S- 9-6 Thiamin Pyrophosphate: Catalytic Mechanism, Role in Protein Turnover

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DISCOVERY AND CHARACTERIZATION OF THIAMIN PYROPHOSPHATE ACTIVATED ACETALDEHE

In 1932 Auhagen [1,2] showed that for the decarboxylation of pyruvate to acetaldehyde with yeast extract, as demonstrated earlier by Neuberg and Karczag [3], a heat resistant coenzyme is necessary. In 1937 Lohmann and Schuster [4] isolated the coenzyme, which they called "cocarboxylase" and identified it as the pyrophosphoric acid ester of vitamin B1, i.e. as thiamin pyrophosphate. Different possibilities were discussed for the structure of an "activated acetaldehyde", i.e. acetaldehyde being attached to thiamin pyrophosphate as an intermediate in the pyruvate decarboxylation reaction: $\text{pyruvate} \rightarrow \text{acetaldehyde} + \text{CO}_2$ (for a summary see [5]). In model experiments with thiamin or thiamin analogs Breslow [6,7] presented evidence from considerations of organic chemical reaction mechanisms that acetaldehyde attached to the C2 of the thiazole ring in thiamin pyrophosphate might be the long sought for "active acetaldehyde". According to Breslow's proposal acetaldehyde formed from pyruvate on the catalytic center of pyruvate decarboxylase "in status nascenti" is bound to the C = N double bond of the thiazole ring corresponding to a "cyan-hydrin" reaction:

A precursor of the "active acetaldehyde" should be "active pyruvate", i.e. the addition product of pyruvate to thiamin pyrophosphate. Holzer and Beaucamp [8,9], using ¹⁴C-labeled pyruvate incubated with purified pyruvate decarboxylase from *S. cerevisiae* were the first to isolate and characterize thiamin pyrophosphate containing intermediates of an enzyme catalyzed reaction which showed the structure proposed by Breslow. First evidence for the nature of the radioactive thiamin pyrophosphate containing intermediates came from experiments with 1-¹⁴C-labeled pyruvate which resulted in the proposed "active pyruvate", i.e. 2-(α -hydroxy- α -carboxyethyl)-TPP, whereas 2-¹⁴C-labeled pyruvate lead to "active acetaldehyde" i.e. 2-(α -hydroxyethyl)-TPP [8,9]. Further characterization of the labeled material was done by using the "sulfite-cleavage" after Williams et al. [10] and by treatment of "active acetaldehyde" with HJ, followed by alkaline hydrolysis which resulted in formation of ¹⁴C-labeled propionic acid. Additional evidence for the correctness of Breslow's proposal of the structure of active acetaldehyde came from experiments of Krampitz et al. [11,12]. These authors had synthesized 2-(α -hydroxyethyl)-thiamin and could show that this synthetic material after incubation with ATP and pyrophosphorylating extracts from yeast was able to act as coenzyme of decarboxylation of pyruvate and of the formation of acetoin from acetaldehyde.

Fig. 1. Enzyme reactions of "active pyruvate" (HCETPP) and "active acetaldehyde" (HETPP).

All enzymatic reactions shown in Fig. 1 starting with "active acetaldehyde" could be demonstrated using as substrate active acetaldehyde prepared from ¹⁴C-labeled pyruvate and thiamin pyrophosphate with pyruvate dehydrogenase from pig heart muscle [13,14] and as catalyst the corresponding enzymes (for summaries and details see [5] and [15]).

THIAMIN PYROPHOSPHATE ACTIVATED GLYCOLALDEHYDE

"Active glycolaldehyde" i.e. glycolaldehyde attached to thiamin pyrophosphate had been postulated as an intermediate in the transketolase reaction, i.e. the transfer of a C₂-piece from one sugar to another [16]. Based on the isolation from enzyme preparations and the structural identification of "active acetaldehyde" (see preceding chapter) it was very probable that "active glycolaldehyde" is a thiamin pyrophosphate activated glycolaldehyde and has the structure 2-(1,2-dihydroxyethyl)-thiamin pyrophosphate (DETTP). According to the reaction

active glycolaldehyde was prepared with pyruvate dehydrogenase complex from pig heart and hydroxypyruvate and TPP as substrates [17]. The structure of the resulting TPP derivative could be shown to correspond to the proposal by Breslow, i.e. to the formula 2-(1,2-dihydroxyethyl)-thiamin pyrophosphate [6]. It could be shown that DETTP is not only the product of decarboxylation of hydroxypyruvate but also a product of incubations of transketolase with TPP and the corresponding sugar-substrates, for example fructose-6-phosphate [17]. In the presence of pyruvate dehydrogenase complex from pig heart, NAD and CoA the isolated radioactive DETTP is oxidized to glycolic acid. Glycyl-CoA is an intermediate [17].

