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Thiamine triphosphatase in the nervous system

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SUMMARY

Thiamine triphosphate is hydrolyzed by a specific triphosphatase in subcellular fractions of rat brain. This enzyme differs from nucleoside triphosphatases in (1) its subcellular distribution pattern; (2) its susceptibility to inhibition by Ca^{2+}; (3) its activation by sodium deoxycholate; (4) its failure to be affected by known inhibitors of other triphosphatases. The specific activity of the thiamine triphosphatase is as high as that of Mg^{2+}-activated ATPase.

The presence of thiamine triphosphate in the total thiamine pool of animal tissues has been recognized for many years, but the function of this vitamin B_1 homolog has remained a mystery [1]. Likewise, the specific involvement of phosphorylated thiamine in nerve conduction has been well established [2], but the nature of this involvement at the molecular level has not yet been elucidated. More recently, the studies of Itokawa and Cooper [3,4] with intact nerves as well as with subcellular fractions of nerve tissue, strongly indicated that ion movements across nerve membranes are associated with the dephosphorylation of thiamine diphosphate and thiamine triphosphate. The possible significance of thiamine triphosphate in nerve tissue is suggested by the recent demonstration [5] that thiamine triphosphate is absent from the brains of patients with subacute necrotizing encephalomyelitis, a fatal disease associated with an abnormality in thiamine metabolism.

We have begun a series of experiments designed to elucidate the molecular mechanisms by which higher phosphate esters of thiamine participate in ion translocation across the excitable membrane. In a preparatory study we found that the dephosphorylation of thiamine diphosphate in rat brain is catalyzed by a general nucleoside diphosphatase, rather than by a specific thiamine diphosphatase [6]. We now have demonstrated that thiamine triphosphate in rat brain, unlike the thiamine diphosphate, is dephosphorylated by a highly specific thiamine triphosphatase. We present evidence here to show that this enzyme is different from any of the previously described nucleoside triphosphatases of brain.

When thiamine triphosphate is incubated with subcellular fractions of rat brain inorganic phosphate is rapidly released enzymatically at a rate proportional to protein concentration. Mg^{2+} is an absolute requirement for this reaction. The apparent K_m for thiamine triphosphate is $5 \cdot 10^{-5}$ M. No addition of ADP is required, even after dialysis of the enzyme preparation, which suggests that the enzyme is not a thiamine triphosphate-ADP phosphotransferase. More than 70% of the total activity appears in the 100 000 × *g* supernatant fraction of a brain homogenate (Fig. 1). The specific activity of this 'soluble' fraction is 800 nmoles of thiamine triphosphate hydrolyzed per mg of protein per min. The remainder of the activity is distributed among other particulate membrane fractions. In contrast, the Mg^{2+}-activated hydrolysis of ATP is catalyzed exclusively by the particulate fractions (Fig. 1).

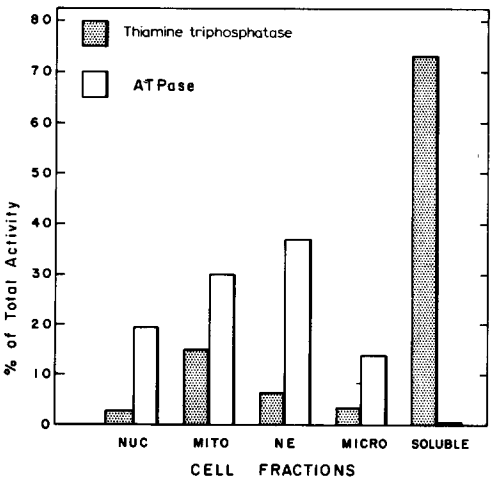

Fig. 1. An homogenate of whole rat brain was fractionated by the method of Seijo and Arnaiz [8]. NUC, nuclei and large cell fragments; MITO, mainly mitochondria; NE, mainly nerve endings; MICRO, microsomes; SOLUBLE, 100 000 × *g* supernatant. The thiamine triphosphatase assay mixture contained thiamine triphosphate (0.5 mM), $MgCl_2$ (5 mM), EGTA (0.1 mM), sodium deoxycholate (0.05%), dithiothreitol (0.5 mM) and Tris maleate buffer (50 mM, pH 7.4) in a final volume of 1.0 ml. After a 15-min incubation at 37° with shaking, cold trichloroacetic acid was added and inorganic phosphate was determined by the procedure of Baginsky *et al.* [9]. Identical reaction conditions were used for Mg^{2+}-ATPase.

