COPURIFICATION OF CHOLINE KINASE AND ETHANOLAMINE KINASE FROM RAT LIVER BY AFFINITY CHROMATOGRAPHY

Peter J. BROPHY and Dennis E. VANCE

Dept. of Biochemistry, University of British Columbia, Vancouver, B. C. Canada V6T 1W5

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1. Introduction

Ethanolamine kinase and choline kinase (EC 2.7.1.32) catalyze the phosphorylation by ATP of ethanolamine and choline respectively. Choline kinase has been purified 200-fold from rabbit brain, yet the enzyme also catalyzed the phosphorylation of ethanolamine [1]. Weinhold and Rethy [2] have partially purified (31-fold) the major ethanolamine kinase from rat liver, ethanolamine kinase II, and this enzyme copurified with choline kinase. The ratio of the ethanolamine and choline kinase activities from rat liver remained constant during chromatography on DEAE-cellulose and Sephadex G-200 and both activities had similar stability properties [2]. The results from these studies on rabbit brain and rat liver suggest that both kinase activities are associated with the same protein. In contrast, Sung and Johnstone [3] have reported indirect evidence that two separate enzymes exist in Ehrlich ascites cells. More recently, Broad and Dawson [4] observed a partial separation of the two kinases from the rumen protozoan *Entodinium caudatum* by gel filtration.

In this communication we wish to report a rapid and highly selective copurification of choline kinase and ethanolamine kinase from rat liver by affinity chromatography.

2. Materials and methods

The affinity column was prepared by the reaction of choline with epoxy-activated Sepharose 6B (Pharmacia (Canada) Ltd., Dorval, Quebec H9P 1H6). The activated Sepharose (10 g) was suspended in an aqueous solution (50 ml) of 1 M choline and the pH adjusted to 12 with 0.1 M NaOH. The reaction mixture was shaken for 18 h at 45°C after which the gel was washed successively with distilled water, 0.1 M sodium borate buffer pH 8.0–0.5 M NaCl, 0.1 M sodium acetate buffer pH 4.0–0.5 M NaCl and finally with 0.1 M Tris-HCl buffer pH 9.0–0.5 M NaCl–10 mM dithiothreitol. The gel was subsequently packed into a column (1.5 × 15 cm). The theoretical capacity of the activated support is 15–20 μmol of ligand per ml of swollen gel and preliminary experiments with [CH_3-^{3}H]choline (spec. act. 5 μCi/mmol) indicated that complete reaction had occurred.

The soluble fraction from rat liver was prepared as described by Weinhold and Rethy [2] and a portion (16 ml, 295 mg protein) was applied to the affinity gel. After 2 h the column was washed with 0.1 M Tris-HCl buffer pH 9.0–0.5 M NaCl–10 mM dithiothreitol, at a flow rate of 11 ml/h until the eluate was free of material that absorbed at 280 nm. The column was subsequently washed with 50 mM Tris-HCl buffer pH 7.5 that contained dithiothreitol (10 mM) and choline (0.1 M). Choline kinase and ethanolamine kinase were assayed as described by Weinhold and Rethy [2] and protein was measured by the method of Lowry [5]. Fractions that contained choline kinase activity were pooled and dialysed against 50 mM Tris-HCl buffer pH 7.5–10 mM β-mercaptoethanol.

3. Results and discussion

Fig.1 shows that choline kinase was retained on the affinity column and specifically eluted with choline.

