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The Response of Plant Mitochondria to Media of High Solute Content

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Abstract

The state-3 rate of respiration of potato tuber mitochondria is inhibited by concentrations of KCl or NaCl above 125 mM, and by concentrations of sucrose, lactose, or maltose above 500 mM, but not at all by mannitol, glucose, glycine, or proline up to a concentration of 1500 mM in the medium. Mitochondria from cauliflower, beetroot, cucumber, rock melon, and watermelon behave very similarly to those from potato tuber. The variable response to different solutes proves that the reduction in respiration is not a simple function of the chemical potential of water in the medium. Disruption of potato mitochondria by ultrasonic vibration does not relieve the inhibition of succinate oxidation caused by KCl or sucrose. However, treatment with detergent abolishes completely the inhibition of respiration by sucrose. Inhibition of succinate dehydrogenase [Succinate:PMS, oxidoreductase (EC.1.3.99.1)] and malate dehydrogenase [L-Malate:NAD oxidoreductase (EC.1.1.1.37)] activities by sucrose is less than the inhibition of succinate- and malate-dependent oxygen uptake by the potato mitochondria. Limited substrate uptake and, alternatively, reduced electron flow as a consequence of a direct effect of solute on the mitochondrial membrane are considered as possible mechanisms of inhibition.

Introduction

Mitochondria from plant sources show a decreased respiration rate when suspended in media in which the chemical potential of water is much below that of the mitochondria themselves. Inhibition occurs when

sucrose or potassium chloride are added to the medium [1, 2]. Inhibition of the respiration of mitochondria from animal tissue also occurs when high concentrations of sucrose, mannitol, potassium chloride, or sodium chloride are added to the medium [3, 4]. Flowers and Hanson [2] studied the inhibition of the respiration of mitochondria from soybean in some detail. They concluded that the inhibition was a direct consequence of a lowered water activity in the medium, and that the site of inhibition was the malate dehydrogenase in the mitochondrial matrix.

In the previous paper [4] we showed that solute inhibition of rat-liver mitochondria varies independently of the chemical potential of water in the medium. In this paper we have re-examined solute inhibition of plant mitochondria in an attempt to confirm the conclusions of Flowers and Hanson [2] and so establish that the mechanism of solute inhibition is not the same for animal and plant mitochondria.

Materials and Methods

Mitochondria from potato tubers, beet roots, cucumber, rock melon, and watermelon fruits, were prepared as described by Raison and Lyons [5], and those from cauliflower buds by the method of Romani et al. [6]. The mitochondria were isolated and resuspended in medium containing 500 mM mannitol [5]. The final mitochondrial suspension contained 10–15 mg of protein per ml. The further treatment of mitochondria, the measurement of oxidation rates, and enzyme reaction rates, and the methods of sonication and detergent treatments were as described in the previous paper [4].

Results

Response of Whole Mitochondria to Change in Solute Concentration

In using succinate as substrate, the rate of state-3 respiration in mitochondria from potato tubers was depressed when the sucrose concentration was above 500 mM (Fig. 1A). The rate of state-4 respiration was essentially unaffected with increasing concentrations of sucrose, as shown in Fig. 1A. Thus, higher sucrose concentrations reduced the respiratory control ratio. With potassium chloride as solute, respiration was reduced when the concentration was above 125 mM and respiratory control was lost at concentrations of 500 mM or above. Mannitol did not inhibit respiration at concentrations up to 1000 mM, and glycine and proline stimulated respiration at concentrations up to 1500 mM.

