Biochimic et Biophysica Acta, 333 (1974) 22–27

BBA 46661

THE COMPARATIVE UPTAKE OF Ba^{2+} AND OTHER ALKALINE EARTH METALS BY PLANT MITOCHONDRIA

R. H. WILSON and G. A. MINTON

The Department of Botany, The University of Texas, Austin, Texas 78712 (U.S.A.)

(Received July 23rd, 1973)

SUMMARY

1. The transport of Ba^{2+} by mung bean (*Phaseolus aureus* L) mitochondria is examined and its uptake properties compared with the accumulation of other alkaline earth metals by plant mitochondria. Uptake is supported by oxidizable substrates but not by ATP under the conditions examined. Ba^{2+} uptake shows an absolute requirement for P_i, whereas AsO_4, acetate and oxalate do not replace P_i. ATP synthesis inhibits Ba^{2+} uptake 50% and both 2,4-dinitrophenol and valinomycin plus K^+ inhibit uptake over 80%.

2. The relative capacity to transport a series of alkaline earth metals showed a preferred order of: $Sr^{2+} > Ca^{2+} > Ba^{2+} \gg Mg^{2+}$. Mitochondria isolated from three plant sources showed net levels of Mg^{2+} uptake 7% or less than the observed Sr^{2+} uptake values. The same relative order of alkaline earth metal supported uptake of P_i was observed with bean (*Phaseolus vulgaris* L) mitochondria. It is suggested that salt transport in plant mitochondria involves a carrier complex which binds both divalent cations and P_i.

INTRODUCTION

Mitochondria isolated from a wide range of organisms possess the capacity to transport divalent cations [1–5]. However some of the properties of the transport system differ depending on the source of mitochondria. Thus mitochondria from plants, in contrast with vertebrate animal mitochondria, show an absolute requirement for P_i which cannot be replaced by other anions [4, 6]. Moreover plant mitochondria do not stimulate respiration nor eject H^+ during divalent cation transport, and appear to lack a high affinity binding site found in a wide range of animal mitochondria [1, 4, 7, 8]. This study examines the uptake of the alkaline earth metal, Ba^{2+}, by mung bean mitochondria. Ba^{2+} is known to be toxic to the growth of several plants including mung beans, and it has been shown to be present in mitochondria isolated from Ba^{2+} exposed plants [10, 11]. This study compares the relative capacity of mitochondria to transport Ba^{2+} and a series of alkaline earth metals. The results are related to the possible mechanism of salt transport in plant mitochondria.

Abbreviation: Tricine, tris(hydroxymethyl)methylglycine.

MATERIALS AND METHODS

Etiolated mung bean hypocotyls (*Phaseolus aureus* L) were harvested from 4–5-day-old plants grown in vermiculite. The isolation of mitochondria was done in a medium containing: 0.4 M mannitol, 50 mM Tris–tris(hydroxymethyl)methylglycine (Tricine), pH 7.4, 1 mg per ml bovine serum albumin, 4 mM cysteine and 5 mM EDTA. The shoots were ground with a Moulinex mixer and isolated by differential centrifugation as previously described [11] except the last centrifugation involved passing the mitochondria through a wash layer of 15 ml of 0.8 M mannitol. The final mitochondrial pellet was resuspended in 2.0 ml of the above medium and had approx. 5 mg/ml protein. Electron micrographs showed mitochondria the predominate organelle with some contaminating vesicular membranes and endoplasmic reticulum. Maize (*Zea mays* L) mitochondria and Kentucky wonder pole bean (*Phaseolus vulgaris* L) mitochondria were isolated as previously described [4, 12].

Divalent cation content was determined on mitochondria which had been incubated in 3.5 ml of medium containing: 0.4 M mannitol, 50 mM Tris–Tricine pH 7.8, 1 mg/ml albumin, 2 mM $BaCl_2$, 2 mM potassium phosphate pH 7.4 and substrate as indicated in the legends. Controls contained 2 mM KCN and showed no respiration on the oxygraph. After 8 min incubation at 26 °C, 3.2 ml of medium containing between 0.6 and 1.5 mg protein per ml were transferred to a cold centrifugation tube and the mitochondria harvested by centrifugation through a layer of 5 ml of 0.8 M mannitol at 31 000 $\times g$ for 10 min. After the centrifugation tubes had been decanted and wiped dry, the mitochondrial pellet was resuspended in 2.5 ml of 10 % trichloroacetic acid plus 1 % La_2O_3. The suspension was centrifuged for 10 min at 35 000 $\times g$ and the divalent cation content of the decanted supernate determined by atomic absorption spectrophotometry [4]. Oxygraph procedures and P_i assay were as previously described [4]. Proteins were determined by the Lowry method [14].

