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AFFINITY CHROMATOGRAPHY OF THIAMIN PYROPHOSPHOKINASE OF RAT BRAIN

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Summary

Affinity column chromatography coupled with thiamin monophosphate absorbs thiamin pyrophosphokinase activity in the crude extract of rat brain, and the enzyme can be eluted from the column by 0.01 mM thiamin with approximately 700-fold purification.

It has been reported that a specific transport system for thiamin is present in the brain of rat and the conversion of free thiamin transported to its pyrophosphate by thiamin pyrophosphokinase [EC 2.7.6.2] plays an important role to accumulate the vitamin in the tissue [1,2].

Thiamin pyrophosphokinase was partially purified (12.5 fold) from rat brain [3], and it may be possible to purify further the enzyme by the conventional method as recently described by Peterson et al. [4] with pig brain which brought about 260-fold purification. However, a more effective purification procedure than the conventional method would be required to isolate the enzyme from a relatively small amount of the tissue such as rat brain. We have previously reported that an affinity column coupled with thiamin pyrophosphate can be successfully applied to purify a thiamin-binding protein of *Escherichia coli* [5], but its application to the purification of corresponding enzymes has not yet been reported.

It has recently been demonstrated by Mitsuda et al. [6] that thiamin pyrophosphokinase purified from parsley leaves was markedly inhibited by thiamin monophosphate, and the similar inhibition was observed with the crude enzyme of rat brain [1]. In this report we describe that thiamin monophosphate-agarose can be effectively applied to purify thiamin pyrophosphokinase from the brain of rat.

Thiamin monophosphate was coupled to activated agarose according to the method for the preparation of thiamin pyrophosphate-agarose as

previously described [7]. Male rats of Wistar strain, weighing 200—250 g were killed by decapitation and the brains were homogenized for 2 min in 10 vol. of 0.02 M Tris·HCl (pH 7.4) containing 2 mM 2-mercaptoethanol and 1 mM EDTA. The homogenate was then centrifuged at 100 000 × *g* for 60 min. The supernatant was used as the crude extract after dialysis against 0.02 M Tris·HCl (pH 7.4) containing 2 mM 2-mercaptoethanol and 1 mM EDTA. 18 ml of the crude extract was applied to a column of thiamin monophosphate-agarose (0.5 × 6 cm) previously equilibrated with the buffer described above. Virtually all of the thiamin pyrophosphokinase activity in the crude extract was adsorbed to the column and the enzyme was eluted with 10 ml of the buffer containing 0.01 mM thiamin after thorough washing of the buffer (Fig. 1). As shown in Table I the specific activity of the enzyme in the eluate represents a purification of approximately 700-fold with a recovery of 71.6 %.

The enzyme was also recovered with thiamin monophosphate of 0.1 mM concentration from the column, but neither with 0.01 mM thiamin monophosphate nor with 2 mM Mg^{2+}-ATP.

The affinity chromatography with thiamin monophosphate-agarose

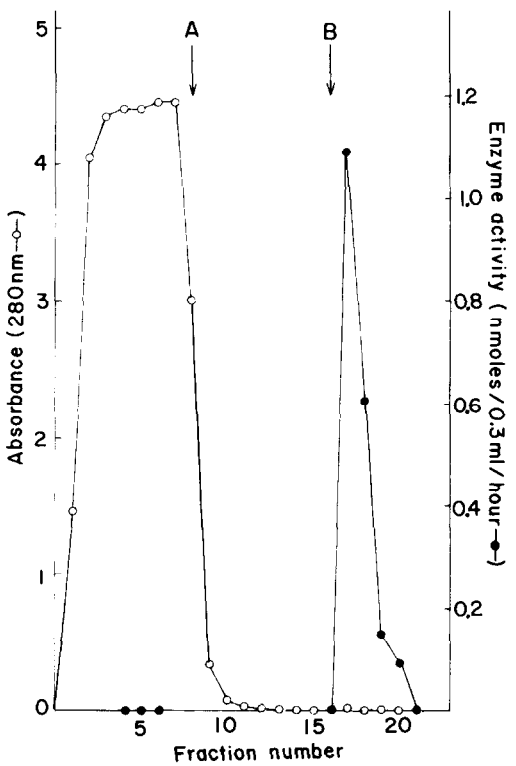

Fig. 1. Affinity chromatography on thiamin monophosphate-agarose column of rat brain thiamin pyrophosphokinase. 18 ml of the crude extract of rat brain was applied to the column (0.5 × 6 cm) which was previously equilibrated with 0.02 M Tris·HCl (pH 7.4) containing 2 mM 2-mercaptoethanol and 1 mM EDTA. After washing with 20 ml of the same buffer (arrow A) the enzyme was eluted with the buffer containing 0.01 mM thiamin (arrow B). Fractions of 2.5 ml were collected at a flow rate of about 10 ml/h at 4°C.

TABLE I

PURIFICATION OF THIAMIN PYROPHOSPHOKINASE FROM RAT BRAIN BY THIAMIN MONOPHOSPHATE-AGAROSE CHROMATOGRAPHY

Thiamin pyrophosphokinase activity was assayed as follows. The reaction mixture consisted of 30 μmol of Tris·HCl (pH 7.4), 3 μmol each of ATP and $MgCl_2$, 0.15 μmol of thiamin and the enzyme preparation in a total volume of 1.5 ml. After incubation for 30 min at 37°C thiamin pyrophosphate formed was determined manometrically as previously described [8]. The protein was measured by the procedure of Lowry et al. [9]. Enzyme activity was expressed as nmol of thiamin converted to thiamin pyrophosphate per hour under the assay conditions.

Step	Volume (ml)	Total protein (mg)	Total activity (nmol)	Specific activity (nmol/mg)
Crude extract	18.0	55.4	22.5	0.405
Affinity chromatography	10.0	0.057	16.1	282.1

described above seems to be useful to purify thiamin pyrophosphokinase from various origins, particularly to purify, in a single step, from a limited amount of the enzyme source.

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