

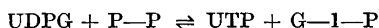
Experiments on studies of the breakdown of other nucleoside triphosphates CTP, UTP, GTP and ITP will be reported.

Some Properties of the Uridine Diphosphate Glucose Pyrophosphorylase

Agnete Munch-Petersen

The Fibiger-Laboratory, University of Copenhagen, Denmark

In a previous communication¹ the purification and isolation of uridine diphosphate glucose (UDPG) pyrophosphorylase from yeast is described. This enzyme catalyses the following reversible reaction:



Experiments were described, which showed that incubation of uridine triphosphate (UTP) and ³²P-labelled pyrophosphate (P—P) with the enzyme rendered ³²P-labelled UTP. Likewise incubation of UDPG with ³²P-labelled glucose-1-phosphate (G-1-P) rendered ³²P-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 pyrophosphorylolytic 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 ³²P—³²P into UTP as described above, the exchange experiments with the yeast enzyme have been repeated under more quantitative conditions. The incorporation rate of ³²P—³²P into UTP has been measured by an assay, similar to that described by Berg³, *i. e.* after incubation of the enzyme with UTP and ³²P-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 spectrophotometrically², 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 μ moles 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.

1. Munch-Petersen, A. *Acta Chem. Scand.* **9** (1955) 1523.
2. Neufeld, E. F., Ginsburg, V., Putman, E. W., Fanshier, D. and Hassid, W. Z. *Arch. Biochem. and Biophys.* *In press.*
3. Berg, P. *J. Biol. Chem.* **222** (1956) 991.

Some Studies on Amino Acid Decarboxylases

Gunnar Steensholt and
P. E. Joner

Biochemical Institute, University of Oslo, Norway

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.