be applied to the values for the fruit with respect to the ethanol insoluble protein and the amino acid pool. For the early stages of development, however, the general sequence of utilization of the three classes of nitrogenous compounds is clear.

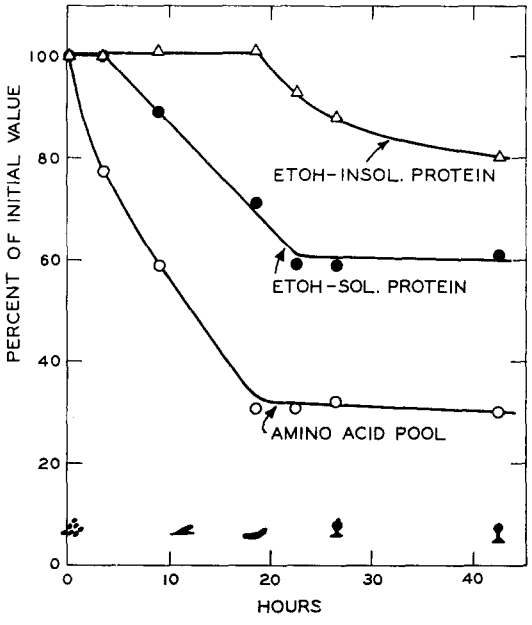

Fig. 1. Utilization of endogenous amino acids and protein during development. The cells were labeled with radioactive methionine and allowed to differentiate in petri dishes on washed agar. The figures on the abscissa indicate the following sequential stages: amoebae, early slug, slug, culmination and fruit. At various times during development, 3 plates (all prepared from the same number of amoebae originally) were harvested and fractionated into the amino acid pool, ethanol soluble and ethanol insoluble protein. Each point is expressed as the percent of the initial radioactivity for that fraction.

DISCUSSION

It is not surprising to find that starvation of the slime molds brings about an early utilization of the amino acid pool. In yeast[19] and *E. coli*[20] the pool amino acids are incorporated into protein during nitrogen starvation. It will be apparent from the accompanying paper that this in part is the fate of "free" amino acids in *D. discoideum*. The preferential utilization of ethanol soluble protein also has its analogy in the metabolism of sulfur-starved *E. coli* cells[16].

In the differentiating slime mold, the utilization of amino acids and protein takes on an added significance, in the sense that it not only accompanies starvation, but is *necessary* in order to obtain the energy and building blocks for multicellular development. Indeed, multicellular development does not proceed except under starvation conditions.

In starving *E. coli* cells, COWIE *et al.*[16] have shown that at least a fraction of the ethanol soluble protein is degraded to amino acids which are incorporated into the residual, ethanol insoluble protein. The soluble protein is "expendable" in the sense that it can be used, without impairing cell viability, to maintain the residual protein under adverse conditions. In the slime molds, too, ethanol soluble protein is apparently used first and, percentagewise, to a greater extent. The next paper in this series will demonstrate that the rate of incorporation of [^{35}S]methionine into ethanol soluble protein is lower than that of the ethanol insoluble protein. Perhaps this relative lack of replenishment accounts at least in part for the early and rapid loss of ethanol soluble protein.

Our data are in general agreement with those of GREGG AND BRONSWEIG on net loss of protein during development[4]. They analysed three stages of differentiation, and found a somewhat greater protein utilization between the pseudoplasmodium and fruit than between the myxamoebae and pseudoplasmodium stages.

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