His 0.03, NH_4 1.11 (3), Arg 0.96 (3), Asp 0.31 (1), Thr 0.16 (1?), Ser 0.52 (2), Glu 0.70 (2?), Gly 0.51 (2), Ala 0.29 (1), Val 0.27 (1) and Leu 1.56 (5).

This preliminary analysis seems to indicate that there are five moles of leucine, three moles of arginine and two moles each of glutamic acid and glycine per one mole of aspartic, acid, alanine and valine, and that proline, cystine, methionine, isoleucine, tyrosin and phenylalanine are absent. Tryptophane is known to be absent in preparations still containing isoleucine, tyrosin and a considerable amount of lysine [5].

At an early stage J. Sjöqvist, Lund, found that the content of leucine increased and that tyrosin disappeared during the purification process. Likewise Swan's reaction for cystine turned negative as found by A. Henschen of this laboratory. The traces of lysine and histidine still present indicate that a slight further purification could be achieved, possibly with a higher number of transfers in the counter-current distribution.

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Acid Phosphatases in *Escherichia coli*

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A cell-free extract of *Escherichia coli* will catalyze the hydrolysis of various monophosphate esters in a sodium acetate buffer at pH 4—5 [1-3]. We have found that the activity of this acid phosphatase is remarkably stable at low temperatures over a very wide pH-range. Thus no loss in enzymatic activity measured against *p*-nitrophenyl phosphate (Sigma 104) was encountered after 24 h at 4° in 0.2 M Na_2CO_3, pH about 11, or in 1 M acetic acid, pH 2.4, in the absence of salt. Repeated freezing and thawing of a crude cell extract was found to cause considerable losses of the phosphatase activity, and only 20—40 % could be recovered after lyophilization. However, solutions could be stored for several months at —10° in the presence of 60 % sucrose without detectable losses.

The interesting stability properties of the phosphatase activity prompted us to attempt its purification with the aid of gel filtration in acetic acid and zone electrophoresis. Sonicated cell-free extracts were prepared from cells grown in a succinate-inorganic salts medium essentially as described earlier by one of us [3]. The phosphatase activity was determined at a 0.01 M substrate concentration in 0.1 M sodium acetate buffer, pH 4.7. Gel filtration experiments [4] were performed on Sephadex G 75 (Pharmacia, Uppsala), and zone electrophoresis was carried out on vertical columns [5].

When an extract of *E. coli* was dialyzed against 0.1 M pyridinium acetate, pH 5, a heavy precipitate containing approximately 20 % of the total acid phosphatase activity was formed. If the supernatant solution was filtered through a column of Sephadex G75, in equilibrium with 1 M acetic acid, an interesting elution pattern was obtained. All the acid phosphatase activity was located in the region containing proteins, which pressumably have a lower molecular weight than those in the main, slightly turbid protein peak. Fig. 1 illustrates a gel filtration experiment in which 12 ml of solution was fractionated on a 22 × 500 mm Sephadex column

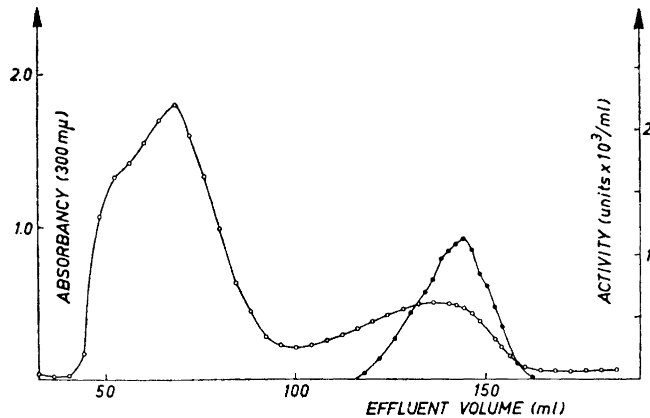

Fig. 1. Gel filtration of a partially purified acid phosphatase preparation on Sephadex G75 in 1 M acetic acid. O absorbancy at 300 mμ; ● enzymatic activity.

