Energy-Dependent Reduction of TPN+ by DPNH with Submitochondrial Particles[1,2]

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Submitochondrial particles, obtained by sonication of mitochondria from rat liver[3] and beef heart [4-6], catalyze an energy dependent transhydrogenase reaction between DPNH and TPN+. The reaction could be demonstrated by incubating particles in a buffered, Mg^{2+}-containing medium, in the presence of KCN, ethanol, alcohol dehydrogenase and a relatively small amount of DPN+. When the reduction of DPN+ (followed spectrophotometrically at 340 mμ) by the alcohol dehydrogenase system was completed, 0.7 μmole of TPN+ was added. No appreciable increase in A_{340} occurred. When 6 μmoles of ATP were now added, A_{340} increased at a linear rate. No similar increase in A_{340} was observed if DPN+ or TPN+ was omitted. The ATP-induced reduction of TPN+ was completely inhibited by oligomycin. It was insensitive to amytal, rotenone and dinitrophenol (0.1 mM). The particles also catalyzed the converse reaction, *i.e.* reduction of DPN+ by TPNH, but this required no added ATP.

Löw *et al.* [5,7,8] have demonstrated that beef heart particles catalyze an ATP-dependent reduction of DPN+ by succinate. The same reaction was observed here with liver particles. The formation of DPNH was ascertained by adding acetoacetate which caused a rapid drop in A_{340} (*via* the β-hydroxybutyric dehydrogenase reaction). When TPN+ was added together with DPN+, the same rate of pyridine nucleotide reduction was observed as with DPN+ alone. However, in this case, addition of acetoacetate caused no drop in A_{340}, indicating that TPNH rather than DPNH was the reaction product. This reduction of TPN+ by succinate proceeded via DPN+, as shown by the fact that, when DPN+ was omitted, no reduction of TPN+ occurred. The ATP-dependent succinate-linked pyridine nucleotide reduction was, in accordance with earlier observations [5,7,8], sensitive to amytal, rotenone and oligomycin, as well as to dinitrophenol (41 % inhibition by 0.02 mM, and 100 % by 0.1 mM dinitrophenol).

Energy-dependent reduction of TPN+ by DPNH could also be achieved under aerobic conditions. In this system, addition of ATP was not required, the energy being supplied by the aerobic oxidation of DPNH. The existence of a possible intermediate in the energy dependent transhydrogenase reaction, consisting of an activated form of pyridine nucleotide, and its relationship to previously reported pyridine nucleotide derivatives [9,10], will be discussed.

1. Abbreviations: ATP, adenosine triphosphate; DPN+ and DPNH, diphosphopyridine nucleotide, oxidized and reduced form; TPN+ and TPNH, triphosphopyridine nucleotide, oxidized and reduced form.
2. Supported by a grant from the *Swedish Cancer Society*.
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6. We are indebted to Dr. Hans Löw for kindly supplying us with the beef heart particles.
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