RESULTS

The time-dependent accumulation of Ba^{2+} is shown in Fig. 1. Net uptake stopped after about 20 min and was followed by a gradual loss possibly due to the partial reduction in coupling as reported with Sr^{2+} accumulation in bean mitochondria [4]. In Table I the uptake of Ba^{2+} is compared using the major oxidizable substrates of mung bean mitochondria. Externally added NADH supported the highest uptake values as well as highest respiration rates. The low values for Ba^{2+} uptake with malate plus pyruvate can be accounted for by the initially low respiration rates which further declined over the time interval when Ba^{2+} accumulation was measured. No net Ba^{2+} uptake was detected with ATP as an energy source. A summary of the characteristics of Ba^{2+} uptake is shown in Table II. Of the anions examined only P_i supported uptake. The presence of dinitrophenol largely inhibited uptake and induced an approx. 50 % loss of Ba^{2+} when added after uptake had occurred (data not shown). One quantitative difference in the results shown in Table II from studies previously reported on divalent cations uptake with plant mitochondria is the 50 % inhibition of Ba^{2+} uptake by an ATP generating system compared with the nearly 80 % inhibition reported with Sr^{2+} and Ca^{2+} uptake [4, 15]. This difference may reflect a greater demand on the use of conserved energy by Ca^{2+} and Sr^{2+} due to the greater uptake rates of these ions compared with Ba^{2+}.

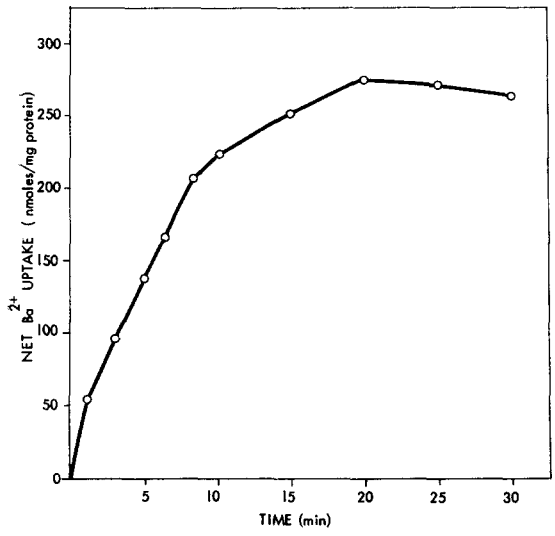

Fig. 1. Time course of uptake of Ba^{2+} by mung bean mitochondria. Aliquots of 3.2 ml from 32 ml were withdrawn at the time intervals shown and Ba^{2+} determined by the procedure given in Materials and Methods. Substrate was 8 mM sodium succinate. Protein was 0.7 mg /ml. Ba^{2+} concentration was 2 mM.

TABLE I

Ba^{2+} UPTAKE AND RESPIRATORY PARAMETERS OF ISOLATED MUNG BEAN MITOCHONDRIA

The medium for the respiratory studies contained: 0.4 M mannitol, 50 mM Tris–Tricine pH 7.8, 1 mg /ml albumin, 5 mM $MgCl_2$, 2 mM potassium phosphate, pH 7.4 and substrates as shown. ADP was added in aliquots of 223 nmoles. The control lacked substrate and contained 2 mM KCN. The medium for following Ba^{2+} uptake was identical to the above with the addition of 2 mM $BaCl_2$ in place of $MgCl_2$. The procedure for following uptake is given in Materials and Methods. Incubation time was 8 min. Net Ba^{2+} uptake is the difference between the uptake in the presence compared with the absence of substrate.

Substrate	State 4 respiration (nmoles O_2/min per mg protein)	Respiratory control*	ADP/O	Ba^{2+} Uptake (nmoles/mg protein)	
				Total	Net uptake
Control	–	–	–	40	–
8 mM sodium succinate	30	3.0	1.4	235	195
1.4 mM NADH	27	3.1	1.3	261	231
8 mM sodium malate + 8 mM sodium pyruvate	15	3.7	1.9	83	43
Control plus 3 mM ATP	–	–	–	40	0

* In the absence of Ba^{2+}.

