ACTIVATION OF NADH OXIDATION BY ATRACTYLATE IN JERUSALEM ARTICHOKE *(HELIANTHUS TUBEROSUS)* MITOCHONDRIA

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1. Introduction

Plant mitochondria contain a more complex NADH dehydrogenase system than their mammalian counterparts [1]. There appear to be at least 3 NADH dehydrogenases associated with the inner membrane. One of these appears to be located on the outer surface of the inner membrane and may be involved in the oxidation of cytosolic NADH. The remaining 2 appear to be located such that they only oxidize endogenous NADH generated in the matrix by the enzymes of the tricarboxylic acid cycle [1]. One of these dehydrogenases appears to be similar to that found in mammalian mitochondria and is coupled to ATP synthesis at the first site of oxidative phosphorylation; this enzyme is sensitive to piericidin A. The other pathway appears to be able to by-pass both the piericidin A-sensitive step and the first site of ATP synthesis [2]. Although little is known about the regulation of electron flux through these alternative dehydrogenase systems it seems reasonable to anticipate that some form of metabolic regulation for each individual pathway must exist. The piericidin A sensitive dehydrogenase was markedly stimulated by AMP in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and oligomycin [3]. Oxidative phosphorylation could not take place under these conditions and it was suggested that AMP directly activated the piericidin A-sensitive NADH dehydrogenase resulting in an enhanced reduction of cytochrome *b* [3]. The ability of AMP to stimulate the NADH dehydrogenase was not sensitive to inhibition by bongkrekic acid and it was concluded that AMP did not have to traverse the inner membrane to bring about the activation [3]. Whilst attempting to repeat this observation using atractylate we made the rather surprising observation that atractylate itself, under certain conditions, was able to replace AMP as an activator of the piericidin A-sensitive NADH dehydrogenase.

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2. Methods

Mitochondria were isolated from the tubers of Jerusalem artichokes as in [4]. Oxygen consumption was measured in a small Rank oxygen electrode (Rank Bros, Bottisham, Cambridge) with internal diam. 9 mm. Reactions were carried out at 25°C in 1.0 ml standard medium containing 0.3 M sucrose, 5 mM phosphate and 5 mM (*N*-tris(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid) (Tes) at pH 7.2. Other additions are as indicated in the figure legends. Mitochondrial protein concentration was determined as in [5] after first solubilizing the protein with deoxycholate; bovine serum albumin was used as the standard. Atractyloside was purchased as the potassium dihydrate salt from Calbiochem Bishops Stortford.

3. Results

The effect of atractylate on the metabolic activity of plant mitochondria has been the subject of some

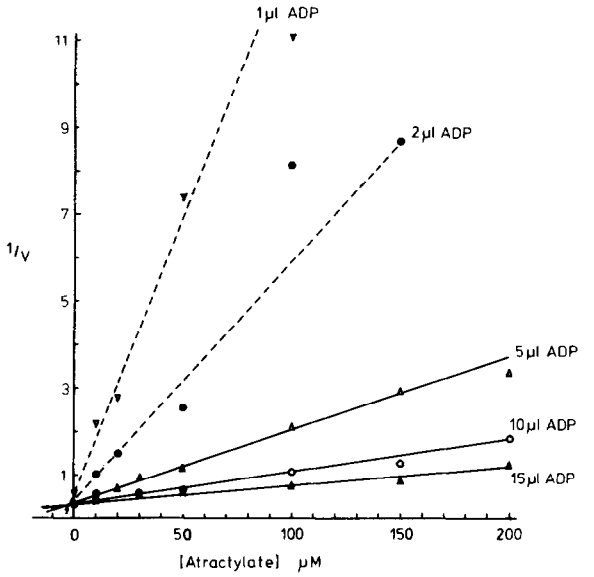

Fig.1. A Dixon plot of the effect of atractylate on the ADP-stimulated NADH oxidase. Mitochondrial protein, 0.58 mg, were suspended in 1 ml reaction medium in the presence of 1 mM NADH, 10 mM glucose and 0.3 mg hexokinase and the appropriate concentration of atractylate. The stimulated rate was induced by adding the stated volume of 18.9 mM ADP. The units of v are nmol O_2 stimulated by ADP min^{-1} mg $protein^{-1}$.

controversy. Although atractylate is known to be a potent inhibitor of the adenine nucleotide translocator in mammalian mitochondria [6], it was ineffective in Jerusalem artichoke mitochondria [7]. Atractylate may inhibit the operation of the adenine nucleotide translocator in plant mitochondria but at considerably higher concentration ranges than those necessary in mammalian mitochondria [8–11]. Atractylate is a competitive inhibitor of the ADP-stimulated oxygen uptake (fig.1) and it can be calculated from these data that the K_i for atractylate falls in the 1–2 µM range which is 10-times greater than that calculated for rat liver mitochondria [12]. An attempt was made to show that the stimulation of oxygen uptake by AMP in the presence of FCCP still occurred in the presence of atractylate (fig.2). The results were unexpected. While FCCP hardly stimulated the oxidation of pyruvate (fig.2, trace a) it resulted in the immediate increase in oxygen uptake in the presence of atractylate (fig.2, trace b). In both of these experiments it is clear that the addition of AMP caused a stimulation of oxygen uptake (32.7 nmol min^{-1} mg $protein^{-1}$ in the absence of atractylate and 19.1 nmol min^{-1} mg $protein^{-1}$ in the presence of atractylate). The effect of atractylate was not diminished if Mg^{2+} was omitted and EDTA added to remove endogenous Mg^{2+} (fig.2, trace c), a treatment known to reduce the concentration of Mg^{2+} in the preparation such that adenylate kinase can no longer function [3]. This observation is significant because the presence of Mg^{2+} in mammalian mitochondria strongly enhances the inhibitory effect of atractylate [13] and increases the affinity between the atractylate and the binding site [14]. AMP failed to have any further stimulatory activity in the absence of Mg^{2+}. It is important to note that AMP is equally active in the presence or absence of Mg^{2+} when atractylate is not added [3]. Atractylate was not able to stimulate the uncoupled rate, either in the presence or absence of Mg^{2+} if added after the FCCP (fig.3). In this situation AMP was able to cause a stimulation in both experiments.

