TEMPERATURE DEPENDENT ALTERATION IN BACTERIAL PROTEIN COMPOSITION

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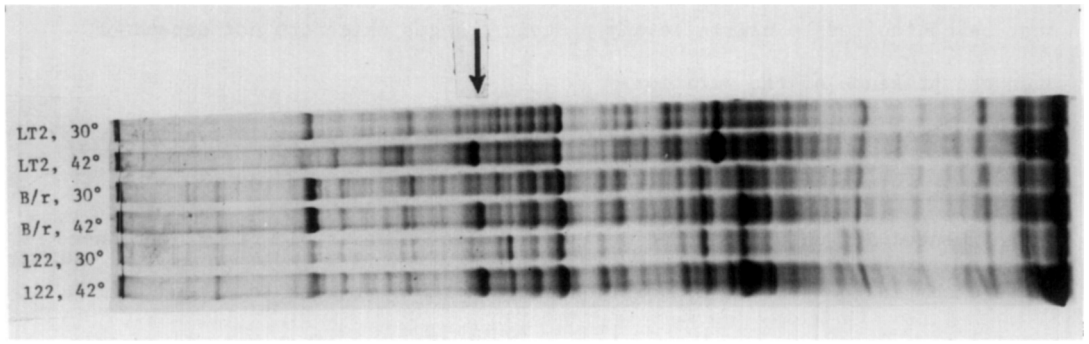
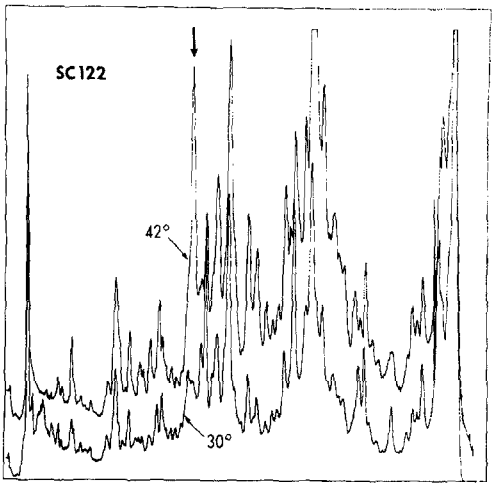
Summary: When different strains of Escherichia coli or Salmonella are grown at 30° or at 42° and their proteins are compared using SDS-polyacrylamide gel electrophoresis, differences are observed in the protein composition of the cells. In particular, one major difference is in a protein of molecular weight of approximately 58,000, which is made at 42° but not at 30°.

During the course of a study of the proteins of various temperature sensitive mutants of E. coli (1) we have had occasion to compare the protein composition of bacteria grown at 30° and 42°. Suprisingly, we find that there are striking differences in the proteins made at these two temperatures. This is illustrated in Figures 1a and 1b. Figure 1a is a photograph of an autoradiogram of a polyacrylamide gel electrophoretic analysis of total bacterial protein of two E. coli strains and one Salmonella strain grown at two temperatures. The arrow notes a protein of a molecular weight of 58,000 which does not appear to be made in any of the bacterial strains at 30° but which is made at 42°. While this difference due to variations in temperature is the most striking change, we have noticed other minor changes in proteins which appear to be present in lesser amount. SC122 contains a temperature sensitive suppressor (1) but the alteration in protein composition is not due to this mutation because other E. coli K12 strains (not shown) without a temperature sensitive suppressor have demonstrated the same temperature effect with a 58,000 MW protein appearing at 42° but not at 30°.

In passing we will merely note two other points. First, the temperature dependent protein is a major protein component of 42° grown cells. Second other experiments have shown that this protein is not a cell envelope protein.



a



b

Fig. 1. Proteins of Bacteria grown at 30° and 42°. (a) Cultures of SC122 (an E. coli K12 strain with the genotype pho$^-$am, lac$^-$am, try$^-$am, su4^{ts},str^r (see Beckman and Cooper, 1973 for description of su4^{ts})), E. coli B/r, and S. typhimurium LT2 were grown at 30° in a glucose minimal medium supplemented with all amino acids except leucine and valine. Fifteen minutes before label was added half of each culture was shifted to 42°. [C^{14}]-Leucine (315 µc/µm; 0.5 uC/ml) were added and after 15 minutes the cells were harvested, washed and lysed (final concentration) in SDS-Mercaptoethanol (4). 10µl of the total cell lysate (containing approximately 2 x 10^5 CPM) were analyzed in a 10% polyacrylamide gel as described by Ames (2). An SE520 apparatus manufactured by the Hoefer Instrument Company (520 Bryant Street, San Francisco, California) was used. After electrophoresis for approximately 4 hours the gel slabs were fixed with 7% acetic acid, dried on 3MM filter paper (Whatman) between two sheets of rubber with heat and a vacuum, and autoradiographed with Kodak No-Screen X-ray film. The arrow indicates the position corresponding to a molecular weight of 58,000 daltons as determined in separate experiments with stained and autoradiographed gels using BSA, pepsin, chymotrypsinogen, myoglobin, lysozyme, trypsin, glutamate dehydrogenase, and aldolase as molecular weight standards as described by Ames (2). (b) Densitometer tracing of the autoradiogram of SC122 at 30° and 42°, using a Joyce-Loebl densitometer. The arrow indicates the location of the protein band denoted by the arrow in (a).

We consider the following points important with regard to this finding:

1. Studies on the proteins synthesized by temperature sensitive mutants

must be controlled to eliminate any protein changes which are not dependent upon the presence of the mutation.

2. Ames (2) in a study of the total proteins of Salmonella typhimurium and Escherichia coli noticed that differences in growth conditions (supplemented and unsupplemented medium, aerobic or anaerobic growth) produced differences in the protein composition of bacteria. We wish to extend her results to temperature which is a less obvious effector of changes in protein composition.

3. Schaechter, Maaløe, and Kjeldgaard (3) presented evidence to suggest that different temperatures did not change the gross composition of bacteria. If their results do hold in general or for the temperature ranges we have studied here, then their generalization does not hold for any specific protein. Some proteins must increase and others must decrease as a percent of the total bacterial mass if the percentage of mass that is protein does not change with temperature.

4. Finally, and most important, the question must be raised as to whether these are functional alterations or non-functional artifacts. Either these protein alterations are important adaptive mechanisms which the cell has evolved to meet altered temperature conditions, or (at the other extreme) the changes are nothing more than alterations due to trivial phenomena, for example, the slight temperature sensitivity of a repressor protein leading to the observed increase in the synthesis of a particular protein.

ACKNOWLEDGEMENTS

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