DIFFERENTIATION BETWEEN THE STRUCTURAL AND REDOX ROLES OF TPNH IN 6-PHOSPHOGLUCONATE DEHYDROGENASE

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SUMMARY

In the tritium exchange reaction catalysed by 6-phosphogluconate dehydrogenase from Candida utilis, the natural reduced coenzyme, 1-4 TPNH, has been substituted efficiently by its non enzymatically oxidizable isomer, 1-4 TPNH. This indicates that, in the tritium exchange reaction, the TPNH has not a redox but a structural role.

We suggest that, as for 6-phosphogluconate dehydrogenase, isomers of the natural coenzymes may be used to differentiate, and study separately, the redox and structural roles of pyridine coenzymes in dehydrogenases.

6-Phosphogluconate dehydrogenase catalyses, in the presence of TPN, the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO_2. The enzyme isolated from Candida utilis is a dimer (1) with a molecular weight of 100,000 (2), has two binding sites for TPNH (1) and two for 6-phosphogluconate (3).

Addition to the enzyme of either the substrate or the coenzyme does not induce changes, detectable by ORD measurements, in the protein conformation (4). The order of addition to the enzyme of the substrate and the coenzyme is not compulsory (5).

It has been reported that the chemical modification of one residue of either lysine (6) or histidine (7-9) or tyrosine (10) or cysteine (11, 12) per enzyme subunit, affects the catalytic activity of the enzyme. Evidence for the involvement of the ionization of one residue of tyrosine (10) and one of histidine (3) in the binding to the enzyme of the coenzyme and of the substrate has recently been presented.

The enzyme catalyses also a tritium exchange between ribulose

5-phosphate and water; for this reaction an absolute requirement for the presence of TPNH has been reported (13).

In the present communication we advance some hypotheses on the role of TPNH in the tritium exchange reaction and suggest the TPNH isomers as usefull reagents to differentiate the redox from the structural role of the reduced coenzyme in the enzyme catalysis.

MATERIALS AND METHODS

The 6-phosphoglucoante dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase, decarboxylating; E. C. 1.1.1.44) crystalline, Type I, was prepared from Candida utilis as previously descrided (14). The TPN and 6-phosphogluconate were purchased from Sigma Chemical Co., St.Louis, Mo. . Tritiated water was purchased from the Radiochemical Center, Amersham, England. Tritiated ribulose 5-phosphate was prepared enzymatically from 6-phosphogluconate in tritiated water and purified by barium precipitation and column chromatography(13). The specific radioactivity of this purified ribulose 5-phosphate was 340 cpm/nmole. The detritiation reaction and assay were carried out as previously reported (13).

Preparation of 1-6 TPNH: The method reported for the preparation of 1-6 DPNH has been followed (15). The 1-6 TPNH so prepared had an absorption spectrum almost identical to that reported (15) for 1-6 DPNH and was found to be free of 1-4 TPNH (by treatment with pyruvate and lactate dehydrogenase) and of TPN (by treatment with 6-phosphogluconate and 6-phosphogluconate dehydrogenase). By phenazine methosulfate oxidation it has been shown that the extinction coefficient at 340 nm of 1-6 TPNH is identical to that of 1-4 TPNH.

Radioactivity measurements were carried out diluting the radioactive sample in Bray's solution (16) and counting in a Packard liquid scintillation counter.

RESULTS

The tritium exchange reaction catalysed by the 6-phosphogluconate dehydrogenase presents an absolute requirement for 1-4 TPNH (13). It appears from Fig. 1 that, at constant enzyme concentration, the rate of ribulose 5-phosphate detritiation depends, up to a given value, on the concentration of the reduced coenzyme.

