Enzymatic Synthesis of Ureidooxuccinic Acid

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Ureidooxuccinic acid (USA) has been shown to be a normal intermediate in the biogenesis of orotic acid from aspartic acid and CO₂ in rat liver slices. After preparation of cell fractions by differential centrifugation of homogenized rat or rabbit liver in 0.25 M sucrose, uridine-pyrophosphate synthetase of the enzymatic synthesis of USA could be localized to the mitochondrial fraction. Aspartic acid, bicarbonate, ammonia, ATP, and glutamate were required for USA synthesis. Glutamine served the double function of regenerating ATP and being the source of a catalyst for USA synthesis. When small amounts of carbamyl glutamate or acetylglutamate were present during the reaction, glutamate could be substituted by succinate. The optimal synthesis was obtained only in the presence of the substituted glutamates. The requirements for optimal USA synthesis are strongly reminiscent of the enzymatic mechanism leading to citrulline synthesis.

USA synthesis could also be obtained in solution after freezing and thawing of the mitochondria followed by high speed centrifugation. These extracts showed optimal USA synthesis from aspartate, ammonia and bicarbonate in the presence of acetylglutamate, Mg⁺, ATP, phosphoglyceric acid and a muscle enzyme fraction (for regeneration of ATP). When ornithine was substituted for aspartic acid, citrulline synthesis could readily be demonstrated in the extract. However, extracts of acetone powder of rat liver prepared according to Cohen and Hayano had almost completely lost the ability to synthesize USA, while showing slightly better capacity to synthesize citrulline than the mitochondria extracts. It is concluded that at least not all the enzymatic steps are the same in USA and citrulline synthesis.


Metabolism of Uridine Triphosphate in Yeast

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Experiments have been performed to investigate the mode of action of the uridyl transferase found in yeast, which catalyzes the reaction:

Uridinediphosphoglucose + pyrophosphate ⇋ uridinetriphosphate + α-glucose-1-phosphate

In accordance with the concept of the enzyme as acceptor of anhydride groups, the reaction could be formulated as follows:

UPPG + enzyme ⇋ enzyme-UP + G-1-P

Enzyme-UP + F-P ⇋ enzyme + UPPP

Attempts to bring P³¹-labelled G-1-P or F-P into equilibrium with UPPG or UPPP respectively failed, when a crude preparation of the uridine transferase was used, but the possibility exists that other reactions may take place here and disturb the equilibrium. If a highly purified enzyme preparation is applied, however, a significant incorporation of the radioactive reactant into the nucleotide can be demonstrated.

The main feature in the purification was a fractionation with ethanol (active fraction obtained between 24—28 % ethanol) succeeded by an ammonium sulfate fractionation (active fraction precipitated at 54—60 % saturation. Activity: 1 μg of protein converted 0.008 mmole UPPG per minute). By incubating this enzyme solution with UPPG and P³¹-labelled FP followed by deproteinization and chromatography of the reaction mixture, a significant amount of the labelled pyrophosphate could be detected in the UPPP, showing that the following reaction has taken place:

UPPP + FP ⇋ UPPP + PP