The 2,4-dinitrophenol-responsive adenosinetriphosphatase of rat-liver mitochondria

Various investigators[1-3] have postulated that DNP activation of mitochondrial ATPase is a manifestation of the system of oxidative phosphorylation. It was of interest in this regard when LARDY[2] showed that desiccation of rat-liver mitochondria gave powders that contained ATPase activity. Extracts of such powders possessed ATPase activity unsedimented at 18,000 $\times$ g and responsive to both DNP and Mg^{++}. This report presents results of further study of the ATPase activities present in such powders.

Early in this work it was found that a significant percentage of the DNP-responsive ATPase of the powders is not sedimented in 60 min at 105,000 $\times$ g. Since such preparations would present advantages in purification attempts, they have been employed routinely. Extracts are prepared by homogenizing the powders in 0.25 M sucrose. The suspension is clarified at 105,000 $\times$ g, and the supernatant is lyophilized for reconstitution as needed. Phosphate formation in the presence of ATP is determined by a modified Martin and Doty procedure[4].

The original assay for DNP-responsive ATPase[2] employed 0.09 M KCl at pH 7.4. This work has shown that pH 8.5 is optimal for the DNP response (P_i formation in presence of DNP less P_i formation in absence of DNP). In addition, the maximum response occurs in the absence of added salts such as KCl or NaCl. In Table I the

TABLE I

ION INTERFERENCE IN RESPONSE OF ATPASE TO 2,4-DINITROPHENOL

Conditions: 0.06 M Tris buffer, pH 8.5; 0.012 M ATP; enzyme containing 1.3 mg protein; additions of KCl and DNP as indicated; in volume of 0.5 ml. Incubation 2 min at 31°. P_i formation was measured directly on the assay system by a modified Martin and Doty procedure[4].

	(µmoles Pi formed/mg protein) × 10²			
Added KCl (M)	—	*0.04*	*0.08*	*0.12*
—	4.2	6.5	5.1	4.3
$5 \cdot 10^{-6}$ M DNP	10.5	7.2	0	0
$5 \cdot 10^{-5}$ M DNP	7.8	7.4	1.6	0
$5 \cdot 10^{-4}$ M DNP	9.4	10.3	2.5	1.6
$1 \cdot 10^{-3}$ M DNP	11.2	14.2	6.9	5.6

results show that added KCl diminishes the response to DNP. Similar results occur with NaCl. Presumably, any monovalent-cation requirement the enzyme has is met by the system as constituted. The item of greatest interest in Table I, however, concerns the first column of figures where no KCl was added. Under these conditions, two concentrations of DNP differing 200-fold possess approximately the same capacity to stimulate P_i release from ATP. In the original assay system[2], in the presence of 0.09 M KCl, $1 \cdot 10^{-4}$ to $1 \cdot 10^{-3}$ M DNP had been found to yield the maximal response.

These findings gave a hint of a multiphase aspect in the response to DNP and

Abbreviations: DNP, 2,4-dinitrophenol; ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane; P_i, inorganic phosphate

therefore prompted reinvestigation of the nature of ATPase response over a wide range of DNP concentrations. Fig. 1 shows extracts that of mitochondrial acetone powders in 0.25 *M* sucrose exhibit characteristic multiphasic curves of ATPase reponse to DNP. Peaks of response occur at $1 \cdot 10^{-8}$, $1 \cdot 10^{-5}$ and $1 \cdot 10^{-3}$ *M* DNP. Curves A and B were obtained with two different powders, the second of which had a lower specific activity at more dilute DNP concentrations. Similar results have been obtained with other acetone powders.

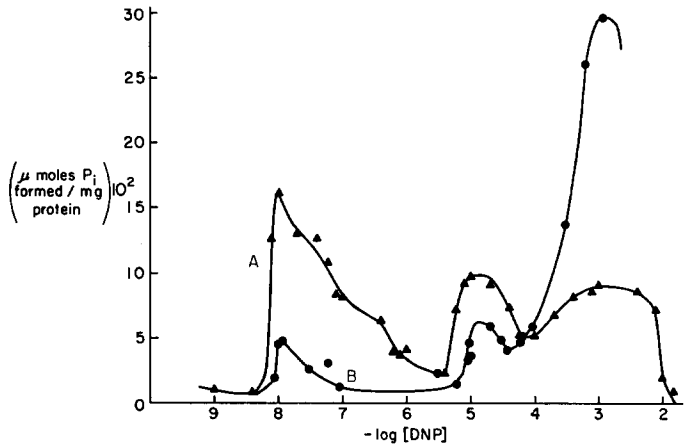

Fig. 1. Effects of variation of DNP concentration on ATPase activity. Conditions: 0.06 *M* Tris, pH 8.5; 0.012 *M* ATP; DNP in 0.5 ml. Curve A: 1.04 mg protein incubated 2 min at 28°. Curve B: 1.25 mg protein incubated 11 min at 28°.

These results may have other interpretations, but it is our feeling that they give direct evidence for the three DNP-responsive ATPases postulated by SLATER[5]. While SLATER's results indicated the presence of three such enzymes with pH optima at 6.3, 7.4 and 8.5, these results were obtained at pH 8.5. Work is in progress to ascertain if the enzymes more sensitive to DNP have greater activity at lower pH values.

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