Enzymic Formation of Uridine-5′-phosphate from Uracil

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Most mammalian tissues studied show a relatively poor utilization of uracil for pyrimidine nucleotide synthesis. In the Ehrlich ascites tumor, however, uracil was incorporated into polynucleotide pyrimidines as rapidly as orotic acid. In a study of the enzymic mechanism of this process it was found that uridine-5′-phosphate(UMP) formation from uracil in acetone powder extracts of the tumor proceeded according to:

$$\text{Uracil} + \text{ribose-1-phosphate} \xrightarrow[\text{phosphorylase}]{\text{nucleoside}} \text{uridine} + \text{phosphate}$$

$$\text{Uridine} + \text{ATP} \xrightarrow[\text{kinase}]{\text{uridine}} \text{UMP} + \text{ADP}$$

Both enzymes involved are known to occur in mammalian organs, *e. g.* liver, which form very little UMP from uracil.

We have now investigated the amount of these two enzymes in the ascites tumor and in rat liver. Furthermore, the breakdown of uracil to CO_2 was also studied, since it had been proposed that this process might determine uracil utilization in different tissues [2].

A significant difference was found in the amount of nucleoside phosphorylase as measured by a spectrophotometric assay. Uridine kinase was consistently *ca.* 10 times more active in extracts of the tumor than in liver extracts. A high concentration of uridine was necessary for optimum UMP synthesis. In accordance with earlier findings of other investigators we observed a quite rapid breakdown of uracil to CO_2 in rat liver preparations. No such degradation was observed in tumor extracts, however. In both cases a high TPNH level was maintained during the reaction.

It seems likely that uracil utilization by different tissues will depend on a competition between catabolic reactions which degrade uracil to CO_2 and the anabolic formation of UMP.

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2. Canellakis, E. S. *J. Biol. Chem.* **221** (1956) 315.

Dephosphorylation of Nucleoside Triphosphates by Rat Diaphragm *in vitro* *

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When the isolated rat diaphragm is incubated in saline phosphate medium at pH 7.4 at 37 °C a marked ATPase and adenylic acid deaminase activity is present. These properties have been studied by determination of the degradation products of ATP in the diaphragm during incubation. The products formed have been separated by an ion exchange resin method on Dowex-1. By the same technique the conversion products of nucleoside triphosphates added to the medium during incubation of rat diaphragm have been studied

The breakdown of ATP in the diaphragm has been studied in experiments where diaphragms from 20 rats, weighing 6—7 g, were incubated in 15 ml saline phosphate medium. During incubation for 30 min in oxygen, 25 % of ATP initially present in the diaphragm (2.2 μmole per g initial level) was broken down and almost quantitatively recovered as IMP and inosine. In anaerobiosis the breakdown of ATP was greatly accelerated, amounting to 90 % in 30 min. The same high rate of breakdown was observed in oxygen if 5×10^{-5} M 2,4-dinitrophenol or 0.01 M NaCN was present in the medium. In these experiments nucleotides were not detected in the medium which, however, contained a considerable amount of inosine.

The breakdown of nucleotides added to the medium was studied by incubating 2 hemidiaphragms weighing 250 mg in 2 ml saline phosphate medium. 10 μmole ATP added at the start of the experiment was rapidly broken down in oxygen atmosphere. Thus after 30 min 95 % of ATP was broken down and accumulation of ADP, AMP, IMP and inosine was demonstrated. The dephosphorylation and deamination probably did occur on the surface of the diaphragm muscle as no enzyme activity was liberated into the medium when 2 hemidiaphragms were incubated for 30 min. The experiments indicate that the following sequence of reactions takes place:

ATP → ADP → AMP → IMP → Inosine

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Experiments on studies of the breakdown of other nucleoside triphosphates CTP, UTP, GTP and ITP will be reported.

Some Properties of the Uridine Diphosphate Glucose Pyrophosphorylase

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In a previous communication [1] the purification and isolation of uridine diphosphate glucose (UDPG) pyrophosphorylase from yeast is described. This enzyme catalyses the following reversible reaction:

$$UDPG + P—P \rightleftharpoons UTP + G—1—P$$

Experiments were described, which showed that incubation of uridine triphosphate (UTP) and ^{32}P-labelled pyrophosphate (P—P) with the enzyme rendered ^{32}P-labelled UTP. Likewise incubation of UDPG with ^{32}P-labelled glucose-1-phosphate (G-1-P) rendered ^{32}P-labelled UDPG. These exchange experiments have been interpreted as an indication of the formation of a free uridyl-enzyme complex as an intermediate in the pyrophosphorolytic transfer of uridyl groups.

In view of the findings of Neufeld *et al.*[2] that UDPG pyrophosphorylase, isolated from seedlings of mungbeans, does not bring about an incorporation of $^{32}P—^{32}P$ into UTP as described above, the exchange experiments with the yeast enzyme have been repeated under more quantitative conditions. The incorporation rate of $^{32}P—^{32}P$ into UTP has been measured by an assay, similar to that described by Berg [3], *i. e.* after incubation of the enzyme with UTP and ^{32}P-labelled P—P, the incubation mixture is treated with charcoal; the charcoal is suspended in 50 % ethanol, and the radioactivity of an aliquot of this suspension is measured. During the purification procedure for the enzyme the different fractions have been tested for exchange activity, measured by the above described charcoal assay, as well as for UDPG pyrophosphorylase activity measured spectrophotometrically [2], and the ratio between these two activities per mg protein remains constant during the different stages (35 fold purification).

If, however, the purified UDPG pyrophosphorylase is treated with crystalline phosphoglucomutase, the exchange activity may be completely abolished; at the same time the enzyme activity as measured spectrophotometrically remains unchanged. This finding together with the fact that addition of G-1-P or UDPG greatly enhances the exchange indicates that the exchange ability of the purified UDPG pyrophosphorylase is not an intrinsic quality of the enzyme, but rather caused by some tightly bound UDPG or G-1-P which cannot be removed by the ordinary purification methods.

The charcoal assay offers an extremely sensitive assay for the pyrophosphorylase reaction. Less than 0.005 μmoles of UDPG or G-1-P may be easily detected and, since this assay, contrary to the spectrophotometric assay, requires no other enzyme system present, it is well suited for kinetic investigations. Measured with this technique the purified UDPG pyrophosphorylase shows specificity for G-1-P as uridyl acceptor among a number of hexose and pentose phosphate esters tested, while crude yeast extract and extracts of fresh spinach leaves contain several uridyl-linked pyrophosphorylases.

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Some Studies on Amino Acid Decarboxylases

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Glutamic acid decarboxylase is known to have pyridoxal-5-phosphate as cofactor. We found it to require in addition a metal cofactor, namely Zn^{++}, Fe^{+++} or Co^{++} for the enzyme from carrots, and Mn^{++} for the rat brain enzyme. SH-groups appear to be essential. Cysteic acid decarboxylase also requires pyridoxal-5-phosphate as cofactor, and also Fe^{++}, Zn^{++} or Mn^{++}. The presence of SH-groups in this enzyme is doubtful. Cysteine sulphinic acid decarboxylase is also a pyridoxal-5-phosphate enzyme, with essential SH-groups and requiring Fe^{++}, Fe^{+++} or Zn^{++}. The authors consider cysteic acid decarboxylase and cysteine sulphinic acid decarboxylase to be two different enzymes. All three enzymes have been purified to some extent.