TABLE II

GENERAL CHARACTERISTICS OF Ba^{2+} UPTAKE BY MUNG BEAN MITOCHONDRIA

The complete reaction medium is given in Table I. The substrate is 8 mM sodium succinate. Protein was between 2.5–4.0 mg per reaction mixture. The results are an average of two or more determinations.

Conditions	Ba^{2+} uptake (nmoles/mg protein)		% of maximum
	Total uptake	Net uptake	
Complete	244	201	100
− succinate + 2 mM KCN	43	–	0
− succinate − P_i	44	1	0
− P_i + 2 mM arsenate	35	−9	0
− P_i + 2 mM acetate	39	−4	0
− P_i + 20 mM acetate	35	−9	0
− P_i + 2 mM oxalate	43	0	0
+ 100 μM 2,4-dinitrophenol	61	18	11
+ 5 mM KCl + 2 μg valinomycin	67	34	17
+ 200 μM ADP + hexokinase trap*	144	101	50

* 20 units of hexokinase (Sigma) + 10 mM glucose + 2 mM $MgCl_2$.

TABLE III

THE UPTAKE OF A SERIES OF ALKALINE EARTH METALS BY PLANT MITOCHONDRIA

The procedure for following the uptake is given in Materials and Methods. The substrate was 0.5 mM NADH. The protein was between 0.5–1.4 mg/ml. The results are an average of two or more determinations. Concentration of divalent cations is 2 mM. Incubation time was 8 min.

Mitochondria source	Ion	Net divalent cation uptake (nmoles/mg protein)	% of Sr^{2+} uptake
Mung bean	Sr^{2+}	308±18	100
Mung bean	Ca^{2+}	264±11	86
Mung bean	Ba^{2+}	234±16	76
Mung bean	Mg^{2+}	13±5	4
Corn shoot	Mg^{2+}	21	7
Kentucky pole bean	Mg^{2+}	8	3

Uptake studies with Ba^{2+} provide an opportunity to examine the relative order of transport of the alkaline earth metal series. The results, shown in Table III, indicate that uptake followed the order: $Sr^{2+} > Ca^{2+} > Ba^{2+} \gg Mg^{2+}$. Mg^{2+} uptake into mitochondria from three plant sources was extremely low; much less than reported for Mg^{2+} uptake into red beet root mitochondria [9].

In a related study with mitochondria from Kentucky wonder pole beans the divalent cation supported accumulation of P_i shows the same relative order of P_i uptake as reported in Table III for divalent cation uptake with mung bean mitochon-

dria. While this appears to support the same order of divalent cation selectivety for the two mitochondrial sources, an underlying assumption is that the metal: P_i ratio is approximately similar for each divalent cation transported.

TABLE IV

THE EFFECT OF A SERIES OF CATIONS ON PHOSPHATE UPTAKE WITH KENTUCKY WONDER POLE BEANS MITOCHONDRIA

The complete reaction mixture of 6.4 ml contained: 0.4 M mannitol, 50 mM Tris–Tricine (pH 7.5), 1 mg/ml albumin, 5 μM rotenone, 2.0 mM concentration of the divalent cation salt, 2.0 mM Tris–phosphate and 8 mM Tris–succinate. Protein was 0.8 mg. Incubation time was 10 min at 27 °C.

Salt added	Total P_i uptake (nmoles/mg protein)	Net P_i uptake (nmoles/mg protein)
None	10	–
$SrCl_2$	190	180
$CaCl_2$	136	126
$BaCl_2$	106	96
$MgCl_2$	21	11
KCl	19	9
NaCl	17	7
LiCl	17	7
NH_4Cl	10	0

DISCUSSION

The relative capacity of mung bean mitochondria to accumulate divalent cations in Group II of the Periodic Table follows the order $Sr^{2+} > Ca^{2+} > Ba^{2+} \gg Mg^{2+}$. Current theories of divalent cation selectivity based on binding studies with non-biological materials including zeolites [16] and glass electrodes [17, 18] have stressed the importance of the free energy difference between cation–site interaction and the free energy of hydration of the divalent cations as decisive factors in determining the relative order of divalent cation binding with these systems. From such studies with non-biological materials a number of permutations of these four divalent cations were predicted as the preferred order to binding.