In order to further differentiate the two membrane-bound enzymatic activities we compared the hydrolysis of thiamine triphosphate and ATP under a variety of conditions (Table I). The membrane-containing cell fraction, rather than the 'soluble' fraction (which contains 70% of the thiamine triphosphatase activity) was used for this experiment in order that a meaningful comparison to ATPase could be made. Ouabain, as expected, had no effect on either Mg^{2+}-activated enzyme; it is known to inhibit only the (Na^+, K^+)-ATPase. Ca^{2+} inhibited the thiamine triphosphatase at a low concentration but had no effect on Mg^{2+}-activated ATPase at this concentration. The presence of Na^+ and K^+ in the reaction mixture failed to activate thiamine triphosphatase, although it greatly

stimulated the breakdown of ATP. The (Na^+, K^+)-ATPase in membrane preparations of brain has been extensively documented in the literature. Tripropyltin chloride, a known inhibitor of Mg^{2+}-activated ATPase in brain microsomes [7] had no effect on thiamine triphosphatase but exhibited the expected inhibition of Mg^{2+}-activated ATPase. The 'soluble' thiamine triphosphatase exhibited the same properties as the membrane-associated thiamine triphosphatase in a parallel experiment.

TABLE I

COMPARISON OF MEMBRANE-ASSOCIATED THIAMINE TRIPHOSPHATASE WITH Mg^{2+}-ACTIVATED ATPase

*Additions to reaction mixture**	*Relative activity***	
	Thiamine triphosphatase	*Mg^{2+}-ATPase*
None (control)**	100	100
Ca^{2+} (0.04 mM)	50	100
Ouabain (1 mM)	100	100
Tripropyltin (0.01 mM)	100	60
Na^+ (110 mM) + K^+ (10 mM)	100	230

*Composition of the reaction mixture and assay conditions are described in Fig. 1. The cell fraction containing nerve endings and other membranes (NE) was used in this experiment.
**A control value of 100 is used to represent the control thiamine triphosphatase (210 nmoles/mg protein per min) and the control Mg^{2+}-ATPase (100 nmoles/mg protein per min).

We also demonstrated that the thiamine triphosphatase is distinct from a more general or non-specific nucleoside triphosphatase by determining the enzymatic activity of both membrane and soluble cell fractions against several other substrates. Hydrolysis of thiamine triphosphate by the soluble fraction occurred 8 times as rapidly as the hydrolysis of CTP, GTP, or UTP. The activity towards the latter three substrates was completely unaffected by Ca^{2+}, whereas the hydrolysis of thiamine triphosphate was strongly inhibited. Similarly, the particulate fraction hydrolyzed all substrates, but only thiamine triphosphatase was inhibited by Ca^{2+}. Furthermore, thiamine triphosphatase in the particulate fraction was stimulated 150% of the control activity by sodium deoxycholate (0.05%), whereas nucleoside triphosphatase was inhibited 40% by this detergent.

We are led by these data to conclude that brain possesses a comparatively active triphosphatase which is specific for thiamine triphosphate and is distinct from ATPase and from non-specific nucleoside triphosphatases. This enzyme may well be the triphosphatase responsible for thiamine phosphate-dependent changes in the permeability of the excitable membrane to sodium ions during the course of the action potential [3,4]. We are currently studying the properties of both the membrane-associated and soluble form of this enzyme and its possible physiological role in nerve conduction.

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