THE ENZYMIC PHOSPHORYLATION OF VITAMIN B_6 DERIVATIVES AND THEIR EFFECTS ON TYROSINE DECARBOXYLASE*

by

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One type of inhibition found with vitamin analogues has been suggested to be via its ability to be further metabolized and thus successfully compete for the same site on an enzyme surface normally occupied by coenzymes. The antagonism found with desoxypyridoxine[1] and pyrithiamine[2] has been attributed to enzymic phosphorylation of these compounds to an inhibitory form. Recently, KEARNEY[3] has demonstrated the direct enzymic phosphorylation of vitamin B_2 antagonists with a purified yeast enzyme. The present investigation demonstrates the direct phosphorylation of vitamin B_6 analogues and their effect on tyrosine apodecarboxylase. The enzyme system used for these studies was an enzyme preparation capable of phosphorylating pyridoxal. The enzyme has been purified about 1000-fold from brewer's yeast strain BSC** by $(NH_4)_2SO_4$ fractionations, alcohol fractionation, isoelectric precipitation of contaminating proteins, and heat inactivation. The enzyme was followed through the above purification by its ability to convert pyridoxal and ATP to pyridoxal phosphate and ADP. Pyridoxal phosphate was assayed by a modification of the tyrosine apodecarboxylase method[4]. The enzyme preparation does not exhibit strict specificity regarding pyridoxal. Pyridoxine, pyridoxamine, desoxypyridoxine, 4,5-dihydroxy-2-methyl pyridine, and 3-amino-4,5-dihydroxymethyl-2-methyl pyridine appear to be phosphorylated since labile phosphate disappears when ATP and the analogues are incubated together in the presence of the kinase. The unphosphorylated analogues have no effect on the union of pyridoxal phosphate with tyrosine apodecarboxylase, while the phosphorylated analogues are found to inhibit tyrosine apodecarboxylase, apparently by blocking the union of pyridoxal phosphate with the enzyme (Table I). The same inhibition is observed when the phosphorylated analogue and pyridoxal phosphate are incubated together as when the phosphorylated analogue is preincubated with tyrosine apodecarboxylase before the addition of pyridoxal phosphate. However, if pyridoxal phosphate is preincubated with tyrosine apodecarboxylase before the addition of the analogue phosphate, no inhibition is found[1]. Both chemically and enzymically synthesized pyridoxamine phosphate are found to stimulate the decarboxylase, and this may be due to its conversion to pyridoxal phosphate. The compound, 2-ethyl-3-amino-

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** Anheuser-Busch, washed, low-temperature dried yeast.

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TABLE I

THE EFFECT OF VITAMIN B_6 ANALOGUES ON TYROSINE DECARBOXYLATION AND PHOSPHORYLATION OF PYRIDOXAL

Compounds tested	*Inhibition of tyrosine apodecarboxylase by product of kinase reaction*	*Inhibition of pyridoxal phosphate formation from pyridoxal plus ATP*
2-methyl-3-hydroxy-4-hydroxymethyl pyridine	—	—
2-ethyl-3-amino-4-ethoxymethyl-5-aminomethyl pyridine	—	+
desoxypyridoxine	+	
3,4-dihydroxymethyl-2,6-dimethyl pyridine	—	—
4,5-dihydroxymethyl-2-methyl pyridine	+	
3-amino-2-methyl-4,5,6-trihydroxy methyl pyridine	—	—
5-hydroxymethyl-2,4,6-trimethyl pyridine	—	—
3-hydroxy-5,6-dihydroxymethyl-2-methyl pyridine	—	—
3-amino-4,5-dihydroxymethyl-2-methyl pyridine	+	
pyridoxine	+	
pyridoxamine	stimulation	

(+) indicates inhibition while (—) indicates no inhibition. Those compounds which were found to inhibit the union of pyridoxal phosphate with tyrosine apodecarboxylase were not assayed for their ability to inhibit pyridoxal phosphorylation since separation of pyridoxal phosphate and the phosphorylated analogue would have been necessary for such tests.

4-ethoxymethyl-5-aminomethyl pyridine, does not inhibit the union of pyridoxal phosphate with tyrosine apodecarboxylase, but it markedly inhibits the phosphorylation of pyridoxal by the kinase when incubated with ATP. The inhibition is competitive and the K_I of this compound is $7.3 \cdot 10^{-5}$ *M* which indicates that this compound has a greater affinity for the kinase than pyridoxal (K_m of pyridoxal is $1.4 \cdot 10^{-4}$ *M*). Thus there are at least 2 possible methods whereby these vitamin B_6 analogues can inhibit: (1) by inhibiting the union of pyridoxal phosphate with the tyrosine apodecarboxylase by virtue of their being phosphorylated, and (2) by inhibiting the phosphorylation of pyridoxal. Also listed in Table I are those compounds which had no effect on the kinase or on the tyrosine decarboxylase after incubation with ATP. A common feature of those compounds which appear to be phosphorylated and affect the kinase is the free 6 position of the pyridine ring and the presence of a hydroxymethyl or methylamine group in the 5 position.

