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The internal concentration of K^+ in isolated rat liver mitochondria

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A simple osmotic method has been developed to determine the internal K^+ concentration of mitochondria by determining the concentration of external K^+ at constant osmotic pressure at which metabolically inhibited mitochondria neither shrink nor swell. This concentration has been found to correspond to approx. 80–85 mM in freshly isolated mitochondria and considerably lower after additional centrifugation procedures. Since mitochondria are in osmotic equilibrium with the suspending medium (in this case, 0.32 osmolal), and K^+ is the primary exchangeable internal ion, a significant proportion of the internal osmotic pressure must be exerted by the sucrose. Results for experiments determining internal K^+ after centrifuging mitochondria at various *G* values confirm the reports of Sitaramam et al. (Sitaraman, V. and Sarma, M.K.J. (1981) Proc. Natl. Acad. Sci. USA 78, 3441–3445 and Sambasivarao, D. and Sitaramam, V. (1983) Biochim. Biophys. Acta 722, 256–270) that centrifugation induces the entry of sucrose in mitochondria isolated in a sucrose medium.

Introduction

There is considerable evidence that sucrose enters the inner space of isolated mitochondria. The influx of sucrose is indicated either by direct studies of the permeability to sucrose [1] or discrepancies between the expected inner mitochondrial space and the measured sucrose impermeable space [2] (see Refs. 3 and 4 for a review). Other evidence for this conclusion is provided by other data. (1) When the total concentration of sucrose in isolated mitochondria is taken into account, the osmotic behavior can only be predicted on the assumption that the sucrose is present in the internal mitochondrial space [4]. (2) The total concentration of sucrose in isolated mitochondria is inversely proportional to the internal K^+. The net influx of sucrose into the inner mitochondrial space during the isolation followed by the corresponding osmotic swelling or the exchange of sucrose with the internal K^+ are the only likely mechanisms that could account for this effect [4]. (3) More recently, Sitaramam et al. [5,6] have shown that enzymes present in the mitochondrial matrix are released at osmotic pressures of the external medium which depend on the concentration of sucrose in which the mitochondria have been isolated. The higher the concentration of the sucrose of the isolation medium, the higher the osmotic sensitivity of the release, a finding consistent with the presence of sucrose in the internal mitochondrial space.

We have chosen an approach derived from these considerations to determine the K^+ concentration

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

in the inner mitochondrial space by determining the mitochondrial changes in volume in isoosmotic media containing varying amounts of K^+, in the absence of metabolism. We have previously shown that the optical density of mitochondrial suspensions is inversely proportional to mitochondrial volume [7,8]. Therefore, the reciprocal of the optical density was used as an indication of mitochondrial volume.

Zhang et al. (Zhang, C.J., Trevouledes, P. and Tedeschi, H., unpublished results) have shown that in the absence of metabolism the net influx of K^+ in mitochondria is independent of the anion present in the external medium (see also Fig. 1). These results demonstrate that under these conditions the entry of K^+ is solely driven by its concentration gradient. Since mitochondria behave like osmometers, it should be possible to estimate the internal K^+ concentration by determining the external K^+ concentration at which isolated mitochondria neither swell nor shrink at constant osmotic pressure. This can be done most effectively from a curve depicting the rate of volume change (i.e., swelling or shrinking) at a variety of K^+ concentrations. The appropriate external K^+ concentration can then be derived from a complete curve. The results are essentially the same in the presence or absence of valinomycin.

Methods

Mitochondria were isolated in 0.25 M sucrose from male Holtzman rats, ranging in weight between 150 and 500 g as previously described [8]. The isolated mitochondria were resuspended in 0.3 molal sucrose containing 10 mM Tris chloride at pH 7.4. Typically the suspensions contained 20–40 mg protein per ml. Unless otherwise specified, the mitochondria were not washed. The isolation and the sedimentations were carried out with an HB-4 swinging bucket rotor of a Sorvall refrigerated centrifuge.

In the experiments, 50 μl of mitochondrial stock suspension were added to 3 ml of the solution. The solutions were maintained at constant osmotic pressure (0.32 osmolal) by mixing potassium salts and sucrose solutions dissolved in 10 mM Tris at pH 7.4. Antimycin A and rotenone were present in a final concentration of 0.3 and 2 μM, respectively. When present, valinomycin was at a concentration of 0.1 μM.

The kinetics shown in these experiments represent the change in the reciprocal of the optical density with time at a wavelength of 540 nm at a temperature of 25 ± 1°C using a Perkin Elmer Junior III spectrophotometer. As indicated above, this parameter is directly proportional to the mitochondrial volume [7,8]. Generally, the changes were followed for 6 min and for 3 min in the presence of valinomycin. In two experiments (Fig. 1), osmotically active mitochondrial volume in relative units (where the volume at 0.320 osmolal was taken as unity) was calculated from photometric measurements, using the method outlined by Tedeschi and Harris [7,8]. In these experiments, antimycin alone was used to block metabolism. The results are expressed as differences in relative volume from the initial value.

