CHOLINE KINASE AS AN ALLOSTERIC ENZYME

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Summary — Choline kinase, enriched over 1,000-fold from the filaments of Cuscuta reflexa Roxb., exhibited sigmoidal response in assays with subsaturating levels of choline. The major part of the enzymic activity in the tissue resided in particulate fractions sedimenting at low centrifugal fields.

INTRODUCTION

First demonstrated in acetone powder of animal tissues and partially purified from yeast (Wittenberg and Kornberg, 1953), choline kinase has since been studied in crude form in rape seed (Ramasarma and Wetter, 1957) and leaves and roots of higher plants (Tanaka, Tolbert and Gohlke, 1966). The present authors have studied the localization of activity in the tissues of an angiospermic parasite and have obtained the enzyme in a highly purified form. Choline kinase is an allosteric protein.

MATERIALS and METHODS

Tissue

Filaments of Cuscuta reflexa Roxb. were harvested fresh from the growth on Lantana camara Linn.

Enzyme assay

Choline kinase activity was determined in an

assay system which was a modification of that of Ramasarma and Wetter (1957), the residual choline being determined by the method of Appleton, LaDu, Levy, Steele and Brodie (1953). In crude fractions, a unit of choline kinase was the utilization of 1 μ mole of choline in one hour at 30° at pH 9.0. The specific activity was calculated as units per mg. protein. In kinetic studies with the purified fraction, the incubation period was reduced to 30 minutes.

Localization

A homogenate of fresh filaments was prepared in 0.5 M mannitol in 0.01 M Tris-HCl, pH 9.0, supplemented with freshly neutralized 0.05 M cysteine hydrochloride and 0.015 M $MgCl_2$. The homogenate was centrifuged at 1,000 x g for 20 minutes, the sedimented fraction rejected and the supernatant centrifuged at 5,000 x g for 20 minutes. The resulting supernatant was centrifuged at 12,000 x g for 20 minutes.

Enzyme purification

The particulate fraction sedimenting at 5,000 x g was treated with 0.35 M NaCl for 20 minutes in the cold, whereupon the enzyme was solubilized. Following heat-treatment of the extract for one minute at 60° C and precipitation with ammonium sulfate at 45 % saturation and dialysis, the enzyme was chromatographed on DEAE-cellulose column equilibrated at pH 7.2 with 0.01 M phosphate buffer. Following elution of inert protein with phosphate buffer of low molarity, the enzyme was eluted with 0.1 M phosphate buffer at pH 7.2.

RESULTS

Localization

The particles sedimenting from the homogenates between 1,000 and 5,000 x g were found to contain 70 to 80 % of the activity. The rest was present in the fraction sedimenting between 5,000 and 12,000 x g.

Enzyme purification

A typical purification schedule is recorded in Table 1.

Table 1. Purification of choline kinase from C. reflexa

Fraction	Volume, ml.	Activity, units	Protein, mg.	Specific activity
Homogenate	300	486	757	0.64
Particles sedimenting at 5,000 x g	10	442	90.8	4.9
NaCl extract of the particles	27	394	13.1	30.1
Extract heated at 60°C for 1 min and centrifuged clear	24.3	429	10.3	41.6
Precipitated with $(NH_4)_2SO_4$ at 45 % saturation and dialysed	3.5	424	1.07	396
Absorbed on DEAE-cellulose column and eluted with 0.1 M phosphate buffer	10	132	0.141	940

The enzyme was obtained in 27 % yield, enriched over 1,000-fold.

Substrate concentration—reaction rate relationship

In 0.370 ml. assay system consisting of 34 μ moles Tris-HCl buffer, pH 8.5, 1.5 μ moles $MgCl_2$, 1.5 μ moles ATP and the enzyme preparation, choline concentration was varied in the range 0.25 to 1.25 μ moles. The allosteric response

will be apparent from figure 1, in which the reaction velocity has been plotted against substrate concentration.

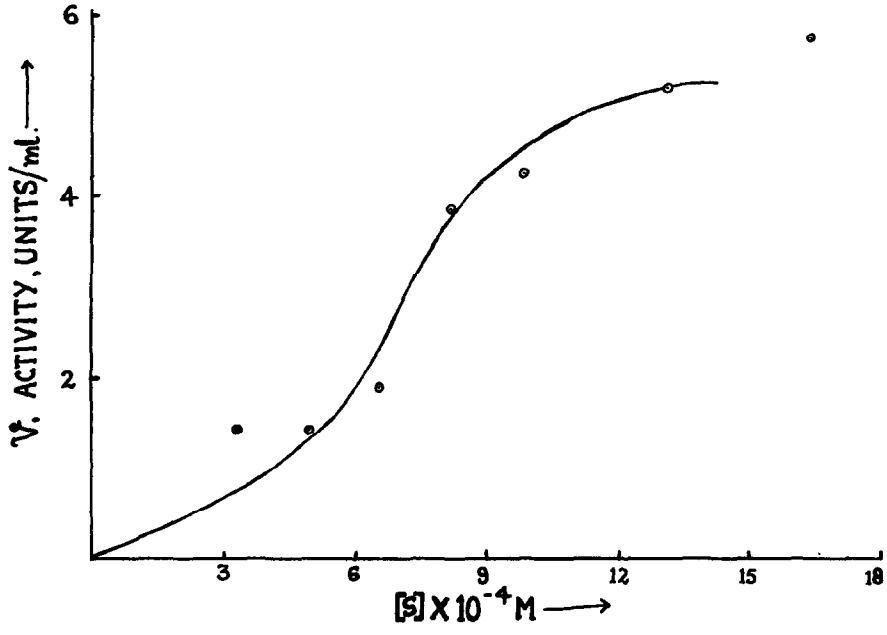

Fig. 1. Reaction rate versus substrate concentration. The rate of of reaction, expressed as units of activity per ml. enzyme preparation has been plotted against the molar concentration of choline.

Heat-activation

Maintenance of enzyme solution at 31°C for 2 hours prior to assay elicited a significant increase in activity.

DISCUSSION

With the demonstration of allosterism, choline kinase is fitted to play a regulatory role in the metabolism of lipids in parasite tissue. The enzyme appeared to be associated with the mitochondrial fraction sedimenting at 5,000 x g, which contained about 60 % of the total cytochrome c oxidase activity associated with the particles sedimenting between 1,000 and 12,000 x g. No

claim can be made as to the purity of the fraction from the plant tissue, especially in view of the unavoidable presence of salts in the dispersion medium. The occurrence of choline kinase in mitochondria does not fit in with the current concept that the de novo synthesis of phospholipid occurs essentially in the microsomal fraction in animal tissue.

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