A FACTOR WHICH INCREASES THE DINITROPHENOL-SENSITIVITY OF THE ATP-ADP EXCHANGE REACTION OF OXIDATIVE PHOSPHORYLATION*

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The preceding report showed that the mitochondrial enzyme catalyzing the ATP-ADP exchange reaction,1-4 which in its soluble purified state is insensitive to dinitrophenol,2 can be recombined again with mitochondrial membrane fragments to restore the dinitrophenol sensitivity7 which this reaction possesses in intact mitochondria.

This communication describes a soluble protein factor (called M-factor), obtained from extracts of mitochondria, which is a component in conferring DNP-sensitivity on the ATP-ADP exchange reaction in digitonin particles. Although the ATP-ADP exchange activity of fresh digitonin particles of rat liver mitochondria is inhibited by $5 \times 10^{-4} M$ dinitrophenol, this inhibition is usually not complete, is rather variable in extent,2 and cannot be increased by increasing the DNP concentration. However, addition of M-factor to such particles greatly increases the fraction of the total ATP-ADP exchange activity of the particles which is sensitive to DNP.

Data from two typical experiments in Table I and a third presented graphically in Figure 1 show that the soluble dialyzed M-factor protein contains no significant ATP-ADP exchange activity itself and has no effect on the rate of the ATP-ADP exchange reaction of rat liver digitonin particles in the absence of DNP. However this fraction greatly increases the sensitivity of the ATP-ADP exchange of the particles to $5 \times 10^{-4}$ DNP, an effect which is most prominent in those preparations of digitonin particles in which the initial sensitivity of the exchange to DNP is relatively low. Increasing concentrations of M-factor produce increasing sensitivity to DNP; a half-maximum effect is given by approximately 5–10 micrograms protein nitrogen of the preparation. The M-factor effect is abolished by heating the soluble fraction 10 min at 80°. The DNP-sensitivity induced by M-factor is completely abolished by 0.002 M azide, as is the unstimulated DNP-sensitivity of the exchange.

The M-factor effect is specific; it is not given by proteins such as bovine serum albumin, yeast hexokinase, or other mitochondrial proteins. The M-factor activity is retained for some days in frozen solutions; further storage causes inactivation.

Using the increase in DNP-sensitivity of the ATP-ADP exchange as a semiquantitative assay method, M-factor has been purified over 40 fold by isoelectric precipitation and ammonium sulfate fractionation, starting either from phosphate extracts of acetone-dried rat liver mitochondria or from ammonium sulfate extracts of fresh, undried mitochondria. High ionic strength evidently detaches M-factor readily from mitochondria. A typical preparation is made as follows: Freshly prepared and washed rat liver mitochondria ($\sim 2500$ mg mitochondrial protein) were extracted at $0^\circ$ with 50 ml of 0.30 M ammonium sulfate adjusted to pH 7.5. After centrifuging at $9000 \times g$ for 15 min, the clear yellow supernatant was dialyzed for
16 hr against 0.1 \( M \) Tris-HCl buffer pH 7.4. The pH was then lowered to 5.5 and the precipitated material removed. Solid ammonium sulfate was added to the supernatant solution and the fraction precipitating between 50 and 70 per cent saturation was collected, dissolved in 0.05 \( M \) Tris buffer pH 7.4 and dialyzed. Such preparations contain little or no ATP-ADP exchange activity, no ATP-Pi\(^{12} \) exchange, adenylate kinase or ATP-ase nor do they stimulate ATP-ase activity of digitonin particles in the presence or absence of DNP or Mg\(^{++} \). However, preparations of M-factor at this stage do contain significant C-factor activity, \(^{6} i.e., \) they promote the ATP-induced contraction of rat-liver mitochondria swollen in the presence of reduced glutathione.

At least two modes of action of M-factor appear possible in increasing the DNP-sensitivity of the ATP-ADP exchange reaction. M-factor may serve as a specific "cement" protein enabling the ATP-ADP exchange enzyme to be bound to specific sites on the digitonin particles in such a way that the exchange enzyme is reactive with presumably adjacent enzyme molecule(s) which catalyze an earlier DNP-sensitive stage of energy-coupling and with which the exchange enzyme is normally reactive in a sequential fashion. If this "cementing protein" is missing, the exchange enzyme is still capable of catalyzing the exchange but in a "disconnected" manner, so that intermediates in the exchange reaction are no longer capable of interacting with the preceding steps in energy coupling. A similar explanation has been offered by Pinchot\(^{9} \) for the role of a heat-stable polynucleotide which causes increased binding of a soluble heat-labile component of the coupling mechanism to the respiratory particles obtained from \textit{Alcaligenes faecalis}.