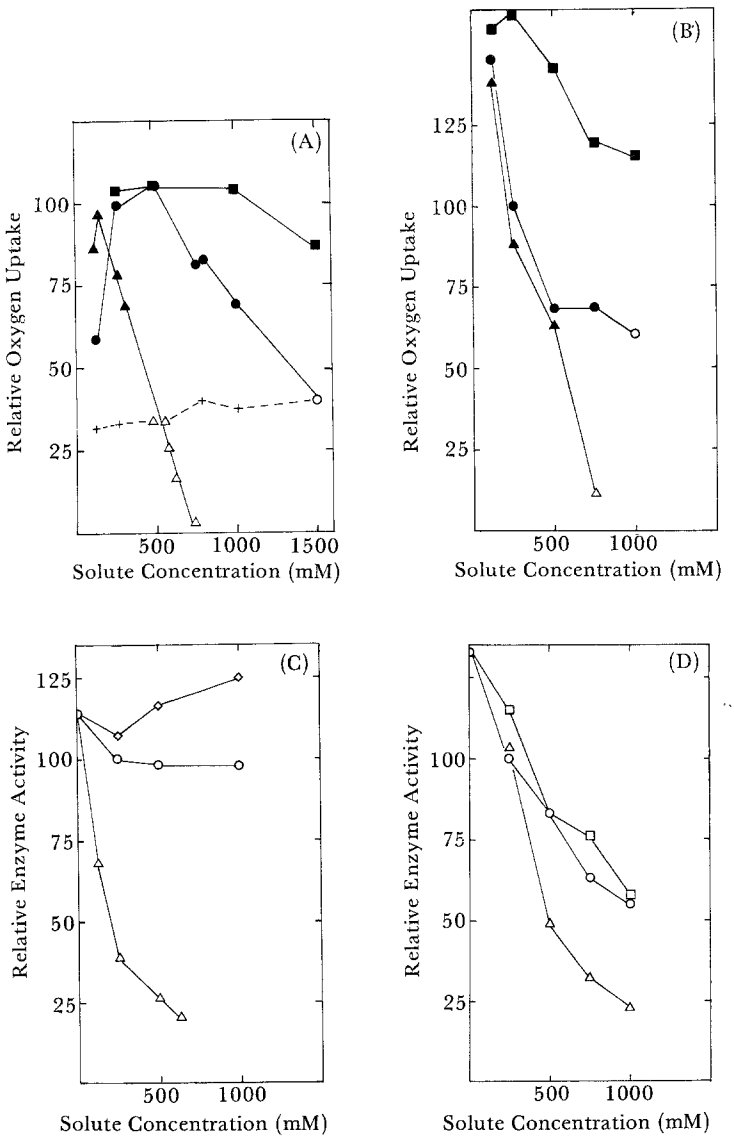

Figure 1. Oxygen uptake by potato mitochondria in the presence of added ADP with succinate 5 mM (A), or malate 20 mM plus glutamate 50 mM (B) as substrate, and the activities of succinate dehydrogenase (C) and malate dehydrogenase (D) of potato mitochondria in the presence of KCl (△), sucrose (○), glucose (□), or mannitol (◇). Closed symbols show where the mitochondria exhibited respiratory control. Rates of succinate oxidation, in the absence of added ADP (state 4) with sucrose as solute (+) are expressed relative to the maximum observed. Maximum rates were 154–186 nmole O_2 min^{-1} mg^{-1} protein for A; 160 nmole O_2 min^{-1} mg^{-1} protein for B; 240 nmole min^{-1} mg^{-1} protein for C and 750–770 nmole min^{-1} mg^{-1} protein for D.

If, as Flowers and Hanson [2] have concluded, inhibition at high sucrose content is due to an inhibition of malate dehydrogenase, the degree of inhibition of oxygen uptake by mitochondria may be expected to vary when different substrates are used. Figure 1B records the oxidation of malate in the presence of glutamate in media containing sucrose, glucose, or potassium chloride. Each solute reduced the rate of oxidation of malate; potassium chloride was the most inhibitory, and inhibition due to sucrose exceeded that due to glucose. A comparison of Fig. 1A and 1B shows that inhibition by potassium chloride was about equivalent, whether succinate or malate was used as the respiratory substrate, and that malate oxidation was more sensitive to sucrose or glucose in the medium. In either case, however, inhibition by sucrose was substantially greater than that due to an equivalent molarity of glucose so that neither succinate nor malate oxidation appeared to be responding to changes in the water activity of the medium. Oxidation of NADH was less severely inhibited (20% by 1000 mM sucrose) than was oxidation of

