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**Changes in cellular proteins during development
in the cellular slime mold
*Dictyostelium discoideum***

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Proteins from whole cells of the cellular slime mold *Dictyostelium discoideum* at the different stages of development were analyzed by SDS-polyacrylamide gel electrophoresis. The electrophoretic band patterns of proteins changed strikingly at the pseudoplasmodial stage of development. When the total proteins were fractionated into acid-soluble and -insoluble proteins, followed by the electrophoretic analysis, the band pattern of the former changed significantly at the pseudoplasmodial stage, whereas that of the latter did not. The change of acid-soluble proteins appeared to be due to that in the amount of total proteins observed at the pseudoplasmodial stage.

The cellular slime mold *Dictyostelium discoideum* has become of interest in recent years as a model system for investigation of cell differentiation. It has been reported that the enzyme activity (ROTH and SUSSMAN, 1968; TELSER and SUSSMAN, 1971; LOOMIS, 1975), synthesis of total proteins (ALTON and LODISH, 1977 a, 1977 b) and membrane proteins (SIU *et al.*, 1977) change during development of this microorganism.

In this paper, the electrophoretic patterns of total cellular proteins at several stages during morphogenetic development of *D. discoideum* were compared with each other. The experimental result showed that several protein bands significantly changed during the course of development and the proteins appearing as the major bands were soluble in acetic acid.

Materials and Methods

Amoeba cells of *D. discoideum*, strain NC-4 (haploid) were grown on an agar medium with *Escherichia coli* (IWABUCHI *et al.*, 1971). The cells cultivated for 36 hr were used as the cells of vegetative stage. In order to obtain a high degree of synchrony of morphogenesis, the cells reaching the late log-phase of growth were harvested, washed several times by centrifuga-

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Abbreviation: SDS, sodium dodecyl sulfate.

tion and suspended in SM buffer (1.5 g KH_2PO_4 and 0.62 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per liter, pH 6.4) at a cell density of $5 \times 10^8/\text{ml}$. Usually 1 ml of the cell suspension was dispensed on non-nutrient agar plates, followed by incubation at 23°C . Cells were harvested at the interphase, aggregation, pseudoplasmodial and culmination stages, respectively. The cells were washed several times with ice-cold SM buffer and then frozen in a deep freezer (-30°C).

The frozen cells were lysed by thawing them in ice-cold extraction buffer (20 mM Tris-HCl, 25 mM KCl and 3 mM magnesium acetate, pH 7.6). The cell lysate was centrifuged at $10,000 \times g$ for 10 min at $2 \sim 4^\circ\text{C}$, and the resultant supernatant fluid divided into two parts. To one of them was added an equal volume of 0.1 M sodium phosphate buffer (4.29 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 25.64 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ per liter, pH 7.0) containing 2% SDS and 2% 2-mercaptoethanol. The mixture was incubated for 2 min at 100°C and electrophoresed on SDS-polyacrylamide gels. To the other one were added two volumes of glacial acetic acid and the mixture was stirred for 1 hr at $2 \sim 4^\circ\text{C}$. The acetic acid-insoluble materials were collected by centrifugation at $10,000 \times g$ for 10 min, dried to remove acetic acid and dissolved in the sample buffer (0.1 M phosphate, pH 7.0, 1% SDS and 1% 2-mercaptoethanol). The acetic acid-soluble material was precipitated with ice-cold 10% trichloroacetic acid, pelleted by centrifugation, dried in air and dissolved in the sample buffer. The acetic acid-insoluble and -soluble materials in the sample buffer were incubated for 2 min at 100°C , and electrophoresed on SDS-gels. Electrophoresis on 10% polyacrylamide gels containing 1% SDS was performed as described previously (KANDA *et al.*, 1974; KANDA, 1977).

Results

When total proteins of interphase amoebae were subjected to the electrophoretic analysis, about 40 protein bands could be identified (Fig. 1). For convenience sake, they were numbered according to the order of their electrophoretic mobility. The protein bands of whole cells will be referred to in the text by the number preceded by the letter "W".

Fig. 1 shows that there were some differences in the band pattern of total proteins among five different stages of development. Although there were no appreciable alterations in the band pattern between the vegetative stage and interphase, the staining intensities of the protein bands of W7, W9, W18, W20 and W22 were increased at the aggregation stage. The most striking change occurred at the pseudoplasmodial stage; the staining intensities of three major protein bands (W9, W16 and W18) and two minor ones (W12 and W13) were increased, whereas those of three major bands

In the case of the acid-insoluble proteins (Fig. 3), there was no difference in the electrophoretic patterns under the experimental conditions tested here.

Discussion

The biochemical studies on enzymes of *D. discoideum* have afforded a lot of information about the change of the enzyme activity during development (LOOMIS, 1975). It has been shown that some of the enzymes involved in carbohydrate metabolism are stage-specific (ROTH and SUSSMAN, 1968; TELSER and SUSSMAN, 1971). However, the increase in activity of these enzymes has not always been shown to require concomitant *de novo* synthesis of proteins (KILLICK and WRIGHT, 1972) or prior synthesis of RNA (MIZUKAMI and IWABUCHI, 1970). Recently, ALTON and LODISH (1977 a, 1977 b) reported the pattern of protein synthesis at different stages of development of *D. discoideum*. In their studies, a major change in the relative synthesis rates of a number of proteins was found in the culmination stage.