The half maximal velocity of this reaction is obtained, in our experimental conditions, when the concentration of 1-4 TPNH is 0.6×10^{-6}M, a value which is very close to the Km of the enzyme for TPNH (0.47×10^{-6}M; ref. 1). It thus appears that only the en-

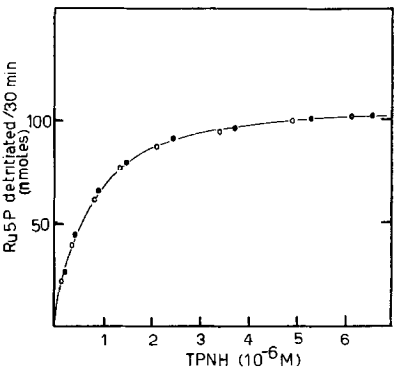

Figure 1. Rate of detritiation of ribulose 5-phosphate as a function of TPNH concentration.

1 ml of the reaction mixture contained: 0.5 nmoles of 6-phosphogluconate dehydrogenase, 800 nmoles of tritiated ribulose 5-phosphate, 50 µmoles of TRIS-HCl buffer, 1000 nmoles of EDTA and 1-4 TPNH (O) or 1-6 TPNH (●) at the concentrations indicated in the abscissa. The final pH was 7.5. After 30 min of incubation at 30°, the exchange of tritium between the ribulose 5-phosphate and water was determined as described (13). Abscissa: TPNH concentration; Ordinate: nmoles of ribulose 5-phosphate detritiated in 30 min.

zyme-TPNH complex is active in the tritium exchange reaction and that the TPNH is active in amounts catalytic with respect to the ribulose 5-phosphate detritiated.

Using for this reaction the non enzymatically oxidizable isomer, 1-6 TPNH, instead of 1-4 TPNH, an identical curve is obtained (Fig. 1). This demonstrates that the two TPNH isomers have the same efficiency in the tritium exchange reaction.

DISCUSSION

An absolute requirement for TPNH in the tritium exchange reaction catalysed by the 6-phosphogluconate dehydrogenase (13) and by isocitrate dehydrogenase (17) has been reported. The hypothesis that in these cases TPNH could have a structural role was put forward but a reversible oxidoreduction function of the pyridine nucleotide, similar to that reported for other enzymes (18), was not considered.

The aim of the present research was to establish the role of TPNH in the tritium exchange reaction catalysed by the 6-phosphogluconate dehydrogenase from Candida utilis.

It is known that, in many instances, the role of the coenzyme in the enzyme catalysis is a dual one, that is 1) exchange of a hydride ion during the oxidoreduction, and 2) structural modification of the enzyme. In order to differentiate these roles, we have employed a TPNH isomer which, altough unable to act as a redox carrier, could neverthless mimic the natural coenzyme in its structural role (19).

The experiments here reported show that 1-4 TPNH is active, in the tritium exchange reaction, in concentrations which are catalytic with respect to these of the ribulose 5-phosphate detritiated. The fact that 1-4 TPNH can be substituted efficiently by its non enzymatically oxidizable isomer 1-6 TPNH, clearly demonstrates that, in this reaction, the coenzyme has not a redox but a structural role.

Since these two TPNH isomers have the same activity, it can be concluded that, at least in this case, the position of the additional hydrogen in the nicotinamide moiety is not critical for the tritium exchange reaction.

The factor which does appear to be essential for the role of TPNH in the tritium exchange reaction is the presence of the uncharged nitrogen atom in the nicotinamide moiety of TPNH. In fact, TPN, which competes with TPNH for the same site, is an inhibitor of the reaction (13); this inhibition could be due to the presence of the positively charged nitrogen in the nicotinamide ring.

The tritium exchange reactions are assumed to be catalysed by basic groups. The hypothesis that the basic uncharged nitrogen of the nicotinamide moiety of TPNH may be directly involved in the tritium exchange reaction is very tempting. A second hypothesis is that the uncharged nicotinamide ring could cause a change in the conformation and/or hydrophobicity of the active site of the enzyme, creating the conditions for the tritium exchange reaction. Studies along these lines are in active progress in this laboratory.

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