THE PARTICIPATION OF INORGANIC PYROPHOSPHATE IN THE REVERSIBLE ENZYMATIC SYNTHESIS OF DIPHOSPHOPYRIDINE NUCLEOTIDE

Sirs:

A purified enzyme preparation has been obtained from an autolysate of dried brewers' yeast by ammonium sulfate fractionation and isoelectric precipitation which catalyzes the reaction: nicotinamide mononucleotide (NMN) + adenosine triphosphate (ATP) ⇄ DPN + inorganic pyrophos-

Substance estimated†	DPN synthesis,* micromoles per ml.			ATP synthesis,* micromoles per ml.		
	0 min.	60 min.	Δ	0 min.	60 min.	Δ
NMN‡	2.50			0.0		
ATP	2.20	1.36	−0.84	0.0	1.02	+1.02
DPN	0.0	0.74	+0.74	1.50	0.61	−0.89
P-P	0.0	0.83	+0.83	1.80	0.79	−1.01
Orthophosphate	0.11	0.13		0.16	0.09	
Phosphate, acid-labile	4.44	4.50		3.60	3.66	

* For DPN synthesis, 1.0 ml. of reaction mixture contained 50 γ of the enzyme preparation, 0.3 micromole of $MgCl_2$, and 50 micromoles of glycylglycine buffer (pH 7.4) in addition to ATP and NMN; for ATP synthesis 1.0 ml. contained 50 γ of the enzyme preparation, 0.75 micromole of $MgCl_2$, and 50 micromoles of glycylglycine buffer (pH 7.4) in addition to DPN and P-P. Constant values were reached after 30 to 40 minutes at 38°.

† ATP was estimated spectrophotometrically by triphosphopyridine nucleotide reduction in the presence of glucose, hexokinase, and *Zwischenferment*, and DPN by reduction with the triose phosphate dehydrogenase system. Inorganic pyrophosphate was estimated as orthophosphate after acid hydrolysis of the precipitated and washed manganous salt and acid-labile phosphate as the orthophosphate released after 10 minute hydrolysis in 1 N H_2SO_4 at 100°.

‡ NMN was prepared by hydrolysis of DPN with nucleotide pyrophosphatase.[1] [2] After purification the ratio, nicotinamide-ribose moiety to organic phosphate, was 1.0.

phate (P-P). In the table are summarized two experiments in which equilibrium was attained starting from the left (DPN synthesis) and the right (ATP synthesis). The equilibrium constant, $K = ((DPN)(P\text{-}P))/((NMN)(ATP))$, calculated from the data of the two experiments is 0.3 in one case and 0.5 in the other. The concentrations of acid-labile phosphate were unchanged and no orthophosphate was produced. Nicotinamide nu-

[1] Kornberg, A., *J. Biol. Chem.*, **174,** 1051 (1948).

[2] Kornberg, A., and Lindberg, O., *J. Biol. Chem.*, **176,** 665 (1948).

cleoside, adenosine diphosphate, and adenylic acid were inactive in DPN synthesis. The reduced form of DPN was split by the purified enzyme preparation in the presence of inorganic pyrophosphate, but triphosphopyridine nucleotide and flavin-adenine dinucleotide were not. The possibility that the latter two nucleotides may participate in analogous reactions with crude enzyme preparations requires further study.

These findings indicate a mechanism for the synthesis of DPN and for the origin and function of inorganic pyrophosphate. Ochoa, Cori, and Cori[3] isolated inorganic pyrophosphate from dialyzed rat liver dispersions in which glutamate, pyruvate, or succinate was being oxidized. It was later identified in washed rabbit kidney particles oxidizing glutamate,[4, 2] in molds,[5] and in yeast.[6] The present findings suggest that the accumulation of inorganic pyrophosphate in fungi and in tissues may be explained by a sequence of three reactions: (1) the irreversible hydrolysis of DPN by nucleotide pyrophosphatase[1, 2] to yield NMN and adenylic acid, (2) the phosphorylation of adenylic acid to ATP in respiration or fermentation, and (3) the combination of NMN with ATP to produce inorganic pyrophosphate and regenerate DPN.

National Institutes of Health
Bethesda
Maryland

ARTHUR KORNBERG[7]

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