AFFINITY CHROMATOGRAPHY OF CREATINE PHOSPHOKINASE ON ORGANOMERCURIAL-SEPHAROSE

R. J. BOEGMAN

Department of Pharmacology, Queen's University, Kingston, Ontario, K7L 3N6, Canada

Received 29 January 1975

1. Introduction

Creatine phosphokinase (CPK, EC 2.7.3.2) occurs mainly in muscle where it catalyses the reversible transfer of phosphate between ADP and creatine phosphate [1]. The enzyme has been isolated by combining alcohol and salt fractionation [1] followed by DEAE-column chromatography [2,3]. Sephadex-block electrophoresis [4] or isoelectric focusing [5]. CPK has a mol. wt of approx. 80 000 [2] and consists of two very similar subunits each with one highly reactive thiol group [3]. A simplified purification procedure based on the reactivity of the thiol groups for an organomercurial affinity column will be described.

2. Materials and methods

The affinity column was prepared by coupling *p*-chloromercuribenzoate to aminoethyl-Sepharose with the water soluble 1-ethyl-3(3-dimethylamino-propyl) carbodiimide [6]. Complete reaction of the amino groups was confirmed by the trinitrobenzene-sulfonate color test [7]. The substituted Sepharose column was packed and equilibrated with Tris–DTE buffer (100 mM Tris adjusted to pH 8.0 with acetic acid and contained 60 mg dithioerythritol per litre). A 10% fowl breast muscle homogenate in Tris–DTE was centrifuged at 100 000 *g* for 30 min and the supernatant applied to the column. The column was eluted with Tris–DTE which was followed by Tris–DTE containing 2.5 mM L-cysteine. Material not displaced by cysteine was removed by 2.5 mM $HgCl_2$ in 100 mM Tris pH 8.0. Before reuse the column was equilibrated with Tris–DTE.

CPK activity was determined fluorometrically [8] and protein according to Lowry et al. [9]. Fractions containing CPK activity were concentrated by pressure dialysis under N_2 against Tris–DTE and analyzed by gel electrophoresis [10]. Gels were stained for protein with Coomassie Blue [11] and for CPK activity [12].

3. Results

Fig.1 represents the elution diagram when 420 mg supernatant protein was placed on the column. No CPK activity could be detected in the first fraction (243 mg protein) eluted from the column with 100 mM Tris–DTE buffer. On elution with the Tris–DTE buffer containing 2.5 mM L-cysteine a second fraction (143 mg protein) was obtained which contained CPK activity (table 1). Material not displaced from the column by cysteine was removed by passing 2.5 mM $HgCl_2$ in Tris–acetate buffer through the affinity column. This resulted in a small protein peak (34 mg) which contained no CPK activity. The fraction containing CPK was concentrated and passed through a Sephadex G-200 column in Tris–DTE buffer; the results are shown in fig.2. Of 143 mg protein (3 X 47.6) applied to the G-200 column, a total of 81 mg was recovered in the major fraction which contained all the CPK activity. A purification of 4.4-fold was obtained from the supernatant of the muscle homogenate to the Sephadex column while the yield was 85% (table 1).

The protein fractions were examined by acrylamide

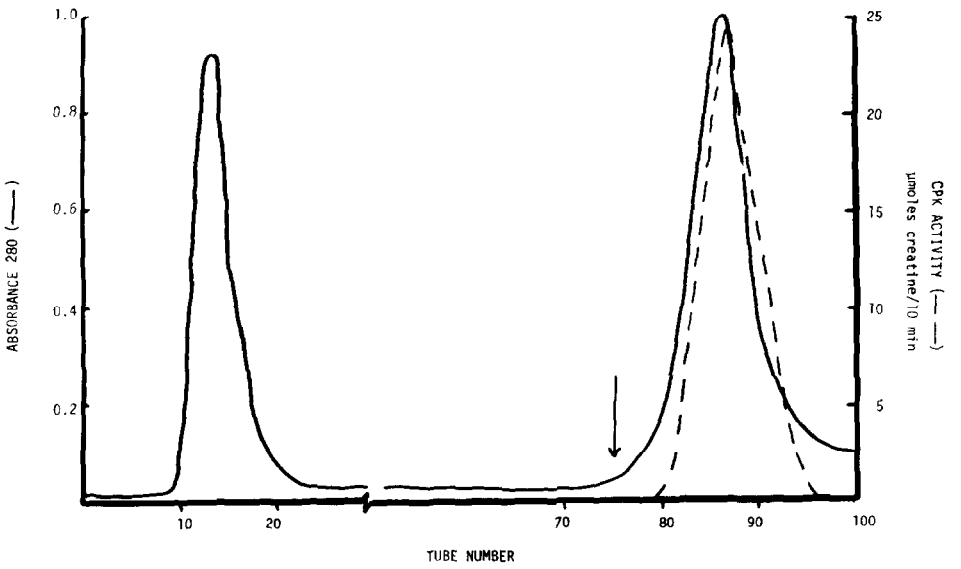

Fig.1. Purification of CPK by affinity chromatography on organomercurial-Sepharose. The column was eluted at 10 ml/hr and the arrow indicates where 2.5 mM cysteine was added to the Tris–DTE eluting buffer. Each tube contained 5 ml.