Results

Independence from the nature of the anion

The kinetic approach used in these experiments depends on the assumption that the entry of K^+ is independent of the anion used. This premise is based on a study of Zhang et al. (Zhang, C.J., Trevouledes, P. and Tedeschi, H., unpublished results), which will be presented in more detail separately. However, since this question is important in the present study, the appropriate results are shown in Fig. 1. Each point represents the difference in the relative mitochondrial volume from the initial volume at one specific time of incubation (see legend of figure) obtained in two independent experiments (indicated by different symbols). The abcissa represents the swelling in KCl, whereas the ordinate represents the swelling in the test solution, either SO_4 or acetate. These results show that there is no significant difference indicated by the small deviations from the ideal line drawn in the figure.

Volume changes at various external K^+ concentrations

The changes in the reciprocal of the absorbance taking place with time are represented in Fig. 2. Curve 1 represents the swelling of mitochondria in 0.15 osmolal K^+, whereas curve 2 represents the

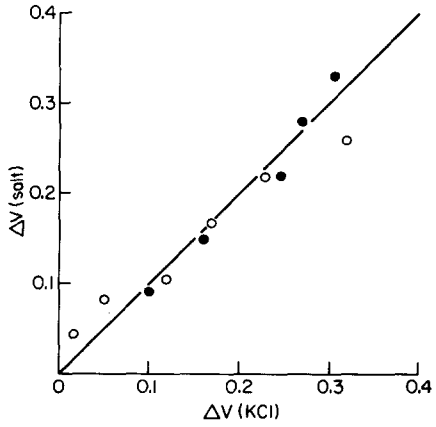

Fig. 1. Changes in relative volume at specific times (see below) during incubation in isoosmotic potassium solutions. The data are expressed as ΔV, the changes in osmotically active volume from the initial value, taken arbitrarily as 1. The solutions were in 10 mM Hepes (pH 7.0). The points represent in ascending order the following times of incubation: open circles, 60, 90, 120, 180, 240 and 300 s; closed circles, 60, 90, 120, 150 and 180 s. The results have been plotted so that volume of the abcissa to the swelling in KCl and the ordinate represents the rate of swelling in either K_2SO_4 (open circles) or potassium acetate (closed circles). The line represents perfect correspondence in the rate of swelling of the two sets. The two sets of data represent independent experiments.

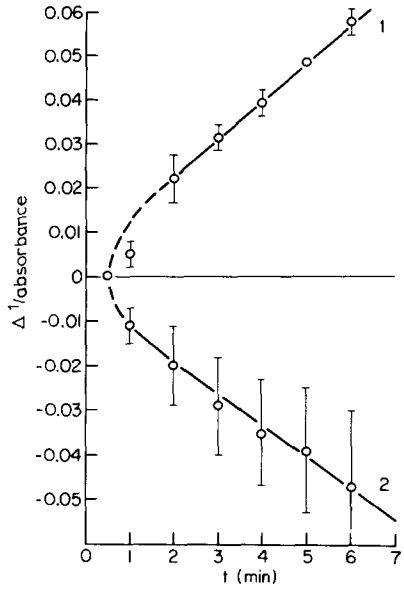

Fig. 2. Changes in the reciprocal of optical density with time at different external K^+ concentrations. The 1/absorbance measured after 30 s was subtracted for the values shown. In effect, this procedure corrects for the differences in refractive index of the various solutions [8]. The deviations correspond to standard deviations. $N = 4$

shrinkage taking place in 0.05 osmolal K^+. Fig. 3 summarizes the results obtained at various external K^+ concentrations. All the points of either figure were fitted using the method of least squares for the linear portion of the curves. All deviations shown are standard deviations. In Fig. 3, the intercept on the abcissa represents the external K^+ concentration at which a there is neither shrinkage nor swelling, and therefore corresponds to the internal K^+ concentration, about 70 mosmolal (68 $\pm$ 8; mean $\pm$ SD). This value is equivalent to approx. 80 mM (with the appropriate correction for activity). The results of five independent experiments are shown in Table I. These indicate that the data are approximately the same regardless of (a) the anion used or (b) the presence or absence of valinomycin. In the latter case, however, the swelling or shrinkage rates occurred 2–5-times faster.

Effect of centrifugation

Sitaramam et al. [5,6] proposed that the high permeability of the mitochondrial inner space results from the gravitational forces exerted during centrifugation. The entry of sucrose would result

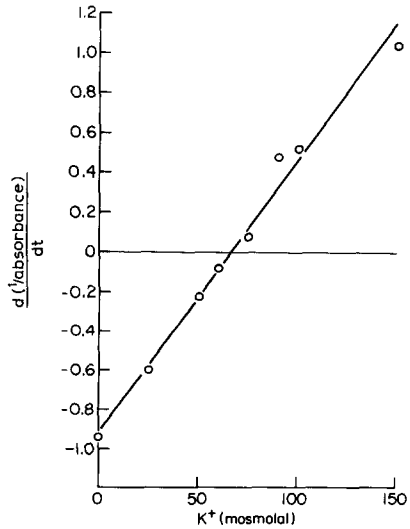

Fig. 3. The rate of change d(1/absorbance)/dt as a function of external K^+ concentrations.