Alternatively, M-factor may itself be an intermediate enzyme in the sequence of reactions which comprise the energy-coupling cycle of oxidative phosphorylation, acting between the terminal DNP-insensitive ATP-ADP exchange enzyme and the preceding DNP-sensitive intermediate. In this event, M-factor would presumably be a group-transferring enzyme through which decomposition of an early intermediate by DNP could cause substantial lowering of the steady-state concentration of a high-energy intermediate in the ATP-ADP exchange reaction. The following reaction sequence, in which E is the ATP-ADP exchange enzyme and M is M-factor, illustrates such a role:

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\begin{align*}
\text{Carrier} + \text{M} & \overset{\text{electron transfer}}{\longrightarrow} \text{Carrier} \sim \text{M} \\
\text{Carrier} \sim \text{M} + \text{P}_i & \rightleftharpoons \text{Carrier} + \text{P} \sim \text{M} \\
\text{P} \sim \text{M} + \text{E} & \rightleftharpoons \text{E} \sim \text{P} + \text{M} \\
\text{E} \sim \text{P} + \text{ADP} & \rightleftharpoons \text{ATP} + \text{E}
\end{align*}
\]
In this formulation, M-factor is suggested to be the component reacting with the carrier to form the primary, DNP-sensitive, high-energy complex Carrier \( \sim M \). Further work is of course necessary to determine whether or not M-factor is in fact an intermediate enzyme.

It appears possible that M-factor may be involved in the conferral of DNP-sensitivity on the soluble, DNP-insensitive ATP-ADP exchange enzyme when it is "recombined" with digitonin fragments, as described in the preceding paper.\(^7\) 

**Crude** preparations of the soluble exchange enzyme, when added to digitonin particles, show restoration of DNP-sensitivity; however, after further purification of the exchange enzyme, such "recombination" or restoration of DNP-sensitivity no longer occurs. This finding suggested that a second factor necessary for the restoration of DNP-sensitivity is removed during purification of the ATP-ADP exchange enzyme. The activity of M-factor in recombining purified ATP-ADP exchange enzyme with digitonin fragments is under examination.

Whatever the mechanism of action of M-factor, it is clear from these findings and also the experiments demonstrating the restoration of DPN-sensitivity of the soluble ATP-ADP exchange enzyme,\(^7\) that the phosphorylating digitonin particles contain some "assemblies" of respiratory chains and energy-coupling enzymes which are incomplete in that they are lacking one or another enzyme or factor such as M-factor or the ATP-ADP exchange enzyme which may have become detached during the course of preparation of the membrane fragments. At least some of the sites which normally bind specific proteins involved in energy-coupling are still intact and able to "rebind" such proteins in a specific manner to reconstitute a functional assembly.

The relationship of the ATP-ADP exchange enzyme and M-factor to the soluble ATP-ase preparation of Pullman et al.\(^{10}\) and the factor of Linnane and Titchener,\(^{11}\) which have been shown to increase the P:O ratio of treated heart mitochondria is a point of some interest. The effect of the exchange enzyme and M-factor on the P:O ratio of digitonin fragments will be considered in detail elsewhere.

**Summary.**—A specific, soluble protein fraction (designated M-factor) obtained from mitochondrial extracts has been found to increase the DNP-sensitivity of
the ATP-ADP exchange reaction of oxidative phosphorylation in digitonin fragments of mitochondria. This factor is postulated to be either a "cementing" protein or an intermediate enzyme of the energy-coupling mechanism linking the terminal enzymatic step catalyzing the ATP-ADP exchange, which is DNP-insensitive, with a preceding, DNP-sensitive reaction. Preparations of M-factor also show C-factor activity, i.e., they promote contraction of glutathione-swollen mitochondria by ATP.

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ON THE MUTAGENIC EFFECT OF ALKYLATING AGENTS*

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Many alkylating agents have been found to exert a mutagenic effect on a great variety of organisms.1 These agents vary in their composition and structure from simple to very complex molecules, which will be classified here in two main groups, the mono- and polyfunctional agents.

Although, for the most part, polyfunctional agents have been used for mutation and cancer therapeutic studies investigations for a molecular explanation of mutation induction with monofunctional agents are more promising, since their reactions are more accessible to chemical analysis. In such investigations it has been found already that methyl groups attack guanine and adenine on their 7-N-position,2 and also adenine on its 1- and 3-position,3 whereas ethylation has been found to esterify primary phosphate groups in DNA.2, 4

One year ago, it was reported that ethylmethanesulfate causes high mutation rates in T4 phages.4 Therefore, investigation of the mechanism of chemically induced mutations by alkylating agents on bacteriophage T4 seemed to us most promising since the rII region of this phage is the most extensively analyzed genetic system6 and because of the experiences acquired in our laboratories during studies on reversion induction with nitrous acid.7