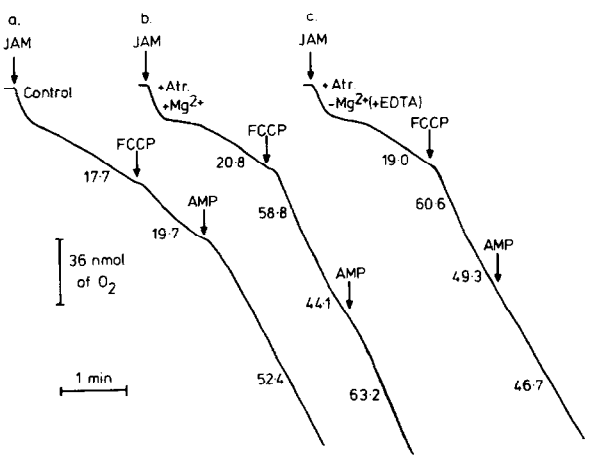

Fig.2. Effects of atractylate on the response of mitochondria to additions of FCCP and AMP. Oxygen uptake was measured in an oxygen electrode. In traces a, b 1.43 mg protein were suspended in 1 ml reaction medium containing 15 mM pyruvate, 1 mM malate and 170 µg thiamine pyrophosphate. In trace c the reaction medium contained no Mg^{2+} and 5 mM EDTA. Atractylate 200 nmol AMP 75 nmol and FCCP 2×10^{-7} M were added where indicated. The numbers under the traces represent rates of oxygen uptake in nmol min^{-1} mg $protein^{-1}$.

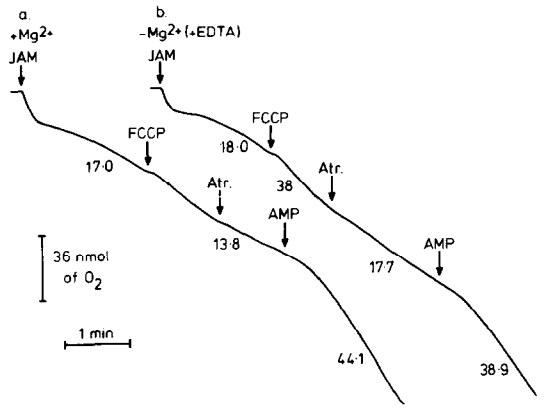

Fig.3. The response of the uncoupled respiration to atractylate and AMP. The conditions of assay were the same as those used in fig.2.

4. Discussion

It can be seen from fig.2,3 that AMP was able to activate the NADH oxidase in the presence of atractylate. Therefore these observations support [3], using bongkrekic acid, which suggested that AMP did not enter via the adenine nucleotide translocator before stimulating the NADH oxidase. It seems likely that AMP binds to a site on the outer surface of the inner membrane in order to bring about the activation. Under certain conditions atractylate can also activate the NADH oxidase and allow it to respond to the addition of FCCP (fig.2). There is no direct proof, but it seems highly likely that atractylate brings about the activation by binding to the same site as the AMP. The probability that the NADH dehydrogenase has separate adenine nucleotide and atractylate binding sites, both of which result in the activation of the enzyme, seems very low. If atractylate can substitute for AMP in the activation the binding site would have to be on the external surface of the inner membrane, since it has been established that atractylate cannot traverse the inner membrane [15]. Studies of atractylate-binding to beef heart mitochondria indicate that atractylate only interacts with the adenine nucleotide translocator which does not bind AMP [16]. There is insufficient information presently available concerning the binding of atractylate and adenine nucleotides to plant mitochondria. However, assuming the situation is similar to that in mammalian mitochondria it would follow that the nucleotide and atractylate binding site associated with the NADH dehydrogenase is distinguishable from the adenine nucleotide translocator. This view is supported by the observation that Mg^{2+} does not appear necessary to activate the NADH dehydrogenase whilst Mg^{2+} greatly increases the affinity of atractylate for the adenine nucleotide translocator. From the data in trace c in fig.2 it seems that in the absence of Mg^{2+} atractylate binds in such a way as to prevent AMP causing any further stimulation. Data in fig.3 show that atractylate can only substitute for AMP if added before the FCCP, atractylate was ineffective if added after the FCCP; AMP is active in both cases. The reason for this dependence on the sequence of addition is not known but it is assumed that the FCCP results in the alteration of the conformation of the dehydrogenase in such a manner that it can bind AMP but not atractylate.

Acknowledgements

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