While the selected series predicted from binding studies to zeolites [16] has been shown to agree with the order observed for binding in a number of biological systems [19], the order reported here for divalent cation transport does not coincide with any of these. This suggests that the relative affinity for the transport binding sites does not in itself determine the selectivety in transport and that other factors influence the order of uptake. While the available evidence does not allow conclusions concerning what factors may be effecting the transport of these cations, it is interesting that studies with divalent cation selective glass electrodes indicate that mobility of the divalent cation across the electrode membrane plays a central role (in addition to binding) in the selectivity with that system [18].

An hypothesis for the mechanism of salt transport in higher plant mitochondria has previously been suggested in which P_i was considered the actively transported species [6]. Support for this conclusion comes from the absolute P_i requirement for divalent cation uptake [4, 6] as well as the identification of a P_i transport system which

functions in the absence of divalent cations [13, 20]. However, it is clear that maximum P_i accumulation occurs only when an appropriate divalent cation is present and transported with the P_i (Table IV). Thus an alternative mechanism might involve the formation of a divalent cation–P_i-carrier complex in which the divalent cation is bound but not transported independently of the P_i [4, 7]. The relative capacity for mitochondria to accumulate different divalent cations may depend on the divalent cation affinities for the transport binding sites as well as other possible factors such as the mobility of the complex across the membrane.

While Ba^{2+} is non-essential for plant growth, and functions only poorly as a "sparing ion" for Ca^{2+}, it can be toxic to plants including etiolated mung beans grown in its presence. Studies with mitochondria isolated from such plants show Ba^{2+} levels of over 100 nmoles/mg protein, higher respiration rates than normal and reduced respiratory control over normal grown plants [11]. These responses, similar to responses seen with classical uncouplers, may be due to the accumulation of Ba^{2+} in mitochondria in vivo and account for the inhibited growth of plants in Ba^{2+} soils.

ACKNOWLEDGMENT

This investigation was supported by Biomedical Sciences Support Grant RR-07091-07 from the General Research Branch, Division of Research Resources, Bureau of Health Professions, Education and Manpower Training, National Institutes of Health.

REFERENCES

1 Carafoli, E. and Lehninger, A. L. (1971) J. Biochem. 122, 681–690
2 Hanson, J. B. and Hodges, T. J. (1967) Curr. Top. Bioenerg. 2, 65–98
3 Bonner, W. D. and Pressman, B. C. (1965) Plant Physiol. 40, p. LV, Abstr.
4 Johnson, H. M. and Wilson, R. H. (1972) Biochim. Biophys. Acta 267, 398–408
5 Lehninger, A. L. (1970) J. Biochem. 119, 129–138
6 Hanson, J. B. and Miller, R. J. (1967) Proc. Natl. Acad. Sci. U. S. 58, 727–734
7 Johnson, H. M. and Wilson, R. H. (1973) Plant Physiol., in the press
8 Reynefarje, B. and Lehninger, A. L. (1969) J. Biol. Chem. 244, 584–593
9 Millard, D. L., Wiskich, J. T. and Robertson, R. N. (1964) Proc. Natl. Acad. Sci. U.S. 52, 966–1004
10 Ballard, E. G. and Butler, G. W. (1966) Annu. Rev. Plant Physiol. 77–112
11 Minton, G. A. and Wilson, R. W. (1973) Plant Soil, in the press
12 Kenefick, D. G. and Hanson, J. B. (1966) Plant Physiol. 41, 1601–1609
13 Wilson, R. H., Dever, J., Harper, W. and Fry, R. (1972) Plant Cell Physiol. 13, 1103–1111
14 Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
15 Hodges, T. K. and Hanson, J. B. (1965) Plant Physiol. 40, 101–109
16 Sherry, H. S. (1968) Ion Exchange II, pp. 89–133, Dekker, New York
17 Truesdell, A. H. and Christ, C. L. (1967) Glass Electrodes for Hydrogen and Other Cations (Eisenman, G., ed.), pp. 293–321, Dekker, New York
18 Eisenman, G., Bates, R., Mattock, G. and Freidman, S. M. (1967) The Glass Electrode, pp. 285–289, Wiley, New York
19 Diamond, J. M. and Wright, E. M. (1969) Annu. Rev. Biochem. 38, 581–646
20 Hanson, J. B., Bertagnolli, B. L. and Shepherd, W. D. (1972) Plant Physiol. 50, 347–354