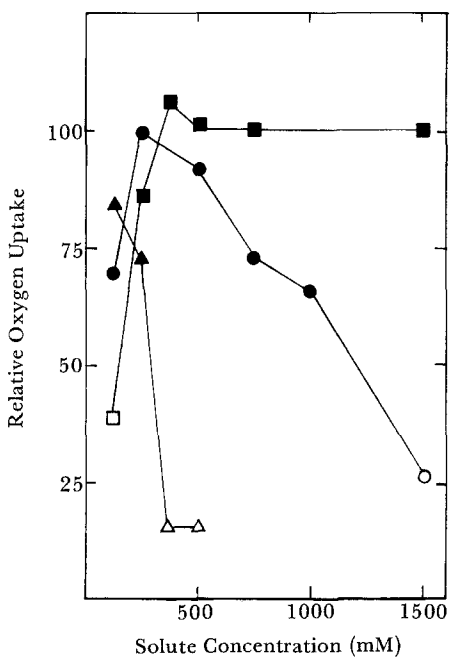

Figure 2. Effect of solute concentration in the medium on succinate oxidation by cauliflower-bud mitochondria. State-3 respiration rate in KCl (▲), sucrose (●) and glucose (■) media is expressed relative to the state-3 respiration rate in 250 mM sucrose (220 and 224 nmole O_2 min^{-1} mg^{-1} protein in separate experiments). Open symbols indicate an absence of respiratory control.

the organic acids. However, when the concentration of sucrose was increased to 1250 mM, the rate of NADH oxidation was reduced by 75%.

The oxidation of succinate by mitochondria from beet root, and cucumber, rock melon, and watermelon fruit, responded to solute concentrations in the same manner as mitochondria from potato tuber. Mitochondria from cauliflower buds were also similar except that these mitochondria were more sensitive to potassium chloride in the medium (Fig. 2).

The effect of some other solutes on succinate oxidation by potato mitochondria was examined. Sodium chloride had the same effect as equivalent concentrations of potassium chloride. The monosaccharides and sugar alcohols, glucose, fructose, xylose, arabinose, mannitol, and sorbitol had little effect on respiration, but each of the disaccharides, sucrose, lactose, and maltose decreased respiration. The amino acids proline, glycine, and alanine all increased the respiration rate. Glycerol up to 2500 mM had no effect, provided at least 100 mM sucrose was present. If sucrose was absent, respiratory control was lost, as it was when no other solutes were added to the basal reaction mixture.

Since it appeared that the degree of inhibition by organic molecules may be related to the rapidity with which they could penetrate the mitochondrial membrane, attempts were made to counteract the inhibition of sucrose by adding amino acids together with sucrose to the medium. In such cases the inhibition to respiration by sucrose was not relieved and the respiration rate was determined by the most inhibiting solute within the medium (Table I).

Substrate Availability and Dehydrogenase Activity

If mitochondrial respiration at high solute concentrations was depressed because of restricted permeability of the membrane to substrate, then the inhibition should not be apparent in sonicated mitochondria.

TABLE I. Influence of adding proline and sucrose to potato mitochondria respiring succinate

Solute concentration (mM)		Relative oxygen uptake
Sucrose	Proline	
0	750	100
750	0	79
750	750	78
1000	750	62

