ACTA CHEMICA SCANDINAVICA 8 (1954) 1095—1110

Proceedings of the Swedish Biochemical Society

Papers read at the meeting in Lund, June 3—4, 1954.

The Biosynthesis of Ribonucleic Acid Pyrimidines in Ehrlich's Ascites Tumor

Ulf Lagerkvist and Peter Reichard

Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

In a previous communication we have proposed the following scheme for the synthesis of orotic acid in rat liver slices [1]. L-aspartic acid + CO_2 + NH_3 → L-ureidosuccinic acid → dihydro-orotic acid → orotic acid.

Reichard [2] has presented strong evidence that orotic acid is a normal intermediate in the biogenesis of polynucleotide pyrimidines and therefore the scheme given above probably represents the actual pathway of pyrimidine synthesis in rat liver. The pyrimidine biogenesis of the Ehrlich ascites tumor [3] has now been investigated for comparison.

Two different experimental approaches were used. In the first one tumor cells were incubated in Krebs-Henseleit medium with any of the following N^{15} labeled substances: Ammonia, aspartic acid, L-ureidosuccinic acid, orotic acid, uracil, dihydrouracil, ureidopropionic acid, β-alanine. The ribonucleic acid pyrimidines were isolated and their isotope content determined. In experiments with N^{15}-ammonia and aspartic acid the distribution of the isotope in the pyrimidine ring was determined [4].

In the second approach the tumor cells were incubated with an isotopically labeled precursor (known to give an incorporation of isotope into the polynucleotide pyrimidines) in the presence of a pool of the non labeled substance suspected to be an intermediate (ureidosuccinic acid, dihydroorotic acid or orotic acid). Both the substance under investigation and the polynucleotide pyrimidines were isolated at the end of the incubation and their isotope content was determined.

The results of these two experimental approaches show that the same precursors as in rat liver slices can be used by the ascites tumor for the synthesis of polynucleotide pyrimidines. The question of these substances being also normal intermediates tends to be obscured by permeability effects and will be discussed.

1. Reichard, P. and Lagerkvist, U. *Acta Chem. Scand.* **7** (1953) 1207.
2. Reichard, P. *J. Biol. Chem.* **197** (1952) 391.
3. Klein, G. and Révész, L. *J. Natl. Cancer Inst.* **14** (1953) 229.
4. Lagerkvist, U. *Acta Chem. Scand.* **4** (1950) 543.

Formation *in vitro* of Uridine Phosphates from Orotic Acid

Robert B. Hurlbert and Peter Reichard

Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

The transformation of orotic acid into different uridine phosphates has been demonstrated in rat liver *in vivo* [1]: Since it was shown that one or several of these nucleotides are precursors of polynucleotide pyrimidines and since orotic acid has been demonstrated to be a normal intermediate in pyrimidine biosynthesis in rat liver [2] an investigation of the enzymatic conversion of C^{14}-orotic acid into uridine phosphates has been started.

The enzymes were obtained either from the dialyzed supernatant of homogenized liver (rat and pigeon) after high speed centrifugation or from extracts of pigeon liver acetone powder. In the presence of ribose-5-phosphate, Mg^{++} and ATP(or fructose-di-phosphate DPN, Mg^{++} and ATP) C^{14}-orotic acid was converted into several radioactive compounds, one of which was identified as uridine-5-phosphate (UMP). The other compounds gave rise to UMP upon acid hydrolysis and were probably pyrophosphates of uridine (*e. g.* uridine-diphosphate and uridinetriphosphate). In some cases enzymatic breakdown of the uridinephosphates to uridine and uracil took place. In a time study it was found that UMP was the first uridine compound formed from orotic acid. This result was confirmed by the finding that C^{14}-UMP was rapidly transformed into radioactive uridinepyro-

phosphates and that the presence of non labeled UMP diminished the incorporation of isotope from C^{14}-orotic acid into the pyrophosphates. Uridine was not an intermediate in the transformation of orotic acid into UMP.

Enzyme fractionation with $(NH_4)_2SO_4$ and methanol are being carried out to investigate the intermediate steps in these reactions.

