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SHORT COMMUNICATION

ION UPTAKE BY MITOCHONDRIA ISOLATED FROM COTYLEDONS OF *PHASEOLUS VULGARIS*

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Abstract—An actively respiring mitochondrial fraction has been prepared from the cotyledons of *Phaseolus vulgaris*. Mitochondria show ion uptake in the presence of succinate as substrate but do not respond to ATP addition. Presence of magnesium is not necessary in the incubation medium for calcium uptake.

INTRODUCTION

SUBSTRATE-dependent ion uptake in animal mitochondria is by now well established.[1–3] Similar observations have been made in recent years with mitochondria isolated from plant sources.[4,5] Various Kreb's cycle intermediates have supported respiration-dependent uptake of cations like calcium and magnesium along with inorganic phosphate. ATP is also able to support ion uptake. Some differences, however, have been observed between the behaviour of animal and plant mitochondria. Hanson[6] has recently reported that with mitochondria isolated from soya bean hypocotyls, no ion uptake was observed in the presence of ATP. Furthermore, a substrate- (succinate) supported uptake was somewhat lowered by ATP. Mitochondria isolated from corn, however, behave in a manner similar to animal mitochondria.

In the present communication, we report the isolation of an actively respiring mitochondrial fraction isolated from the cotyledons of *Phaseolus vulgaris*. Substrate-dependent calcium and phosphate ion uptake has been demonstrated.

RESULTS

In the presence of succinate as a source of energy, the mitochondrial fraction has shown the capacity for uptake of calcium and inorganic phosphate. Results in Table 1 indicate that the net uptake of calcium is about 300 mμmoles/mg N and that of phosphate about 1000 mμmoles/mg N giving Ca/P_i ratio of 0·3. When ATP was added along with succinate, the net uptake was lowered to 188 mμmoles calcium and 375 mμmoles of phosphate. Values near control (with no energy source) were obtained in the presence of 1·5 μmoles of DNP

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indicating the substrate-dependent nature of ion uptake. No uptake was observed when ATP was used as the energy source, as can be seen from Table 1, experiment II. Values reported in experiment III indicate that presence of externally added magnesium was not essential for ion uptake. However, the ratio of Ca/P_i which, in presence of magnesium is about 0·3, rises to 0·8 when magnesium is omitted.

TABLE 1. SUBSTRATE-DEPENDENT ION UPTAKE BY MITOCHONDRIA ISOLATED FROM THE COTYLEDONS OF *Phaseolus vulgaris*

Additions	Ca	P_i	Net uptake Ca	Net uptake P_i	Ca/P_i
	mμmoles/mg N		mμmoles/mg N		
I.					
1. Control	331	875	—	—	—
2. +Succinate	625	1813	294	938	0·33
3. +Succinate+DNP	356	1063	25	188	0·11
II.					
1. Control	375	1438	—	—	—
2. +ATP	375	1500	—	62	—
3. +Succinate	688	2625	313	1187	0·25
4. +Succinate+ATP	563	1813	188	375	0·50
III.					
1. Control	506	1063	—	—	—
2. +Succinate	1000	2500	494	1437	0·33
3. +Succinate ($MgCl_2$ omitted)	1063	1750	557	687	0·80

DISCUSSION

Mitochondrial fraction isolated from cotyledons of *Phaseolus vulgaris* has shown the characteristic property of ion uptake in the presence of an oxidizable substrate, succinate. Some differences from the animal mitochondrial system are, however, evident. Lehninger[2] and Brierley[1] have reported Ca/P_i uptake ratios between 1·5–1·8 with mitochondria isolated from rat liver and beef heart respectively, whereas with plant systems, this ratio in some cases has tended to be below one. Millard *et al.*[4] have reported Mg/P_i ratio of 1·5 for red beet mitochondria. With maize mitochondria, the ratio of Ca/P_i was of the order of 0·5, although when divalent cation (Ca + Mg) to phosphate ratio is considered, the figure is about one. In the present study, with mitochondria from cotyledons of *P. vulgaris*, we observe ratios between 0·25–0·33 in the complete system. When the calcium concentration in the medium was lowered to 0·18 mM, the ratio was still the same. But, in the absence of magnesium and with 1·8 mM calcium chloride, this ratio increases and approaches one. This is more due to less P_i uptake in the absence of magnesium than increased calcium uptake. It is rather premature at this stage to attach any biochemical significance to this observation. These results, however, indicate the non-requirement of externally added magnesium in the medium for calcium uptake.

The nature of energy-dependent uptake is shown by the addition of DNP (1·5 μmoles) when the uptake values are near about control.

In the presence of ATP as the source of energy, no uptake could be demonstrated under

our experimental conditions. A similar observation has been reported by Hanson[6] for soya bean mitochondria. Besides, when ATP was added along with succinate, no increase over succinate-dependent values was observed. Nature of this lack of response is under study.

Magnesium was not an obligatory requirement unlike with animal mitochondria.[2] Endogenous magnesium level might be sufficient to meet the requirement, but it may be mentioned that the mitochondrial fraction was isolated in medium containing EDTA. In the absence of magnesium, where the phosphate uptake is in response to calcium uptake unlike when both calcium and magnesium are present, the Ca/P_i ratio approaches one but is still lower than 1·5–1·8 as reported for animal mitochondria.

EXPERIMENTAL

Isolation of mitochondria. About 80 g of cotyledons free of outer seed coat were collected from freshly harvested beans which were precooled at 4°. Homogenate was prepared in the Waring blendor using the following medium: 0·33 M sucrose–0·001 M EDTA, pH 7·4, containing 20 mg L-cysteine hydrochloride, 300 mg PVP-40 (polyvinylpyrrolidone) and 100 mg egg albumen per 100 ml. The homogenization was carried out for 1 min during which time the pH was maintained by the addition of 0·33 M sucrose–1 M NaOH. After removing the unbroken cells and nuclei at 1000 *g* for 10 min, the mitochondria were sedimented at 13,000 *g* for 20 min. The pellet was suspended in 0·33 M sucrose and again centrifuged at 1000 *g* for 10 min. The sediment was discarded and the mitochondrial fraction was collected at 13,000 *g* for 20 min. After one washing, the pellet was suspended in 4 ml of 0·33 M sucrose. All the operations were carried out at 0–4°. The $Q(O_2)_N$ of the isolated mitochondria was found to be about 400 with succinate as substrate. The mitochondria were taken up for the ion uptake studies within 2 hr of isolation.

Ion uptake studies. Incubation medium consisted of: NaCl, 66·6 mM; $MgCl_2$, 4·6 mM; tris, pH 7·4, 8·3 mM; NaH_2PO_4, pH 7·4, 8·75 mM; sodium succinate, 8·3 mM; $CaCl_2$, 1·8 mM + $Ca^{45}Cl_2$ 65,000 cpm, mitochondria, 1·0 ml (9 mg protein). Final volume was 6·0 ml. ATP, 0·83 mM and DNP, 1·5 μmoles were added as indicated. After incubation for 10 min at 30°, the mitochondrial pellet was sedimented and taken up in water. After 30 min, the protein was precipitated with TCA and the supernatant was freed of TCA by ether extraction. Ca^{45} was counted in a windowless gas-flow counter. P_i was determined by the method of Taussky and Shorr.[7] Protein was estimated by the method of Lowry *et al.*[8]

ATP and PVP 40 (Sigma), L-cysteine hydrochloride (Nutrition Biochemicals) and egg albumen (Merck) were used. All the other reagents were BDH Analar grade. $Ca^{45}Cl_2$ was purchased from Atomic Energy Establishment, Trombay, Bombay.

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