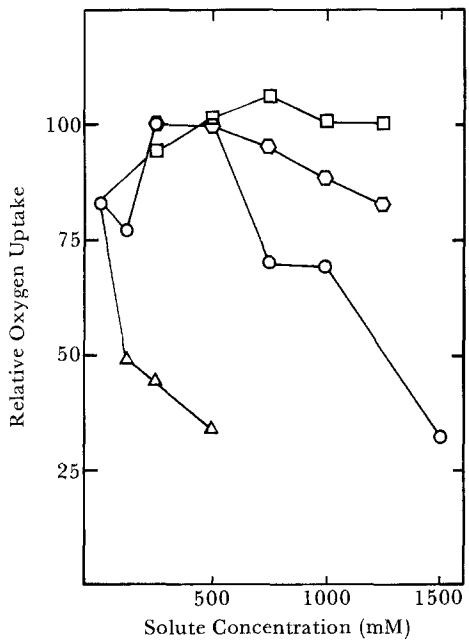

Figure 3. Effect of solute concentration in the medium on succinate oxidation by sonicated potato mitochondria. Oxygen uptake, determined in media containing succinate (5 mM) and ADP (2 mM) with sucrose (○), glucose (□), KCl (△), or glycine (⬭) is expressed relative to the oxygen uptake in 250 mM sucrose; this varied between 54 and 87 nmole O_2 min^{-1} mg^{-1} protein in different experiments.

However, the decrease in oxygen uptake by sonicated mitochondria respiring succinate and exposed to increasing concentrations of sucrose, was very similar to that observed for intact mitochondria (Fig. 3). Neither glucose nor mannitol inhibited succinate oxidation by the sonicated mitochondria, while glycine and proline neither inhibited nor stimulated oxygen uptake. Potassium chloride concentrations in excess of 50 mM markedly depressed oxygen uptake. Electron micrographs of the sonicated preparations showed that the mitochondria were extensively disrupted.

Inhibition of succinate-dependent oxygen uptake by 1000 mM sucrose (Fig. 1A) contrasts with a lack of inhibition of the succinate dehydrogenase shown in Fig. 1C. On the other hand, the activity of the dehydrogenase enzyme and the succinate oxidase system were both severely inhibited by 500 mM potassium chloride, as shown in Fig. 1C.

Potato mitochondrial malate dehydrogenase was inhibited by in-

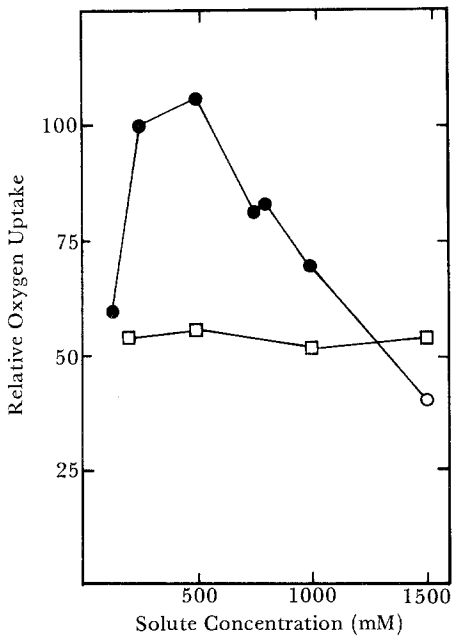

Figure 4. Effect of detergent on oxygen uptake by mitochondria at various solute concentrations. Oxygen uptake of potato mitochondria in media with succinate (5 mM), excess ADP, and varying sucrose content was determined, before (○) and after (□) the addition of Terex-X-8 (0.03% v/v). Oxygen uptake is expressed relative to that in 250 mM sucrose 187 nmole min^{-1} mg^{-1} protein.

creasing concentrations of sucrose, glucose and potassium chloride as shown in Fig. 1D. Glycine at any concentration from 250 to 1500 mM produced a 30–40% stimulation of enzyme activity relative to controls (data not shown). The specific activity of the enzyme as assayed exceeded the rate of oxidation of malate by the mitochondria about six-fold, and so the degree of inhibition of the dehydrogenase would not of itself account for the observed inhibitions of oxygen uptake.

Detergent relief of inhibition by high solute concentration

As shown in Fig. 4, the addition of the nonionic detergent Terex-X-8 to potato mitochondria abolished the depression of respiration caused by high sucrose concentration. The detergent concentration needed to alleviate inhibition was 0.03% and this produced a clear solution.

