The Interaction Between Adenine Nucleotide Transport and Phosphorylation in Intact Rat Liver Mitochondria

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Some of the reactions catalysed by intact rat liver mitochondria under state 3 conditions are:

\[
\begin{align*}
\text{ADP}_o & \rightarrow \text{ADP}_{in} \rightarrow \text{ATP}_{in} \rightarrow \text{ATP}_o
\end{align*}
\]

(o stands for outside, in stands for inside). The specific enzyme systems are the adenine nucleotide translocator and the mitochondrial ATPase (adenosine triphosphatase), F₁.

According to the experiments of Klingenberg and coworkers (Klingenberg, 1970; Heldt & Pfaff, 1969), the ADP_in and the ATP_in participating in these reactions consist of the total intramitochondrial pool of ADP and ATP. Though Vignais and coworkers (Vignais et al., 1973; Duée & Vignais, 1969) state that the intramitochondrial pool of adenine nucleotides is homogeneous, they demonstrated that the phosphorylation of intramitochondrial ADP and of externally added ADP occur independently.

We reported evidence (Bertha & Out, 1973) that the intermediates between the separate components of a single enzyme complex containing respiratory chain, F₁, and adenine nucleotide translocator are not in rapid equilibrium with the total intramitochondrial pool of those intermediates.

We have now obtained further evidence for a compartmentation of the intramitochondrial adenine nucleotides by the use of the 'irreversible' inhibitors of the adenine nucleotide transport, bongkrekic acid and gummiferine. The latter inhibitor has been shown by Klingenberg et al. (1973) to be irreversible. In co-operation with Mr. K. Duin, we have found that [3H]bongkrekic acid bound to specific binding sites (0.17nmol·mg of protein⁻¹) in rat liver mitochondria equilibrates only extremely slowly with added unlabelled bongkrekic acid and is not removed by serum albumin. Binding to the specific binding sites parallels inhibition of adenine nucleotide transport.

The exchange of added [14C]ADP with the intramitochondrial adenine nucleotides shows in the presence of bongkrekic acid or gummiferine inhomogeneous kinetics, namely (1) a fast reaction that can be ascribed to the exchange between added adenine nucleotides and those in the inside 'compartments' that are not excluded by the inhibitor from direct equilibration with the medium, and (2) a slow reaction, possibly revealing intramitochondrial exchange between 'compartments' that are and those that are not in direct interaction with the external adenine nucleotides. The extent to which the first reaction labels the intramitochondrial adenine nucleotides is decreased by increasing inhibitor concentrations, the half time being hardly influenced.

It can also be demonstrated that, during steady-state state 3 phosphorylation at 0°C, after addition of [14C]ADP with high specific radioactivity, [14C]ATP appears outside without an appreciable lag time that should occur if the ADP transported inwards or the ATP transported outwards equilibrates with the intramitochondrial adenine nucleotides (Bertina & Out, 1973). However, the rate of equilibration of external with internal adenine nucleotides (3.2nmol·min⁻¹·mg of protein⁻¹) is nearly as high as the rate of ATP synthesis (3.9nmol·min⁻¹·mg of protein⁻¹).

In Table 1, results are given of measurements of the transport of ADP into the mitochondria. It seems clear that when the mitochondria are incubated in a system that maintains a high energy pressure, the rate of adenine nucleotide transport is about half the rate under conditions of low energy pressure. In this respect it should be mentioned that both Souverijn et al. (1973) and Vignais et al. (1973) have shown that the maximal rate of ADP transport is dependent on the metabolic conditions.
Table 1. Effect of metabolic conditions on the ADP exchange in rat liver mitochondria

Rat liver mitochondria were incubated at 0°C in a medium containing 75mM-sucrose, 15mM-KCl, 50mM-triethanolamine hydrochloride (pH 7.2), 4mM-EDTA, 0.4mM-ADP and 2.65mg of protein·ml⁻¹, 7.5µg of oligomycin·mg of protein⁻¹ (Expt. 1) or 2.9mg of protein·ml⁻¹, 9.2µg of oligomycin·mg of protein⁻¹ (Expt. 2). After 10min [¹⁴C]ADP was added. Samples were taken during 20min and the reaction stopped with the combined atracyloside stop and rapid-filtration technique of Souverijn et al. (1973). v and t₉ were calculated by the method of Souverijn et al. (1973). Abbreviation: CCCP, carbonyl cyanide m-chlorophenylhydrazone.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Additions</th>
<th>t₉ (min)</th>
<th>v (nmol·min⁻¹·mg of protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rotenone (0.4µg·mg of protein⁻¹)</td>
<td>1.4</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Rotenone, CCCP (0.5µM)</td>
<td>1.4</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Rotenone, succinate (12mM)</td>
<td>2.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Rotenone, succinate, CCCP, (0.7µM)</td>
<td>1.6</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>Glutamate (5mm), malate (5mm)</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Rotenone (0.23µg·mg of protein⁻¹)</td>
<td>1.9</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Rotenone, succinate (12mM)</td>
<td>3.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

A possible explanation is that under conditions of high energy pressure approximately half of the enzyme system that is able to catalyse the transport of adenine nucleotides becomes closely linked to the F₁, and as a consequence becomes disconnected from fast equilibration with the intramitochondrial adenine nucleotides. This would imply that the inner mitochondrial membrane acts as an enzyme system that supplies the extramitochondrial space with ATP, without participation of the intramitochondrial adenine nucleotides.


A Comparison of the Catalysis of Chloride–Hydroxyl Ion Exchange Across the Inner Membrane of Rat Liver Mitochondria by Several Substituted Diphenyleneiodonium Derivatives and Their Effects on Mitochondrial Oxidations in Chloride-Free and Chloride-Containing Media

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Diphenyleneiodonium and some of its substituted derivatives catalyse an exchange of Cl⁻ and OH⁻ ions across the inner mitochondrial membrane, and they cause strong inhibition of the mitochondrial oxidation of succinate in Cl⁻-containing media as a