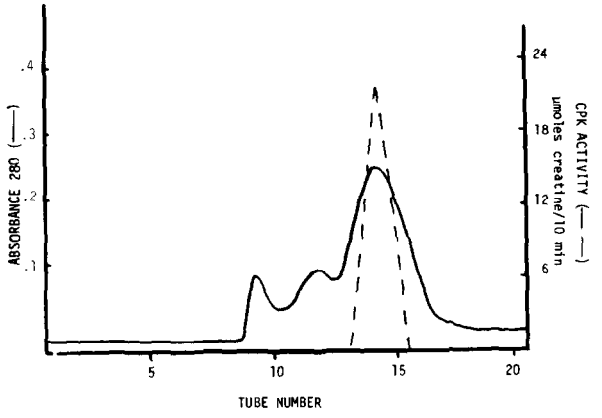

Fig.2. Sephadex G-200 chromatography of the CPK fraction obtained from the organomercurial-Sepharose column. The column was eluted with Tris–DTE at 3 ml/hr and each tube contained 5 ml.

disc electrophoresis employing 7% gels and a Tris–glycine buffer [10]. The protein fraction not retained by the affinity column showed numerous bands (fig.3) none of which reacted with the CPK enzyme stain. The fraction displaced by cysteine contains at least five protein bands of which only one stained with the enzyme stain. The CPK peak obtained from the Sephadex column gave a single major band containing all the CPK activity. A minor contaminant with no CPK activity could also be detected.

4. Discussion

Affinity chromatography of a muscle homogenate on an organomercurial-Sepharose column affords a

Table 1
Purification of creatine phosphokinase (CPK) from fowl breast muscle

Fraction	Total protein (mg)	CPK activity X 10^3 (μmol creatine formed/10 min/mg protein)
Supernatant	420	2.6
Tris–DTE–Cysteine	143	9.4
G-200	81	11.5

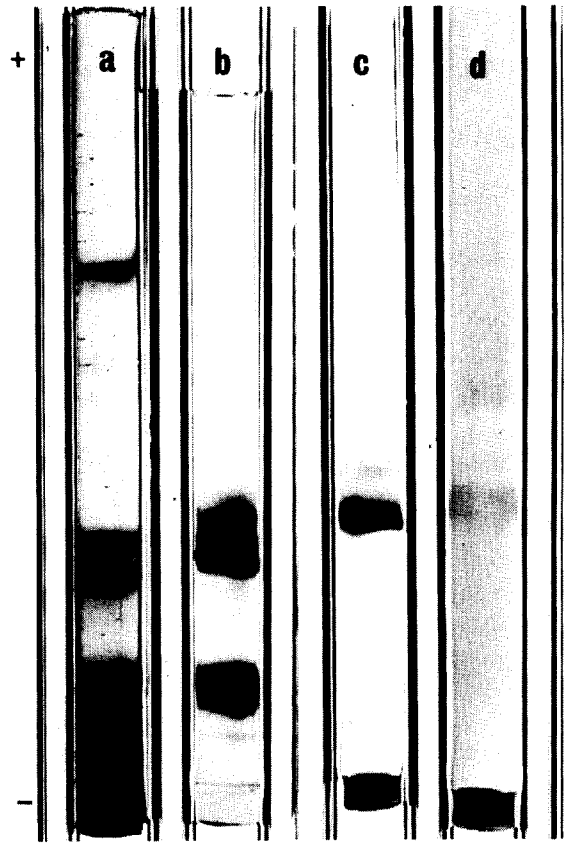

Fig.3. 7% Polyacrylamide gel electrophoresis of CPK during isolation: a) 100 000 *g* supernatant of the muscle homogenate; b) CPK fraction from the organomercurial column; c) and d) CPK fraction from the Sephadex G-200 column. a) – c) stained with Coomassie Blue; d) stained for CPK enzyme activity. Approx. 100 μg proteins was applied to each gel.

rapid separation of sulfhydryl from non-sulfhydryl proteins. Differential elution of the sulfhydryl proteins adsorbed to the column by a cysteine gradient resulted in a gradual release of CPK and other proteins from the column and was therefore not used. Most of the non-CPK protein displaced from the column by 2.5 mM cysteine could however, be removed by single passage through a Sephadex column.

The 11-fold purification reported by Kuby et al. [1] is most likely due to the initial low speed (1000 *g*) centrifugation used which would result in more non-specific protein being present in the first fraction and therefore by comparison a higher purification. By employing the traditional salt and ethanol fractionation procedures a yield of 40 to 60% was obtained however, by using an affinity and Sephadex column the yield was 85%.

Acknowledgements

This work was carried out with the technical assistance of Rose Page and with financial support from the Canadian Muscular Dystrophy Association.

References

[1] Kuby, S. A., Noda, L. and Lardy, H. A. (1954) J. Biol. Chem. 209, 191–201.
[2] Roy, B. P., Laws, J. F. and Thomson, A. R. (1970) Biochem. J. 120, 177–185.
[3] Mercer, D. W. (1974) Clin. Chem. 20, 36–40.
[4] Focant, B. and Watts, D. C. (1973) Biochem. J. 135, 265–276.
[5] Warren, W. A. (1973) Prep. Biochem. 3, 199–208.
[6] Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065.
[7] Inman, J. K. and Dintzis, H. M. (1969) Biochem. 8, 4074–4082.
[8] Sax, S. M. and Moore, J. J. (1965) Clin. Chem. 11, 951–958.
[9] Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[10] Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427.
[11] Chrambach, A., Reisfeld, R. A., Wyckoff, M. and Zaccari, J. (1967) Anal. Biochem. 20, 150–154.
[12] Sherwin, A. L., Norris, J. W. and Bulcke, J. A. (1969) Neurol. 19, 993–999.