Changes in Protein Composition of *Escherichia coli* during the Active Growth Phase

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It is a well known fact that the changes in enzyme activities during the batch culture of bacterical growth differ markedly from the increase in total protein content during the same cultivation time.[1] It has previously been shown in *E. coli* that during the batch culture cultivation the amino acid composition remains practically the same.[2]

The purpose of this work is to study, using disc and starch electrophoresis as well as gel filtration method, if there are changes in protein composition during the active growth phases of *E. coli*.

Methods. Transfer and cultivation of *E. coli* has been described previously.[1] The inoculum medium contained 1.0 % glucose, 1.0 % sodium citrate, 0.5 % Difco's Bacto-tryptone, and 0.5 % Difco's yeast extract. Samples were harvested during the batch culture incubation from a single amino acid and vitaminfree medium containing 2.0 % glucose, 0.2 % ammonium sulfate, 0.6 % dipotassium phosphate, 0.3 % monopotassium phosphate, and 0.02 % magnesium sulfate heptahydrate. The cells were washed twice with 0.15 M NaCl and homogenized for 5 min with MSE ultrasonic disintegrator (60 W, small head). The milky extract obtained was treated with streptomycin sulfate (20 mg/50 mg of dry weight of cells) in the cold for 15 min in order to precipitate the bulk of the nucleic acids. The extract was centrifuged in a refrigerated superspeed Servall RC-2 at 20 000 *g* for 20 min at 0–5°C. The supernatant obtained was tested for protein content using three different methods: gel filtration, starch and disc electrophoresis.

Starch electrophoresis was carried out horizontally using starch hydrolyzed according to Smithies (Connaught Medical Research Laboratories, Toronto, Canada) and the size of the bed was 130 × 7 × 45 mm. The fractionation was carried out using 0.025 M veronal-HCl buffer, pH 8.5 and 15 mA per gel. The gels containing 12 % starch were stained with amido black by the Smith procedure.[3]

Disc electrophoresis was carried out as described originally by Ornstein and Davies and modified by Chang *et al.*[4] The acrylamide content of the gels was 7.5 % and the pH 8.9. The corresponding figures in the spacer gel were 5.0 and 6.7.

Gel filtration experiments were carried out using Sephadex G 100 Superfine dextran (Pharmacia, Uppsala, Sweden). The column was 50 cm high with a diameter of 2 cm. The protein samples containing 10–15 mg of dry weight cells were eluted at 4° with 0.01 M Tris-HCl buffer, pH 8.0 containing 0.1 M KCl. The flow rate was 10 ml/h.

Protein was estimated in gel filtration experiments by Lowry *et al.* method.[5]

Results and discussion. The results obtained from the starch gel electrophoresis experiments are presented in Fig. 1. The samples were harvested at the beginning of the growth (0 h), at the end of the acceleration phase (2 h) and at the beginning of the retardation phase (3.5 h). Seven bands are observed visually. The intensity of the color of amido black increases in bands a, b, d, e, and g during the active growth phases. The protein content of bands c and f remains practically on the same level during the incubation.

Fig. 2 shows the fractionation of proteins in samples 0, 2, and 3.5 h in the disc electrophoresis experiments, which gives a

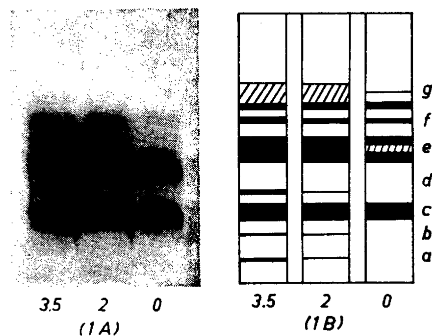

Fig. 1. Fractionation of *Escherichia coli* proteins with starch gel electrophoresis. The samples were harvested during the active growth 0, 2, and 3.5 h from inoculation from a simple amino acid and vitamin-free medium. The letters a-g indicate bands observed by visual inspection from the starting line (1B). On the left is a photo obtained from original bands (1A).

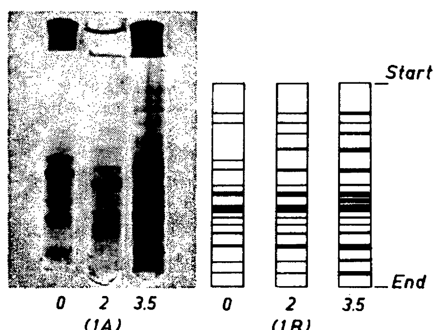

Fig. 2. Fractionation of *Escherichia coli* proteins with disc electrophoresis. On the left are the photos from the separated bands (1A) and on the right a visual reading of original bands (1B). Samples were the same as in Fig.1.

better resolution of bands than starch gel electrophoresis. Distinct differences can be seen in the protein bands using this method. The sample harvested in the retardation phase contains more bands than the previous samples. Upon visual inspection 16 bands are observed, especially high is the appearance of new bands at both ends of the electropherogram. A similar phenomenon is observed in Fig. 1, where the resolution conditions were similar.

The results obtained in the gel filtration experiments are presented in Fig. 3,

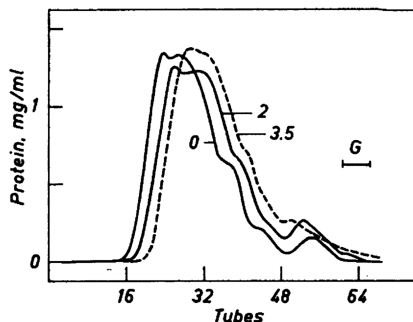

Fig. 3. Molecular sieving of proteins of *Escherichia coli* cells harvested from the active growth phases of growth. 2-ml fractions were collected and protein was estimated. G = tubes containing D-glucose. It was added to the samples before fractionation and tested by the use of Test-tape papers.

where Sephadex G 100 Superfine gel was used. No remarkable differences were found using this method. This result favors the opinion that no remarkable differences are found in molecular weights of the protein components.

The following conclusion can be drawn from the above results: during the active growth phases of *E. coli* remarkable differences can be found in protein composition in certain bands after electrophoretic separation. However, these differences are not reflected in the molecular weight spectrum obtained with the molecular sieve method.

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Molecular Weight of the Albumin Fraction of Barley

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In textbooks the molecular weight of barley albumin is still stated to be 35 000. To any one working on barley proteins, however, it is evident that this cannot be correct, as the water-soluble protein fraction of seeds is very heterogeneous. The heterogeneity of barley albumin has been confirmed by a great number of workers, using modern methods such as ultracentrifugation,[1] various types of electrophoresis, [2-7] chromatography on DEAE cellulose,[8] and exclusion chromatography on Sephadex.[9,10]