Microbial Phosphorylation of Pyridoxine

— A New Phosphotransferring Reaction —

Sir:

It has been demonstrated in mammalian tissues and microorganisms that the enzymatic phosphorylation of vitamin B₆ was catalyzed with pyridoxal phosphokinase in the presence of ATP.¹⁾

In the course of our investigations on the microbial phosphorylation of vitamin B₆, it was found that a new phosphotransferring system which phosphorylated pyridoxine with *p*-nitrophenylphosphate was widely distributed in various kinds of microorganisms, especially in fungi.

TABLE I. PHOSPHORYLATION OF PYRIDOXINE

Strains tested	<i>p</i> -NPP***	Vitamin B ₆ -P formed (γ /4 ml)			
		PIN-P*		PAL-P**	
		+	—	+	—
<i>Aspergillus tamarii</i>		22.8	0	2.4	1.6
<i>Aspergillus candidus</i>		29.2	1.2	4.4	1.4
<i>Aspergillus oryzae</i> var. <i>globosus</i>		28.4	0	3.6	1.2
<i>Aspergillus flavus</i>		37.8	0.9	5.4	1.3
<i>Penicillium oxalicum</i>		4.1	0	0.2	0
<i>Neurospora crassa</i>		2.7	0.3	1.7	0.8
<i>Endomyces fibuliger</i>		1.1	0.3	1.3	0.8
<i>Pichia polymorpha</i>		2.1	0	0.8	0.8
<i>Saccharomyces cerevisiae</i>		0.8	0.4	0	0

The reaction mixture containing, per 4 ml of 10 μ moles of pyridoxine hydrochloride, 50 μ moles of disodium *p*-nitrophenylphosphate, 200 μ moles of potassium phosphate buffer of pH 7.0, 20 μ moles of magnesium sulfate and 20 mg of resting cells as dry matter was shaken for 14 hours at 29°C.

* PIN-P: Pyridoxine phosphate

** PAL-P: Pyridoxal phosphate

*** *p*-NPP: *p*-Nitrophenylphosphate

1) D. B. McCormick, M. E. Gregory and E. E. Snell, *J. Biol. Chem.*, **236**, 2076 (1961).

Table I shows the formation of vitamin B₆ phosphates from pyridoxine and *p*-nitrophenylphosphate by the cells of several microorganisms. This system was remarkably present in such genera as *Aspergillus*, *Penicillium*, *Neurospora*, *Endomyces*, *Pichia*, and *Saccharomyces*. In the case of *Saccharomyces*, the activity increased when the dried cells were used. Vitamin B₆ phosphate formed by these microorganisms was mainly pyridoxine phosphate. In some microorganisms, pyridoxine phosphate formed was partially oxidized to pyridoxal phosphate by pyridoxine phosphate oxidase.

TABLE II. THE SPECIFICITY OF PHOSPHATE DONOR

Donor substrate	Vitamin B ₆ -P formed (γ /4 ml)	
	Pyridoxine-P	Pyridoxal-P
<i>p</i> -Nitrophenylphosphate	3.6	1.9
ATP	0	0.4
Glucose-1-P	0.1	0.2
α -Glycerophosphate	0.1	0.2
Ribotide*	0	0.3
FMN	0	0
None	0	0.2

The reaction mixture containing, per 2 ml of 2 μ moles of pyridoxine hydrochloride, 4 μ moles of phosphate donor, 100 μ moles of potassium phosphate buffer of pH 7.0, 10 μ moles of magnesium sulfate and 10 mg of dried resting cells of *Saccharomyces cerevisiae* was incubated for 4 hours at 37°C.

* Ribotide: Inosinic acid 40.8, Guanylic acid 33.1, Cytidylic acid 1.8, Uridylic acid 1.4%.

When dried cells of *Saccharomyces cerevisiae* were incubated with pyridoxine and various phosphate donors, *p*-nitrophenylphosphate was more effective for the formation of vitamin B₆ phosphates than the other phosphate donors.

(Table II)

These results suggest the occurrence of an enzyme which catalyzes the phosphorylation of pyridoxine with *p*-nitrophenylphosphate as an unphysiological phosphate donor.

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