1. Hurlbert, R. B. and Potter, V. R. *J. Biol. Chem. In press.*
2. Reichard, P. *J. Biol. Chem.* **197** (1952) 391.

Quantitative Determination of Phenylthiohydantoins from Amino Acids

John Sjöquist

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Phenylthiohydantoins are separated on a chromatographic column on a microscale and the optical density of the effluent is continuously recorded. Details of the method and the apparatus will be described.

1. Edman, P. *Acta Chem. Scand.* **4** (1950) 283.
2. Sjöquist, J. *Acta Chem. Scand.* **7** (1953) 447.

The Mode of Action of Mn^{++} on Oxidative Phosphorylation

L. Ernster and O. Lindberg

Wenner-Gren's Institute, Stockholm, Sweden

In a recent communication [1] it has been shown that Mn^{++} forms a prosthetic group of the hydrogen transferring catalysts involved in the generation of energy rich phosphate ($\sim$ P) bonds. The role of Mn^{++} is to link these enzymes with ATP which serves as a cofactor in coupled phosphorylation. Non-phosphorylative (as it, *e. g.*, occurs in the Keilin-Hartree heart-muscle preparation) and phosphorylative oxidation may thus be described by equations 1 and 2 respectively:

$$DPNH_2^* + 1/2\ O_2 \xrightarrow{E-CoE} DPN + H_2O \quad (1)$$

$$DPNH_2 + 1/2\ O_2 + n\cdot P \xrightarrow{(E-CoE)-Mn-ATP} DPN + H_2O + n\cdot\sim P \quad (2)$$

* DPN = diphosphopyridine nucleotide
ATP = adenosinetriphosphate

(where CoE stands for the hydrogen bearing coenzymes involved), the two enzyme complexes being correlated according to the equation:

$$(E-CoE)-Mn-ATP \rightleftharpoons (E-CoE) + Mn^{++} + ATP.$$

The question was left open whether the ATP-containing enzyme complex which catalyzes reaction 2 is responsible for the ultimate synthesis of ATP, or whether its function is merely restricted to the oxidative formation of the primary $\sim$ P bond. It has now been shown that the latter is the case.

2,4-dinitrophenol (DNP), which is known to inhibit ATP synthesis connected with hydrogen transfer between $DPNH_2$ and oxygen, stimulates respiration in mitochondrial systems (see Table 1). The latter action is, however, dependent on the concentration of ATP prevailing in the medium. Thus the stimulation can be turned into an inhibition when the concentration of ATP is kept low by adding hexokinase. Moreover, Mn^{++} ($0.5—1.0 \times 10^{-3}$ *M*) is able to prevent this inhibition. These facts indicate that $\sim$ P is formed in this system according to equation 2, while the action of DNP is confined to the blockage of the subsequent transfer of the formed $\sim$ P to the adenylic acid system.

Table 1. Effect of Mn^{++} on mitochondrial respiration inhibited by DNP. Each Warburg vessel contained: mitochondria (prepared in 0.25 M sucrose containing 0.01 M versene), 1/12 rat liver; glycylglycine, 200 µmoles; KCl, 150 µmoles; glucose, 180 µmoles; D,L-*glutamate, 60 µmoles; orthophosphate, 90 µmoles; AMP, 3 µmoles; Mg^{++}, 10 µmoles. Additions (where indicated): yeast hexokinase (prepared as described previously* [1]*), 0.1 ml; DNP, 0.4 µmoles; Mn^{++} 1.2 µmoles.—Final volume, 2.5 ml. pH, 7.8. Temp. 30° C. Gas phase, air. Time of incubation, 45 min.*

Additions	Respiration µatoms O	Phosphorylation µmoles P
None	15.2	9.6
DNP	44.0	4.9
Hexokinase	36.5	85.5
DNP + hexokinase	8.4	9.7
DNP + hexokinase + Mn^{++}	45.5	12.2

1. Lindberg, O. and Ernster, L. *Nature* **173** (1954) 1038.