MECHANISMS OF TRANSKETOLASE (E.C. 2.2.1.1) REACTIONS

Transketolase (TK) plays an integral and rate-limiting role in the operation of the hexose monophosphate shunt pathway for the metabolism of glucose-6-phosphate. The key reaction is the transfer of an activated C₂-fragment (activated glycolaldehyde, or 2-(1,2-dihydroxyethyl)-thiamin pyrophosphate, DETPP) from an appropriate ketosugar donor to an acceptable aldose sugar acceptor [18-20]. In this thiamin pyrophosphate-catalyzed transfer, the leaving group is an aldose moiety with two fewer C-atoms instead of CO₂ as in the case with pyruvate decarboxylase and pyruvate dehydrogenase.

Active ketol donors must have the OH groups on C₃ and C₄ trans to each other with the C₃-OH in the L-configuration and the C₄-OH in the D. Thus D-Xu-5-P, D-fructose-6-P and D-sedoheptulose-7-P are the most active donors. However L-erythrulose, D-xylulose, hydroxypyruvate and D-glycero-D-idooctulose have also been found to act as donors under certain circumstances [18-20]. As acceptors, D-GA-3-P, D-erythrose-4-P and D-R-5-P are the most efficient, but D-G-6-P, D-allose-6-P, D-arabinose-5-P, glycolaldehyde, D-glyceraldehyde and formaldehyde may also act in certain systems.

THIAMIN PYROPHOSPHATE ACTIVATED FORMALDEHYDE

Incubation of pyruvate dehydrogenase complex from pig heart with thiamin pyrophosphate and glyoxylate results in the formation of 2-hydroxymethyl-thiamin pyrophosphate, i.e. thiamin pyrophosphate-activated formaldehyde [21]:

With the methods used for structural identification of active acetaldehyde, active pyruvate and active glycolaldehyde, it was shown that the structure of the active formaldehyde corresponds to the proposal of Breslow [5,6]. Krakow et al. [22,23] have isolated and characterized an enzyme from *Escherichia coli* which catalyzes the condensation of 2 moles of glyoxylate to give 1 mol of tartronic semialdehyde plus 1 mol of CO₂:

The dependence on thiamin pyrophosphate of this condensation reaction suggested that thiamin pyrophosphate activated formaldehyde is an intermediate in the reaction. With the active formaldehyde prepared from glyoxylate and pyruvate dehydrogenase complex (see above) it could be shown that the thiamin pyrophosphate activated formaldehyde is indeed an intermediate in the "glyoxylic acid carboligase" reaction [21,24].

CONTROL OF PROTEIN TURNOVER BY THIAMIN PYROPHOSPHATE

Addition of ammonia to glucose-oxidizing yeast cells leads to an increase in the content of pyruvate decarboxylase [25]. The increase of pyruvate decarboxylase activity is prevented by addition of actinomycin or other inhibitors of protein synthesis [25,26]. The addition of thiamin causes similarly to ammonia an increase in the synthesis of pyruvate decarboxylase. The pyruvate decarboxylase-inducing activity of thiamin is clearly shown in experiments with the thiamin-deficient mutant of *S. cerevisiae* [25]. The most probable explanation for the action of thiamin on the level of pyruvate decarboxylase is that either thiamin is an inducer of the synthesis of this enzyme or that thiamin pyrophosphate stabilizes the enzyme before proteolytic degradation, i.e.

inhibits proteolytic degradation. Such a stabilizing effect is well known for other coenzymes [27]: pyridoxal phosphate [28,30,31], NAD [29,30], FAD [32].

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