ACYL COENZYME A INHIBITION OF *Leuconostoc mesenteroides* GLUCOSE-6-PHOSPHATE DEHYDROGENASE: A COMPARISON OF THE TPN AND DPN LINKED REACTIONS

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The inhibitory effects of ATP, coenzyme A, and acetyl, malonyl, and oleyl derivatives of coenzyme A on the TPN and DPN dependent activities of *Leuconostoc* glucose-6-phosphate dehydrogenase are compared. At pH 7.8, 24°, saturating levels of DPN or TPN, and inhibitor concentrations of 2-4 mM only ATP has an appreciable effect on the TPN dependent reaction, but all were potent inhibitors of the DPN dependent reaction. Oleyl coenzyme A was the most effective (K_i ~ 0.15 mM against glucose-6-phosphate) while acetyl coenzyme A was least effective (K_i ~ 1.0 mM). A possible regulatory role of this inhibition in fatty acid synthesis is suggested.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from *Leuconostoc mesenteroides* is known to function with both TPN and DPN as hydrogen acceptors.[1] From isotope distribution studies, Kemp and Rose[2] deduced that TPNH and DPNH generated by this enzyme are reoxidized via distinctly different pathways, the DPNH donating its hydrogen to the end products of fermentation, and the TPNH providing hydrogen for reductive syntheses, particularly fatty acid synthesis. In 1967, Olive and Levy[3] crystallized the enzyme and from comparative inhibition studies concluded that the reactions with TPN and DPN were both catalyzed by a single enzyme and that both coenzymes are bound to the same site. More recently, Hsu[4] observed that both reactions were inhibited by ATP; this inhibition is competitive with respect to glucose-6-phosphate, but the inhibition of the DPN-linked reaction is the more severe, the K_i being 0.3-0.5 mM compared to 1.5-2.0 mM for the TPN-linked reaction. This suggested that an increasing ATP level would tend to shift the hydrogens generated by this enzyme away from reduction of fermentation products toward reductive syntheses.

A possible complementary control mechanism was suggested by the observation that long-chain acyl coenzyme A derivatives inhibited the TPN-linked glucose-6-phosphate dehydrogenase activities of yeast, rat liver, rat adipose tissue, and human erythrocytes.[5,6] This inhibition was also competitive with respect to glucose-6-phosphate and was evident at very low concentrations of the coenzyme A derivatives. With the yeast enzyme, the K_i values for the palmityl[6] and stearyl[5] derivatives were 0.003 and 0.004 mM, respectively. Hence, it appeared possible that acyl-coenzyme A could "turn off" the TPNH generating function of the *Leuconostoc* enzyme and thereby act in opposition to the ATP effect. To test this possibility the *Leuconostoc* enzyme was assayed in the presence of several coenzyme A derivatives. A comparison of the effect on the TPN-linked and DPN-linked activities are shown in Table I, and it may be seen that, contrary to expectations, coenzyme A and acyl coenzyme A exert their major effect against the DPN-linked system. In fact, ATP appears to be the most effective inhibitor of the TPN-linked system. The approximate K_i values (DPN system only) are calculated on the assumption that the inhibition is competitive with respect to glucose-6-phosphate; the data are consistent with this assumption, although in some instances they are not sufficiently precise to exclude other types of inhibition. From these approximations, however, it is apparent that even the long chain oleyl-coenzyme A is much less effective against the *Leuconostoc* enzyme than the palmityl or stearyl-coenzyme A is against the yeast or mammalian dehydrogenases. It is also apparent that the oleyl-coenzyme A is the most effective inhibitor for the DPN dependent reaction.

Although the original hypothesis that TPNH generation by the dehydrogenase is selectively turned off by acyl-coenzyme A is shown to be invalid by these results, an alternate possibility for regulation is suggested by the observation of Ilton et al.[7] that the presence of DPNH substantially decreases the TPNH level required for optimal activity of the fatty acid synthetase from *Mycobacterium phlei*. If the systems from *Leuconostoc* and *Mycobacterium* are

TABLE I

Relative Inhibitions of TPN and DPN Dependent Glucose-6-Phosphate Dehydrogenase Activities by ATP and Coenzyme A Derivatives

INHIBITOR	Conc. mM	% Inhibition TPN	% Inhibition DPN	Approx. K_i (with DPN) mM
ATP	3.9	50	90	0.5
CoA	3.4	12	82	0.7
AcCoA	2.6	0	70	1.0
MalCoA	2.4	0	86	0.4
Oleyl CoA	2.0	9	90	0.15

CONDITIONS USED FOR ASSAY:

REACTION MIXTURE: 40 mM tris buffer, pH 7.8; 0.4-0.5 mM DPN or 0.2 mM TPN; 0.02-0.2 mM glucose-6-phosphate; 0.07 μ/ml glucose-6-phosphate dehydrogenase; 23-24°; concentrations of ATP, coenzyme A derivatives as indicated. Absorption increase at 340 nm followed with a Gilford recording spectrophotometer.
SOURCES: Leuconostoc mesenteroides enzyme, Worthington Biochemical Corp., Freehold, New Jersey; ATP, glucose-6-phosphate, and Tris, Sigma Chemical Co., St. Louis, Mo.; coenzyme A and acyl-coenzyme A derivatives, P-L Biochemicals, Inc., Milwaukee, Wisconsin.
KINETIC PARAMETERS OF Leuconostoc ENZYME: Km for TPN, 0.014 mM; for DPN, 0.13 mM; for glucose-6-phosphate, 0.17 mM with either coenzyme.

comparable in this regard, then accumulation of long chain acyl-coenzyme A derivatives might indirectly curtail fatty acid synthesis by decreasing the DPNH level, thereby lowering the rate of reaction with TPNH. Such a mechanism would be more selective than a simple suppression of TPNH generation in that it could redirect the continuing supply of TPNH into other biosynthetic pathways.

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