A Liver-Microsomal Enzyme System Liberating Orthophosphate from Nucleoside Di- and Triphosphates

Lois C. Jones and Lars Ernster

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

Freshly prepared microsomes from 0.25 M sucrose homogenates of rat liver exhibit a high "ATPase" activity. This activity resembles the "latent" ATPase activity [1] appearing in liver mitochondria upon structural damage in the following respects: 1) it requires added Mg^{++} [1]; 2) it is stimulated by $Na_2S_2O_4$ [2,3]; 3) it is inhibited by atebrin [3], chlorpromazine [4], azide [5,6]; 4) the enzyme is not strictly specific (like the dinitrophenol-induced ATPase of intact mitochondria) for ATP, but reacts, in a non-additive manner, with the five nucleoside triphosphates, ATP, GTP, UTP, CTP and ITP [7].

In the course of attempts to more closely characterize this enzyme it was observed that the activities with the different nucleoside triphosphates responded in different ways to the presence of sodium deoxycholate (DOC) in the test system. Thus, at 0.05 % DOC, the GTPase and UTPase activities became markedly stimulated, whereas the activities with the other three triphosphates either decreased or remained unchanged; at higher DOC concentrations, all five activities were strongly inhibited. Simultaneously it was also found that a nucleoside diphosphatase activity, which was relatively low in the absence of DOC, revealed a strong increase at both 0.05 and 0.1 % DOC in the case of GDP, UDP or IDP as substrates, whereas it remained unchanged or became inhibited with ADP or CDP as substrates.

Treatment of microsomes with 0.26 % DOC in the cold, and subsequent centrifugation at 105 000 *g* for 2 h, resulted in a clear supernatant fraction, which exhibited a high activity toward GDP, UDP and IDP as compared with the two remaining diphosphates and the five triphosphates. Table 1 illustrates the nucleoside di- and triphosphatase activities of this fraction as compared with those found in the original microsomes. The activities of the three active diphosphatases recovered in this fraction corresponded to about 90 % of the total activities found in the DOC-treated microsomes. The five triphosphatase activities were concentrated, 2—8 fold on the protein basis, in a loose pellet appearing in this fractionation [8] on the top of the tightly packed ribonucleoprotein particles. This loose pellet has recently been found [9] to contain the microsomal DPNH-cytochrome c reductase and glucose-6-phosphatase activities in a concentrated form. The ribonucleoprotein particle fraction exhibited only marginal di- and triphosphatase activities.

When the clear DOC supernatant was tested with the three active diphosphates in combination the resulting activity did not exceed the maximum found with the individual diphosphates; addition of the two remaining diphosphates lowered this activity slightly. The di-

Table 1. Nucleoside di- and triphosphatase activities of rat liver microsomes, as determined in fresh preparations and in deoxycholate-extracts. The test system contained microsomes or microsomal extract from 40 mg wet weight liver, 2.5 μmoles substrate, 2 μmoles $MgCl_2$, and 25 μmoles TRIS buffer, pH 7.5, in a final volume of 0.5 ml. Incubation at 30°C. The values are expressed in terms of μmoles orthophosphate liberated in 10 min. per gram microsomal protein.

Substrate	Microsomes	Deoxycholate-extract
ATP	846	158
GTP	865	525
UTP	702	420
CTP	747	281
ITP	1 026	368
ADP	387	105
GDP	594	2 370
UDP	576	2 012
CDP	207	123
IDP	640	1 595

phosphatase activities required added Mg++ and were inhibited by atebrine, chlorpromazine and azide to about the same extent as the triphosphatases.

The above findings seem to indicate that freshly prepared rat liver microsomes exhibit a nucleoside triphosphatase activity which resembles that of structurally damaged rat liver mitochondria in regards to behavior toward certain activators and inhibitors, lacking nucleoside specificity, and firm association with the membrane structure. In addition, microsomes seem to contain a "latent" nucleoside diphosphatase activity, which can be rendered manifest by disruption of the microsomal membranes. This enzyme resembles the nucleoside triphosphatase in its behavior toward certain activators and inhibitors, but differs from the latter in possessing a more restricted array of nucleoside specificity, as well as in being more loosely associated with the microsomes.

An enzyme with a similar array of specificity has been known for some time [10-13], but its association with microsomes and its latent character have not been reported.

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By-Pass of the Amytal-Sensitive Site of the Respiratory Chain in Mitochondria by Means of Vitamin K_3

T. E. Conover* and L. Ernster

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

A soluble diaphorase (DT diaphorase) from liver has been isolated which can couple the oxidation of extramitochondrial reduced pyridine nucleotide through vitamin K_3 to the respiratory chain of mitochondria [1]. The cellular distribution of this enzyme has been shown[2], and significant amounts can be extracted from isolated mitochondria [3].

The presence of this enzyme in intact mitochondria and its interaction with intramitochondrially-generated reduced pyridine nucleotide is demonstrated in this paper by the bypassing of the point of amytal inhibition in the respiratory chain with the addition of vitamin K_3.

Mitochondria freshly prepared from rat liver as previously described [4] were incubated in a isotonic, buffered medium containing substrate, phosphate, Mg++, adenosine triphosphate, hexokinase and glucose. In these conditions the pyridine nucleotide-linked oxidation of substrates showed the usual high rates and strong sensitivity to amytal as is shown for glutamate in Table 1. The addition of low concentrations of vitamin K_3 (5×10^{-6} M) to the amytal-inhibited system gave a complete recovery of the maximal respiration. It can also be seen from Table 1 that this respiration was highly sensitive to antimycin A, KCN, and dicumarol. The high degree of inhibition obtained by low concentrations of dicumarol strongly implies the role of DT diaphorase, as the high sensitivity of this enzyme to dicumarol is considered to be very characteristic[2].

It was found that the concentration the of vitamin K_3 was important in this reaction as concentrations under 5×10^{-6} M were limiting in the oxidation of glutamate in these conditions, while higher concentrations were often inhibitory. This inhibition seemed to be related to a loss of pyridine nucleotide and could be protected at least partially by added nucleotide or nicotinamide.

* Fellow of the National Foundation, New York, N. Y.