The enzyme is competitively inhibited by ADP, adenylic acid, adenosine, and adenine while hypoxanthine, inosine, and inosinic acid have no effect on the reaction. ITP competitively inhibits the reaction only when high concentrations are used and the inhibition can be completely reversed by increasing the concentration of Mg^{++}, suggesting that it is inhibitory by virtue of its ability to chelate Mg^{++}[5]. It appears that the 6-amino group is responsible for the union of the enzyme and purine ring. ADP and ITP are unable to participate in the phosphorylation of pyridoxal, and the system is inactive in the absence of a divalent metal.

A recent report[6] indicated that an enzyme preparation obtained by repeated $(NH_4)_2SO_4$ fractionation of an extract of yeast, phosphorylated pyridoxine and also phosphorylated thiamine leading to pyridoxine phosphate and diphosphothiamine, respectively. The preparation described here does not appear to phosphorylate thiamine as determined by cocarboxylase activity.

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Protocol

Inhibition of tyrosine apodecarboxylase: $5 \cdot 10^{-3}$ *M* analogue, $1.87 \cdot 10^{-4}$ *M* ATP, 0.04 *M* maleate buffer pH 6.9, $4 \cdot 10^{-3}$ *M* Mg^{++}, and 1.5 mg of enzyme in a total volume of 2 ml were incubated 12 hours at 33.5° C. After hydrolyzing the remaining ATP with apyrase, the reaction products were assayed for their ability to inhibit tyrosine apodecarboxylase union with $5.5 \cdot 10^{-8}$ *M* of pyridoxal phosphate.

Inhibition of pyridoxal phosphate formation: $2 \cdot 10^{-3}$ *M* analogue, $1 \cdot 10^{-2}$ *M* ATP, 0.02 *M* phosphate buffer pH 6.9, $3 \cdot 10^{-3}$ *M* Mg^{++}, 0.64 mg of protein, incubated in presence of 3 different concentrations of pyridoxal ($1 \cdot 10^{-3}$ *M*, $2 \cdot 10^{-3}$ *M*, and $1 \cdot 10^{-2}$ *M*) in a total volume of 2 ml. These reaction mixtures were incubated 2 hours at 33.5° C and then assayed for pyridoxal phosphate formation.

SUMMARY

A kinase system purified from yeast is capable of phosphorylating pyridoxal in the presence of ATP. This system has been found capable of phosphorylating other vitamin B_6 derivatives provided the 6 position of the pyridine ring is free and the 5 position contains a hydroxymethyl group. The compounds which are phosphorylated and reported in this paper are also found to inhibit the union of pyridoxal phosphate with tyrosine apodecarboxylase. The requirements for inhibition of this union and other methods of inhibiting the kinase are discussed.

RÉSUMÉ

Un système de kinase purifiée peut phosphoryler le pyridoxal en présence d'ATP. Ce système est également capable de phosphoryler d'autres dérivés de la vitamine B_6, pourvu que la position 6 du noyau pyridinique soit libre et que le position 5 contienne un groupe hydroxymethyl. Les composés qui sont phosphorylés et qui se trouvent cités dans ce mémoire empêchent aussi l'union du phosphate de pyridoxal à la tyrosine-apodecarboxylose. Les conditions d'inhibition de cette union et d'autres méthodes d'inhibition de la kinase sont discutées.

ZUSAMMENFASSUNG

Ein von Hefe gereinigtes Kinase-System kann Pyridoxal in Gegenwart von ATP phosphorylieren. Dieses System kann auch andere Vitamin-B_6-Derivate phosphorylieren, vorausgesetzt, dass die Stellung 6 im Pyridinring frei ist und in Stellung 5 eine Oxymethyl-Gruppe sitzt. Die Verbindungen, welche phosphoryliert werden und die in dieser Arbeit aufgezählt sind, verhindern auch die Bindung von Pyridoxal-Phosphat an Tyrosin-Apodecarboxylase. Die Bedingungen, unter welchen diese Verbindung gehemmt wird und andere Hemmungs-Methoden der Kinase werden erörtert.

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