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Chromatographic purification of yeast hexokinase

In a preliminary communication, it was reported that a highly purified hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) preparation could be obtained by chromatography of a crude commercial preparation on CM- and DEAE-cellulose[1]. A more detailed account of the procedure is given in the present paper.

Enzyme activity was measured according to KUNITZ AND MCDONALD[2]. Protein concentration was estimated by measuring the ultraviolet absorbancy per cm at 280 mμ, or by nitrogen determination with a micro-Kjeldahl procedure or the ultra-micro-method of KIRSTEN[3]. As starting material, we used Sigma yeast hexokinase,

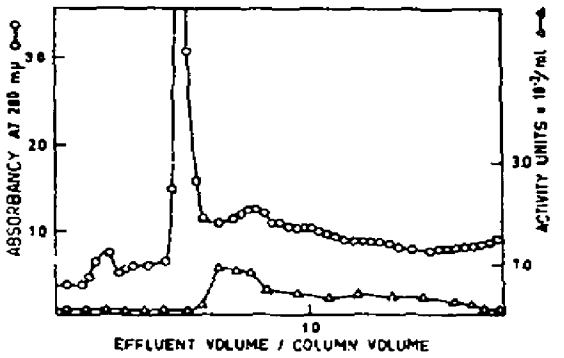

Fig. 1. Chromatography of 1 g of crude yeast hexokinase (Sigma Type III) on a 300-ml CM-cellulose column (3 × 45 cm). Gradient elution with 400 ml of 0.02 *M* sodium acetate–acetic acid buffer (pH 5.0) in the closed mixing chamber and 0.30 *M* acetate buffer (pH 5.0) in the reservoir flask. Both buffers contained 0.5% of glucose. Elution rate: 30 ml/h, collected in 10-ml fractions. ○- ○, absorbancy at 280 mμ; △—△, enzyme activity.

Type III (100–150 enzyme units/mg). The pooled fractions from each chromatography were concentrated by ultrafiltration according to EVERALL AND WRIGHT[4], using Visking tubing 8/32 in flat width as filtering membrane. A negative pressure of about 400 mm Hg was used.

The first purification step was chromatography of 1-g samples of the crude

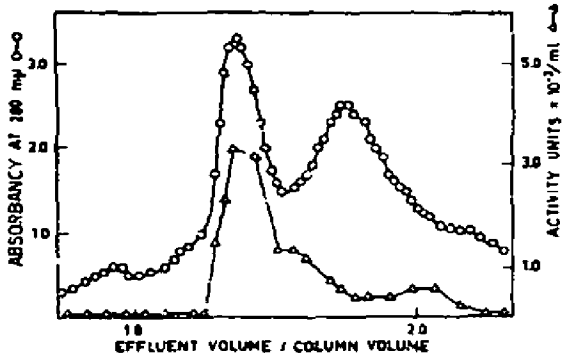

Fig. 2. Rechromatography on CM-cellulose of the main enzyme peak from 5 chromatographies as in Fig. 1. Same column and elution system as in Fig. 1. ○—○, absorbancy at 280 mμ; △—△, enzyme activity.

enzyme on 300 ml CM-cellulose columns (Fig. 1). The main enzyme peak corresponded to 30–40% of the activity put on the column, and at least 5-fold purification was usually obtained. Enzyme from 5 chromatographies was purified by rechromatography in the same way (Fig. 2). About 60–70% of the enzyme activity was recovered in the main peak, with a 2-fold increase in specific activity. The final step was carried out on DEAE-cellulose. The enzyme from the last CM-cellulose chromatography was run through a 50-ml column. A large inactive protein peak was eluted in the first part of the chromatogram (not shown in Fig. 3). About half the enzyme activity was

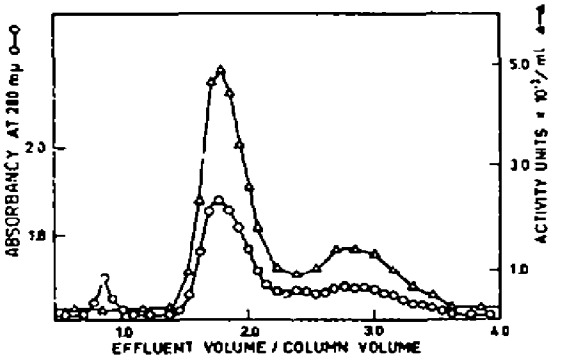

Fig. 3. Chromatography of the main enzyme fraction in Fig. 2 on a 50-ml DEAE-cellulose column (2.1 × 15 cm). Gradient elution with 300 ml of a 0.05 M potassium phosphate buffer (pH 7.0) in the closed mixing chamber and 0.30 M phosphate buffer (pH 7.0) in the reservoir flask. Both buffers contained 0.5% glucose. Elution rate: 4 ml/20 min. ○ – ○, absorbancy at 280 mμ; △ – – △, enzyme activity.