Discussion

If solutes regulate mitochondrial respiration, by changing the chemical potential of water in the medium, then the degree of inhibition by nonionizing solutes would be closely related to their molar concentrations and that by ionizing solutes to the integral of the concentrations of their ionic species. As with rat-liver mitochondria [4], this relationship is not seen with plant mitochondria from a variety of sources and the conclusion that inhibition is not due to changes in water potential is clear. Inhibition by salt may obviously involve mechanisms which are quite distinct from those involving nonionizing solutes. Among the latter there is a general inverse relationship between the rate of penetration of the mitochondrial membranes, as revealed by swelling studies [7], and the degree of inhibition of respiration. However, solute inhibition does not reflect the time course of solute penetrations, nor do rapidly penetrating solutes afford any protection to mitochondria from inhibition by disaccharides (Table I), hence inhibited respiration does not seem to be a direct consequence of the osmotic responses of the mitochondria.

Flowers and Hanson [2] concluded from studies of soybean mitochondria that mitochondrial respiration was inhibited by solutes due to an inhibition of malate dehydrogenase. For the latter, enzyme inhibition is about equivalent for sucrose and glucose at equivalent concentrations (Fig. 1D), and for organic solutes, inhibition of this enzyme may be a consequence of changes in water potential. However, malate dehydrogenase activity exceeds, by a factor of six, the observed rates of malate oxidation by mitochondria and oxygen uptake by the mitochondria is differentially affected by glucose, mannitol, and sucrose. This indicates that reactions between malate dehydrogenase and oxygen determine the rates of oxidation and it is the effect of solutes on these limiting reactions which govern the response of intact mitochondria.

Solutes cause comparable inhibitions of both succinate- and malate-dependent oxidation. However the corresponding dehydrogenases are not equally affected; succinate dehydrogenase activity is not inhibited at 1000 mM sucrose whereas inhibition of malate dehydrogenase is similar to malate oxidase. Malate oxidase and malate dehydrogenase also show a differential response with glucose. This evidence again argues against the dehydrogenase as a specific locus of inhibition and suggests that the organization of the electron-transport system might be modified in the presence of high concentrations of solutes. The abolition of inhibition by a detergent (Fig. 4) is consistent with this view.

Sonicated mitochondria remain susceptible to inhibition by sucrose, so inhibition is probably not due to limited substrate uptake [4]. A plant-mitochondrial ATPase has been shown to be progressively inhibited as the sucrose concentration is raised [8], and this suggests the

possibility that the availability of ADP within the mitochondria may be a factor limiting state-3 respiration when the solute concentration is high. The evidence concerning substrate uptake, nucleotide translocation, and the direct effects of solutes on membrane structure, as areas of inhibition of mitochondrial function is discussed in the preceding paper [4].

A comparison of the susceptibility of animal and plant mitochondria to organic solutes in the medium reveals that the animal organelles are the more susceptible in sucrose medium—a concentration of 500 mM inhibited succinate oxidation by rat-liver mitochondria by 30%, but did not inhibit succinate oxidation by plant mitochondria. Higher concentrations of sucrose inhibited the plant mitochondria. Mannitol proves as inhibitory as sucrose on rat-liver mitochondria [4], but mannitol did not inhibit succinate oxidation by potato mitochondria at a concentration of 1000 mM. Animal mitochondria in their natural environment are less subject to large variations in solute content than are many plant mitochondria, and consequently animals may have been able to set finer limits of tolerance to solutes for their mitochondria.

The evidence, presented here, that solute inhibition is not primarily a consequence of an inhibition of substrate dehydrogenases, and is not a function of lowered water activity in the medium, means not only that we fail to confirm the conclusions of Flowers and Hanson [2], but also that we remove the implication that plant and animal mitochondria are fundamentally different in their responses to medium solutes. We find that different solutes cause about the same relative inhibitions in mitochondria from both sources, and that the evidence does not suggest a different mechanism. A conclusion as to the significance of the quantitative differences in the response of mitochondria from the two sources may be apparent when a more complete explanation of the mechanisms involved has been made.

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