My experiment indicated that the electrophoretic band pattern of total cellular proteins changed strikingly at the pseudoplasmodial stage. This fact is not substantially inconsistent with the results of the experiments on the enzymes and protein synthesis described above. SIU *et al.* (1977) reported that several membrane proteins with molecular weights of 38,000, 36,500 and 10,000 to 12,000 rapidly accumulated between late interphase and early aggregation stage. At least a major protein, W22, which was increased at this stage, may be the same as the membrane protein of 36,500 daltons.

The present experiment also showed that some of the acid-soluble proteins changed uniquely. The acid-soluble proteins will certainly include a number of ribosomal proteins (KANDA *et al.*, 1974). It seems possible to say that the alteration of the acid-soluble proteins is due to the change of ribosomal proteins. However, preliminary experiments have showed that there were no detectable changes of the ribosomal proteins during *D. discoideum* development. Further experiments are now in progress to clarify the relationship between the morphological change and the quantitative change of the proteins contained in the cell organelle during development of this microorganism.

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(W20, W22 and W23) were decreased. Furthermore, several minor bands (W19', W19'' and W24') appeared newly at this stage. A band pattern similar to that at the pseudoplasmodial stage was observed at the culmination stage.

The molecular weights of proteins seen in the band pattern were determined by co-electrophoresis of several marker proteins as described previously (KANDA *et al.*, 1974). Some of the major proteins, W9, W16 and W18, which were remarkably accumulated at the pseudoplasmodial stage, had the molecular weights of 87,000, 56,000 and 46,000 daltons, respectively. The other major proteins, W20, W22 and W23, which were decreased at the pseudoplasmodial stage, had the molecular weights of 41,000, 36,000 and 31,000 daltons, respectively.

The total cellular proteins were separated into the acid-soluble and -insoluble fractions by the fractionation method with acetic acid, and the two protein fractions were analyzed by SDS-gel electrophoresis. Generally speaking, the acid-soluble proteins are basic proteins including the histone or

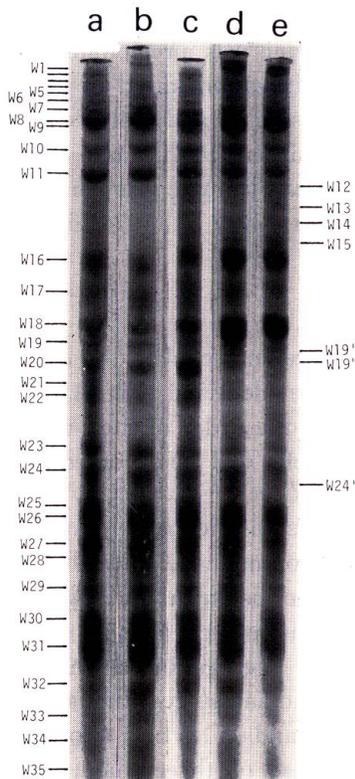


Fig. 1. Electrophoretograms of whole-cell proteins in the developmental cycle. Cells were harvested at the vegetative stage, interphase, aggregation stage, pseudoplasmodial stage and culmination stage. Whole-cell proteins were prepared as described in the text and electrophoresed on 10% polyacrylamide-gels containing 1% SDS as described previously (KANDA *et al.*, 1974; KANDA, 1977). Electrophoresis was performed at a constant current of 5 mA/gel and room temperature. After electrophoresis, gels were stained with 0.2% Comma'sie brilliant blue in 7% acetic acid-50% ethanol and destained in the same acetic acid-ethanol mixture. a, vegetative stage; b, interphase; c, aggregation stage; d, pseudoplasmodial stage; e, culmination stage.

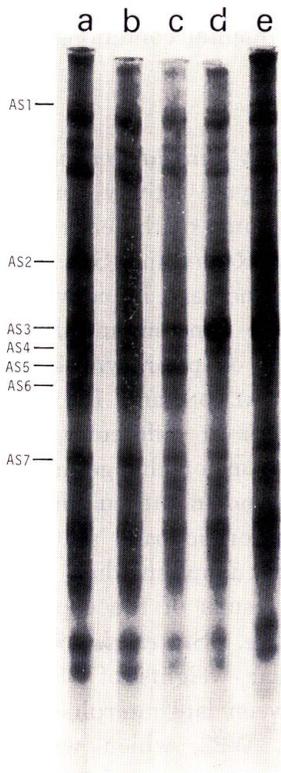


Fig. 2. Electrophoretograms of acid-soluble proteins at the different stages of development. The whole-cell proteins were treated with 67% acetic acid. After centrifugation, the supernatant was electrophoresed on SDS-polyacrylamide gels. a, vegetative stage; b, interphase; c, aggregation stage; d, pseudoplasmodial stage; e, culmination stage.



Fig. 3. Electrophoretograms of acid-insoluble proteins at three different stages of development. Acid-insoluble proteins were obtained from three different stages and electrophoresed on SDS-polyacrylamide gels. a, vegetative stage; b, aggregation stage; c, culmination stage.

ribosomal proteins which surely have an important role in the transcriptional and translational machinery. The protein bands of the acid-soluble fraction were referred to by the number preceded by the letter "AS". As shown in Fig. 2, the staining intensities of the protein bands AS2 and AS3 in the acid-soluble fraction were increased at the pseudoplasmodial stage, whereas that of AS5 was decreased. In addition, the molecular weights of AS2, AS3 and AS5 were the same as those of W16, W18 and W20, respectively.