2. Quantities of amino acids containing between 0.05 and 5 μg of α-amino nitrogen can be determined. The accuracy of a single determination is of the order of $\pm$ 5% at levels of 0.05–1 μg N, but is $\pm$ 2% when quantities of 1.5–5 μg N are determined.

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Short Communications

The *in vitro* inhibition of acetyl phosphatase by thyroxine

A study of the properties of purified acetyl phosphatase[1], an enzyme first described by LIPMANN[2], demonstrated that it is a hydrolase with specificity for acyl phosphates. Radioisotope and chemical studies indicated that it does not possess acetyl- or phosphotransferase activity. The possibility that an enzyme whose function leads to the loss of high energy phosphate compounds may take part in the cellular mechanisms regulating growth and metabolism has led to a study of this enzyme in varying metabolic states. It was also thought to be of interest to study the effect of hormones on the activity of purified preparations. This communication deals with the inhibition of acetyl phosphatase by L-thyroxine.

Enzymic activity was measured by incubating 20 μmoles acetyl phosphate, 100 μmoles sodium acetate buffer, pH 5.4, and enzyme in a final volume of 1.0 ml for 20 min at 36° C. One unit is defined as that amount of enzyme catalyzing the hydrolysis of 10 μmoles of acetyl phosphate, under these conditions, as measured by the hydroxamic method of LIPMANN AND TUTTLE[3]. Little effect of $5 \cdot 10^{-5} M$ L-thyroxine on the enzymic activity could be obtained if both substrate and L-thyroxine were added at the same time. However, upon preincubation of 0.32 units of the enzyme with $5 \cdot 10^{-5} M$ L-thyroxine for 30 min at 36° C, complete inhibition of the hydrolysis of added acetyl phosphate resulted, as shown in Table I. The enzyme used was purified 400 fold from a water extract of horse skeletal muscle.

TABLE I

EFFECT OF PREINCUBATION OF L-THYROXINE ON ACETYL PHOSPHATASE ACTIVITY

	% *activity*
Enzyme	100
Enzyme + L-thyroxine	92
Enzyme + L-thyroxine preincubated 30 min, 36° C, pH 6.0	0

Concentrations: L-thyroxine = $5 \cdot 10^{-5} M$; Enzyme = 0.32 units.
Test conditions: 20 μmoles acetyl phosphate, 100 μmoles sodium acetate, pH 5.4, vol. 1.0 ml, 36° C, 20 min.

Further experiments with varying concentrations of enzyme and L-thyroxine indicate an apparent stoichiometric inhibition of the enzyme. As can be seen in Table II, approximately 0.06 unit of enzyme was inhibited by 0.01 μmole L-thyroxine under the test conditions used. Both DL-3,3',5-triiodothyronine and DL-3,5-diiodothyronine at the same concentration inhibited the enzyme but were 60% as effective as thyroxine. No effect was obtained from $5 \cdot 10^{-5} M$ NaI. Benzoic acid, 2,4-dinitrophenol, L-tyrosine, DL-phenylalanine did not inhibit at a concentration of $5 \cdot 10^{-5} M$. At $6 \cdot 10^{-4} M$ an inhibition resulted from these compounds which was 30% as effective as $5 \cdot 10^{-5} M$ L-thyroxine. Except in the case of benzoic acid, this inhibition did not depend upon preincubation with the enzyme.

TABLE II

APPARENT STOICHIOMETRIC RELATIONSHIP BETWEEN L-THYROXINE AND ACETYL PHOSPHATASE

Enzyme added units	*L-thyroxine added μmoles*	*% inhibition*	*units inhibited by 0.01 μmoles L-thyroxine**
0.85	0.05	37	0.064
0.59	0.05	52	0.062
0.32	0.05	100	0.064
0.32	0.025	44	0.056
0.32	0.009	20	0.070

* Preincubation for 30 min at 36° C, pH = 6.0. Test conditions as in Table I.

The mechanism of the inhibition is under investigation. Inhibition by binding of a necessary metal was suggested by the strong metal-complexing properties of thyroxine[4]. However, all attempts to show a metal requirement for this enzyme have been unsuccessful. The following did not inhibit the enzyme: 0.01 M ethylenediaminetetraacetate, 0.01 M NaF, 0.1 M NaCN, 0.0016 M α,α-dipyridyl, 0.001 M sodium diethyldithiocarbamate and 0.001 M o-phenanthroline. The last two compounds were reported by VALLEE[5] to inhibit alcohol dehydrogenase presumably by complexing with the zinc tightly bound to the enzyme. Dialysis of acetyl phosphatase against distilled and demineralized water resulted in a loss of 20% of the specific activity, which could not be restored by 0.001 M $MgCl_2$, $MnCl_2$, $Cu(NO_3)_2$, $CoCl_2$, $FeCl_3$, Zn (acetate)$_2$, $FeSO_4$, $CaCl_2$ or by the concentrated dialysate. Addition of 0.001 M Zn (acetate)$_2$ to the preincubation mixture at zero time prevented inhibition by thyroxine, but addition after the preincubation period did not reverse the already established inhibition, nor was 0.001 M $MnCl_2$ or $MgCl_2$ able to reverse the inhibition.

Additions of up to 40 μmoles acetyl phosphate after preincubation of thyroxine and enzyme without substrate had no effect in reversing the already established inhibition. However, addition of 5 μmoles acetyl phosphate in the preincubation period was effective in preventing 50% of the inhibition. The substrate therefore protects the enzyme, perhaps by combining with the susceptible site and preventing inactivation by thyroxine. Zinc, on the other hand, may protect the enzyme by binding with the thyroxine and preventing it from reacting with the enzyme.

Little can be said about the physiological role of the inhibition *in vitro*. Further elucidation of the mechanism of the inhibition and of the possible role of acetyl phosphatase in regulating glycolysis and respiration may cast further light on these problems.

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