eluted as a second peak, with nearly constant specific activity from fraction to fraction, followed by another smaller peak with about the same specific activity. At least 2-fold purification was obtained. The specific activity of the main peak was 3000–4000 units/mg. The average yield after the 3 purification steps was about 10%.

On rechromatography of each peak on DEAE-cellulose only one activity peak was obtained in the expected position, demonstrating microheterogeneity of the

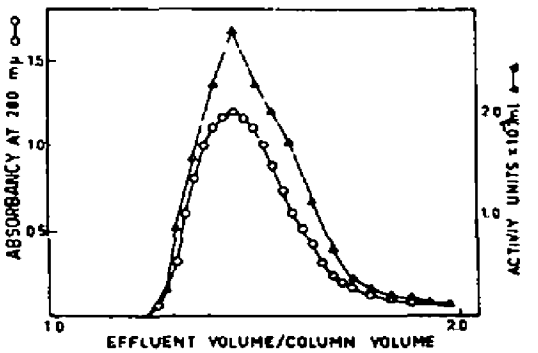

Fig. 4. Rechromatography on DEAE-cellulose of the main enzyme peak from chromatography as in Fig. 3. Column dimensions: 3 × 45 cm (300 ml). 500 ml of 0.05 M phosphate buffer (pH 7.0) in the closed mixing chamber. Elution rate: 9 ml/20 min. ○ – ○, absorbancy at 280 mμ; △ – – △, enzyme activity.

enzyme. The main enzyme peaks from different DEAE-cellulose chromatographies were combined and rechromatographed on a 300-ml column (Fig. 4). The enzyme activity ran fairly parallel with the protein content, suggesting a comparatively high degree of purity. A sedimentation analysis was carried out with a Spinco Analytical Centrifuge, Model E. Only one sharp sedimentation boundary was obtained, with an observed sedimentation coefficient of 4.0 S (Fig. 5). A single determination of the

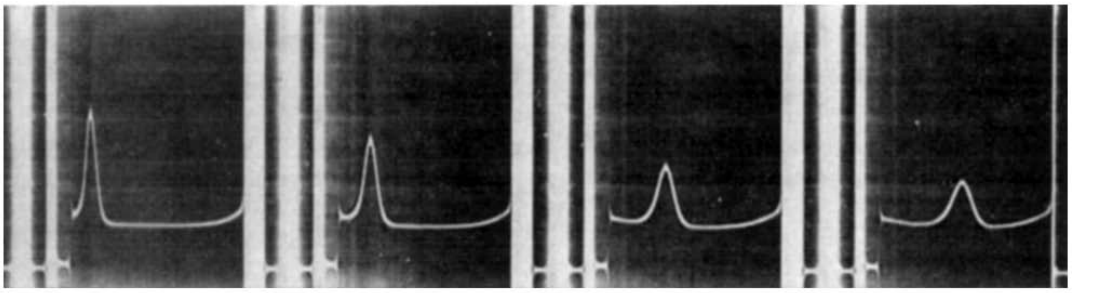

Fig. 5. Sedimentation pattern of purified hexokinase (Fig. 4). Buffer: 0.05 M phosphate (pH 7.0) also containing 0.5% glucose. Protein concentration about 0.6%. The exposures were taken 26, 42, 74 and 106 min after reaching 59 780 rev./min. Direction of sedimentation: from left to right. Temp. 7.2°.

molecular weight according to ARCHIBALD[5] gave a value of about 50 000. KUNITZ AND MCDONALD[2] have previously reported a value of 96 000. A possible explanation of this discrepancy is cleavage of the enzyme molecule, in the presence of glucose[6]. Probably, the specific activity of our preparation is not quite of the same order as that obtained by DARROW AND COLOWICK[7]. Contrary to previous findings[8], our highly purified preparation did not form an intermediate phosphorylenzyme when incubated with ATP, as also reported by other workers[9,10].

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