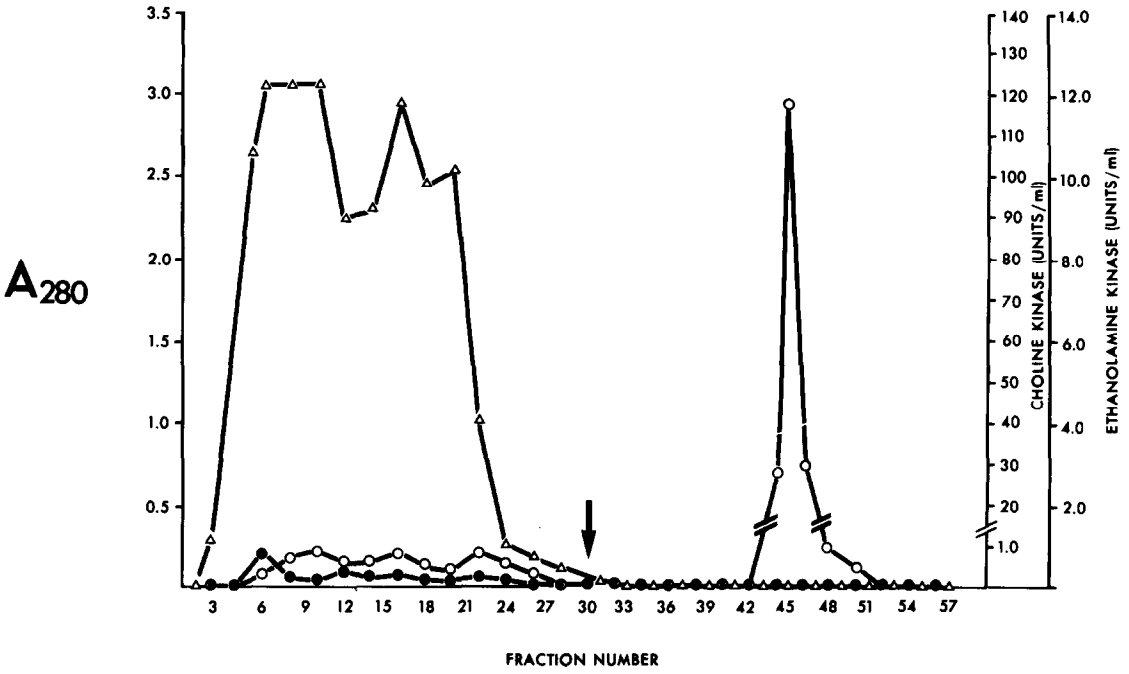

Fig.1. Affinity chromatography of rat liver soluble fraction on a choline-Sepharose 6B column. The 100 000 *g* supernatant was applied to the column and after 2 h eluted with 0.1 M Tris-HCl buffer pH 9.0–0.5 M NaCl–10 mM dithiothreitol. The arrow indicates the start of elution of the column with 50 mM Tris-HCl buffer pH 7.5–10 mM dithiothreitol–0.1 M choline. One unit of enzymatic activity represents the formation of 1 nmol of product/min. (△—△) A_{280}; (○—○) choline kinase activity; (●—●) ethanolamine kinase activity. Fraction size was 1.3 ml.

No ethanolamine kinase activity was detected in these fractions, however, this was due to the presence of choline which is a strong inhibitor of ethanolamine kinase (K_i, 30 μM) [2]. After removal of choline from the pooled fractions by dialysis, ethanolamine kinase activity could indeed be detected. The purified choline kinase preparation was completely inactivated during dialysis unless bovine serum albumin was added; neither glycerol (20%) nor thiol protecting reagents were effective for stabilization of the enzymatic activity. This inactivation was presumably due to the low protein concentration in the purified preparation.

Table 1 shows that the affinity column yielded a 550-fold purification with a 19% recovery of choline kinase activity. The ratio of ethanolamine kinase to choline kinase in the purified preparation (0.48) was

Table 1
Purification of choline kinase and ethanolamine kinase

Stage	Protein (mg)	Choline kinase			Ethanolamine kinase			EK/CK
		Specific activity (nmol·min^{-1}·mg^{-1})	Purification	Yield (%)	Specific activity (nmol·min^{-1}·mg^{-1})	Purification	Yield (%)	
100 000 *g* supernatant	1180	1.60	1	100	0.84	1	100	0.52
Dialyzed eluate from affinity column	0.38[a]	875	550	19	422	500	17	0.48

[a] A pooled preparation from four purifications.

very close to that in the rat liver soluble fraction (0.52) and this ratio was not altered drastically by storage at −70°C for 10 days (0.42) or 4°C for 17 days (0.46). In another preparation which had been purified 965 times the ratio of ethanolamine kinase to choline kinase was 0.42.

The choline is attached to the affinity gel by its hydroxyl group with the quaternary ammonium function available for the binding of choline kinase; this arrangement apparently conferred a high degree of specificity to the column. Preliminary experiments demonstrated that in the absence of 0.5 M NaCl much non-specific binding of proteins occurred. In the presence of 0.5 M NaCl the column retained its specificity whilst other proteins non-specifically adsorbed by the positive charge of the ammonium group of choline were eluted.

In an attempt to displace preferentially ethanolamine kinase from the gel, we eluted the column with ethanolamine (0.1 M) instead of choline. However, both activities were eluted by ethanolamine and the ratio of activities was unchanged. Similarly, the ratio of ethanolamine kinase to choline kinase was unaltered in a preparation obtained after pre-elution of the column with 0.5 M NaCl followed by 1 M NaCl.

These results suggest that both ethanolamine kinase and choline kinase activities may be associated with the same protein. We intend to purify these activities on a large scale by the method described in this communication and subsequently we hope to determine unambiguously whether these activities are associated with the same or discrete proteins.

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