TABLE I

INTERNAL K^+ CONCENTRATION

Exp. number	Salt/condition	K^+ concentration (mosmol)
1	KCl	75
2	Potassium acetate	66
3	Potassium acetate	77
4	Potassium acetate/valinomycin	60
5	Potassium acetate/valinomycin	60

in a K^+ dilution (either because of an osmotic swelling or the replacement of K^+ by sucrose in the presence of K^+ leakage; see Discussion). We therefore examined the dependence of the internal K^+ on centrifugation. The results are summarized in Fig. 4 which expresses the internal K^+ concentration as a function of the $G \times$ min of centrifugation. In all cases the centrifugations were carried out for 10 min. For $G \times$ min above 200 000, two separate centrifugations were carried out after resuspension of the mitochondria.

The results leave no doubt that the internal K^+ sharply decreases with centrifugal force. We have found (not shown) that centrifuging for the same length of time using an angle rotor produces less K^+ loss. This is probably the result of the fact that the sedimentation path of the mitochondria is much shorter (approx. 1/3) and therefore the sedimentation time is also correspondingly shorter.

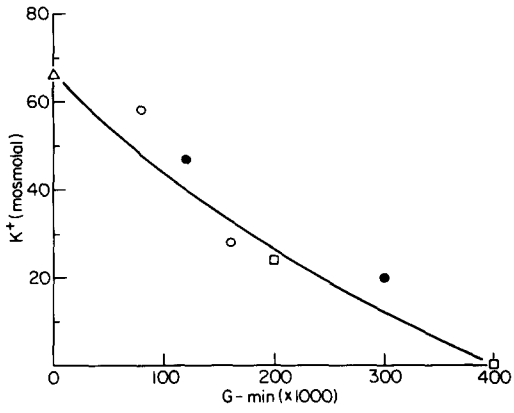

Fig. 4. The internal K^+ calculated as a function of centrifugal force. The different symbols indicate different experiments. The 0 value in the abcissa corresponds to freshly isolated mitochondria with no additional centrifugations and was obtained from Table I.

Discussion

This approach provides a simple way of determining the internal K^+ concentration (as well as the space penetrated by the suspending medium, since the two are inversely proportional). In addition, it confirms that the mitochondria become leaky to sucrose as the result of the gravitational forces exerted by centrifugation.

The internal concentration of K^+, 80 mM, as estimated by this method in freshly isolated mitochondria is approximately the same as that reported by others (e.g., Ref. 10), 100 mM being a common estimate when a correction is made for sucrose space determined with ^{14}C- or ^3H-sucrose (although as noted above, these values tend to be erratic). This agreement is not surprising because, in effect, in the experiments using labelled sucrose a correction is made for the sucrose which has entered into the inner space during the sedimentation procedure which followed the addition of the label. The true concentration, however, would be much lower than that calculated in this manner because the inner mitochondrial volume is much greater. It should be noted that even values of 100 mM are likely to indicate considerable entry of sucrose. The concentration of K^+ in the cytoplasm, at least in mice, is approx. 170 mM [11]. This concentration is likely to correspond to the mitochondrial concentration, since K^+ is the most abundant mitochondrial ion and the system is at osmotic equilibrium. In fresh mitochondria, K^+ appears to constitute approx. 70% of the cation content [12]. Other cations, such as Ca^{2+} and Mg^{2+}, occur in much lower concentration. Furthermore, they are not likely to play a significant role. The concentration of acid-soluble phosphates are well in excess of either the Mg^{2+} or the Ca^{2+} concentration [13,14], suggesting that these ions will not be present in their free form. Furthermore, phospholipids have been shown to bind as much as 25–30 μmol divalent cations per g protein in submitochondrial particles [15]. In order to decrease the K^+ concentration to the values estimated by these techniques, a considerable amount of sucrose must enter. Other considerations support this view. An internal concentration of 80 mM represents a total ion concentration (cations + anions) of no more than 160 mM (assuming

that a monovalent anion balances the K^+). Since the osmotic pressure of the suspending medium corresponds to 0.320 osmolal, the rest of the osmotic pressure (equivalent to approx. 160 mM) would have to be made up by sucrose. This proportion corresponds to a penetration of approx. 50%. Because of the osmotic behavior of mitochondria, the leakage of K^+ alone would not be able to decrease the K^+ concentration in the inner mitochondrial space, since the mitochondria would shrink osmotically to compensate exactly for the exit of K^+.

The 80 mM K^+ concentration we calculate is likely to correspond to a slight underestimate. Internal mitochondrial Na^+ in freshly isolated mitochondria corresponds to approx. 6% of the K^+ content [12]. The permeability to Na^+ is greater than that to K^+ (Refs. 16 and 17, see also Zhang, C.J., Trevouledes, P. and Tedeschi, H., unpublished results). Consequently, a leakage of Na^+ during our incubation would in effect shift the intercept of Fig. 3 to a lower value. The precise value is difficult to estimate in view of the reported binding of monovalent cations to mitochondrial membranes [15].

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