with 1 M acetic acid as eluent. The recovery of the enzymatic activity was usually quantitative within the limit of experimental error. The acid phosphatase activity was also assayed with substrates other than *p*-nitrophenyl phosphate, and the liberated orthophosphate was then determined according to Dryer *et al.*[6] An identical distribution of the enzymatic activity was observed with glucose-1-phosphate, glucose-6-phosphate and *o*-carboxyphenyl phosphate. When the gel filtration was carried out at a higher pH, for instance in 0,2 M pyridinium formate, pH 3,2, or in

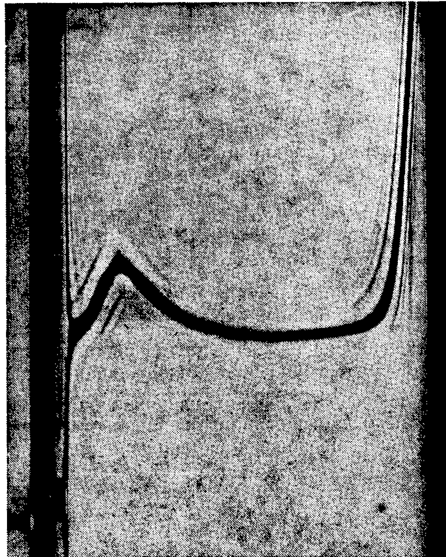

Fig. 2. Sedimentation pattern of enzyme concentrate from the experiment illustrated in Fig. 1. Spinco ultracentrifuge Model E, anal. D-rotor, 130 min after full speed (59 780 r.p.m.)

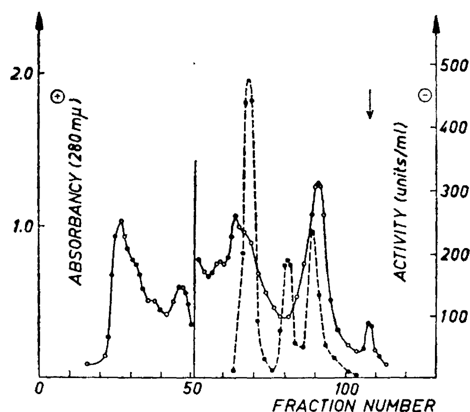

Fig. 3. Zone electropherogram of a crude extract of *E. coli* on a 20 × 250 mm column packed with formaldehyde treated cellulose in 0.05 M Tris-HCl, pH 8.2. 17 h, 27–30 mA, 600–610 V. Fractions 0–50 eluted concomitant with the electrophoresis (Ref.[5]). Position of the starting zone (2 ml) is indicated by an arrow. Fraction volume 2 ml. O absorbancy at 280 mμ; ● enzymatic activity.

Tris-HCl buffer, pH 8.2, the phosphatase activity was eluted with only slight retardation.

Ultracentrifugation of the pooled phosphatase-containing fractions obtained after gel filtration in acetic acid and subsequent concentration by ultra-filtration [7] gave the sedimentation pattern shown in Fig. 2. In addition to some gelatinous material accumulating on the bottom of the centrifuge cell, only one sedimenting boundary was observed. The uncorrected sedimentation coefficient of this component was approximately 1.0 S. We have reason to believe that the heavy gelatinous material is formed by aggregation of protein molecules in concentrated solutions.

Zone electrophoresis of the acid phosphatase preparation obtained after gel filtration revealed several different electrophoretic components. For instance, in a 0.05 M Tris-HCl buffer, pH 8.2, two phosphatase activity peaks were observed in addition to three or four inactive components. This was not surprising, since electrophoresis of the crude sonicated extract under similar conditions gave three acid phosphatase components having different electrophoretic mobilities as illustrated in Fig. 3.

Our experiments thus demonstrate that the acid phosphatase activity of *E. coli* is associated with several different proteins in the cell-free extract. This is in agreement with the results of Rogers and Reithel [2], who have partially purified five different proteins with phosphatase activity from the same organism. The gel filtration experiments in 1 M acetic acid show that the major part of the acid phosphatase activity exists as, or can be converted to, a protein of relatively low molecular weight. We are now attempting to isolate and characterize such a low-molecular weight phosphatase, in order to gain further information about the chemical background of the enzyme multiplicity.

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