ACTA CHEMICA SCANDINAVICA 11 (1957) 1077—1096

Proceedings of the Danish Biochemical Society

Papers read at the joint meeting of the Scandinavian Biochemical Societies in Copenhagen, June 3—4, 1957

The Enzymic Amination of Xanthosine-5-phosphate to Guanosine-5-phosphate

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In a previous communication [1] the occurrence of two enzyme-catalyzed reactions in extracts of pigeon liver acetone powder was reported, leading from inosine-5-phosphate to guanosine-5-phosphate *via* xanthosine-5-phosphate. The first reaction took place only in the presence of DPN * while the second reaction required ATP, Mg^{++}-ions and L-glutamine as an aminogroup donor. NH_4^+-ions or L-aspartate could not substitute for L-glutamine while L-glutamate was active at higher concentrations owing to the presence of glutamine synthease in the crude extract.

Inosine-5-phosphate $\xrightarrow{\text{DPN}}$ Xanthosine-5-phosphate (1)

Xanthosine-5-phosphate $\xrightarrow[\text{L-glutamine}]{\text{ATP}}$ Guanosine-5-phosphate (2)

At the same time Abrams and Bentley [2,3] demonstrated the occurrence of similar reactions in extracts of rabbit bone marrow while Gehring and Magasanik [4] reported on the partial purification of an enzyme catalyzing reaction (1) from *Aerobacter aerogenes*. Magasanik *et al.*[5] have later reported briefly on the purification of an enzyme from *E. coli* that aminated XMP by a different mechanism using NH_4^+-ions as aminogroup donors.

The enzyme (or enzymes) catalyzing reaction (2) has been purified 80—90 fold over the crude extract. Acetone powder of pigeon liver was extracted with dilute phosphate buffer pH 7.4 and after acidification to pH 6 fractionated with ammonium sulfate between 30 and 50 % saturation. The precipitate was dissolved in phosphate buffer pH 7.4 and inactive protein removed by adding $MnCl_2$. The centrifugate was again fractionated with ammonium sulfate between 39 and 55 % saturation. The precipitate was dissolved in dilute phosphate buffer pH 7.4, dialyzed shortly and chromatographed on DEAE-cellulose in PO_4-form by gradient elution with phosphate buffer pH 6.0. A typical preparation is shown in Table 1.

Table 1. Purification of the enzyme.

Purification step	mg protein	Spec. act.	Total units
Crude extract	8 600	0.21	1 810
1st ammonium sulfate precipitate	3 660	0.54	1 970
Adsorption on manganous phosphate	1 830	0.93	1 700
2nd ammonium sulfate precipitate	500	1.70	850
Chromatography on DEAE-cellulose	14	19	266

Using the purified enzyme no amination of XMP was obtained in the presence of L-glutamate instead of L-glutamine. Balance experiments indicate the following stoichiometry for reaction (2):

XMP + L-glutamine + ATP → GMP + L-glutamate + AMP + pyrophosphate

* The following abbreviations are employed: AMP = adenosine-5-phosphate, ATP = adenosine-5-triphosphate, DEAE = diethylaminoethyl, DPN = diphosphopyridine nucleotide, XMP = xanthosine-5-phosphate.

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2. Abrams, R. and Bentley, M. *J. Am. Chem. Soc.* **77** (1955) 4179.
3. Bentley, M. and Abrams, R. *Federation Proc.* **15** (1956) 218.
4. Gehring, L. B. and Magasanik, B. *J. Am. Chem. Soc.* **77** (1955) 4685.
5. Magasanik, B., Moyed, H. S. and Karibian, D. *J. Am. Chem. Soc.* **78** (1956) 1510.