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TEMPERATURE EFFECTS ON THE KINETICS OF CALCIUM TRANSPORT BY CARDIAC MITOCHONDRIA

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SUMMARY: The relationship between the initial rates of respiration dependent calcium transport by isolated rabbit cardiac mitochondria and the free calcium concentration of the reaction media has been examined at 10°C and 25°C. Initial rates of calcium transport were determined using an adaption of the EGTA/ruthenium red quench technique described by Reed and Bygrave (5). At 10°C the initial rate of calcium transport was found to be a sigmoidal function of free calcium concentration, with a Hill coefficient of approximately 1.9. Elevation of the temperature to 25°C produced a less sigmoidal relationship, the Hill coefficient being lowered to approximately 1.3.

The initial rates of respiration dependent calcium transport by mitochondria have been investigated by a number of groups, employing a variety of techniques (1, 2, 3). Some disagreement appears to exist concerning the relationship between the initial rates of calcium transport and free calcium concentration and it seems possible that these differences may reflect the variety of methods used to produce them.

Reed and Bygrave (2), using an E.G.T.A./ruthenium red quench technique and working at 0°C, suggested that the initial rate of calcium transport by rat liver mitochondria was a sigmoidal function of calcium concentration. Recent experiments by Crompton et al. (4), using both calcium sensitive electrode and inhibitor stop methods to determine the initial rates of calcium transport by rat heart mitochondria at 25°C, have described a hyperbolic relationship between initial rates of transport and calcium concentration.

Abbreviation: N.T.A., nitrilo tri acetic acid.

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In the present study we have investigated the possibility that the temperature at which calcium transport is allowed to proceed might produce alterations in the relationship between the initial rate of calcium transport and the free calcium concentration.

MATERIALS AND METHODS

Mitochondrial preparation

New Zealand white rabbits (2 - 2.5 kg) were killed by a blow to the head, the hearts excised and placed in an ice cold medium containing 180 mM KCl, 10 mM EDTA and 0.5% bovine serum albumin, pH 7.2 (Medium 1). The ventricles were washed to remove excess blood and finely chopped with a razor blade. The tissue was then homogenised, in medium 1, using an Ultra turrex homogeniser at speed setting 4.5 for ten seconds. The homogenate was centrifuged at 1000 g at 4°C in an M.S.E. high speed 18 for five minutes. The supernatant was filtered and recentrifuged for ten minutes at 9000 g at 4°C. The resulting crude mitochondrial pellet was resuspended in a medium containing 180 mM KCl and 0.5% bovine serum albumin, pH 7.2 (medium 2), and centrifuged for ten minutes at 5000 g at 4°C. The mitochondrial pellet obtained from this centrifugation was washed again in medium 2 and the final pellet collected and resuspended in medium 2. The protein content of this suspension was determined by the method of Bradford (6).

Initial rates of calcium transport at 10°C

Mitochondria, (1.25 mg protein), were suspended in one ml of a medium containing 250.0 mM sucrose, 10.0 mM Tris, 10.0 mM nitrilotriacetic acid, 3.0 mM succinic acid and 4.5 µg rotenone adjusted to pH 7.4 at 10°C with HCl. The medium was continually stirred via a magnetic stirrer and was surrounded by a jacket of circulating water maintained at 10°C. Following one minute's incubation, calcium transport was initiated by the addition of a known quantity of calcium chloride containing 0.2 m Curies $^{45}Ca^{2+}$/ml. The rate of calcium transport was monitored by withdrawing 200 µl samples of the mixture after 6, 15, 25, 35 and 45 seconds. Each sample was immediately mixed with 500 µl of a quench solution containing 40.0 mM E.G.T.A. and 3.0 µM ruthenium red, pH 7.4 at 4°C.

The samples were then filtered through 0.45 µ pore size millipore filters and washed with a total of 10.0 ml of 250 mM sucrose, 10 mM Tris, pH 7.4 at 4°C. The filters were dried, mixed with a scintillant and counted in a Packard 3380 liquid scintillation counter. Samples were filtered within five minutes of mixing with the quench solution. Pilot experiments showed that no significant alterations in the calcium content of the mitochondria occurred during this time.

Initial rates of calcium transport at 25°C

These experiments were carried out in a similar manner to those conducted at 10°C. However, at this temperature, the rates of calcium transport were monitored by withdrawing samples 6, 15 and 20 seconds after the addition of calcium.

Calcium concentration of reaction media

The free calcium concentrations of the reaction media were controlled by the inclusion of a calcium buffering system, (10.0 mM NTA). The free calcium concentrations were calculated using an ion-complexes-in-solutions computer programme and the available stability constants (7).

RESULTS AND DISCUSSION

Initial rates of calcium transport at 10°C

The free calcium concentrations of the reaction media used in these experiments were calculated to range from 7.4×10^{-7} molar to 1.74×10^{-4} molar. For a given calcium concentration the quantities of calcium transported into the mitochondria after 6, 15, 25, 35 and 45 seconds were calculated. The data was then subjected to a simple linear regression analysis and the slopes of those lines with t values $\geq$ 10 were taken to represent the initial rate of calcium transport at that free calcium concentration. At all the calcium concentrations investigated, transport was found to be linear with respect to time over the 45 second sampling period.

A typical plot of initial rate of calcium transported against free calcium concentration at 10°C, pH 7.4 is shown in figure 1. At this temperature the initial rate of calcium transport is a sigmoidal function of free calcium concentration, with a mean maximal rate of 27.6 ± 1.8 n moles calcium transported/minute/mg protein (S.E.M., n = 6). The mean km calculated for the six mitochondrial preparations is 10.9 ± 0.6 (S.E.M.) μ molar calcium. The data shown in figure 1 has been replotted in the form of a Hill plot in figure 3 and the mean Hill coefficient at 10°C and pH 7.4 is 1.92 ± 0.08 (S.E.M., n = 6).

Initial rates of calcium transport at 25°C

At 25°C the rates of mitochondrial calcium transport were found to be linear over the first 20 seconds of transport at all calcium concentrations

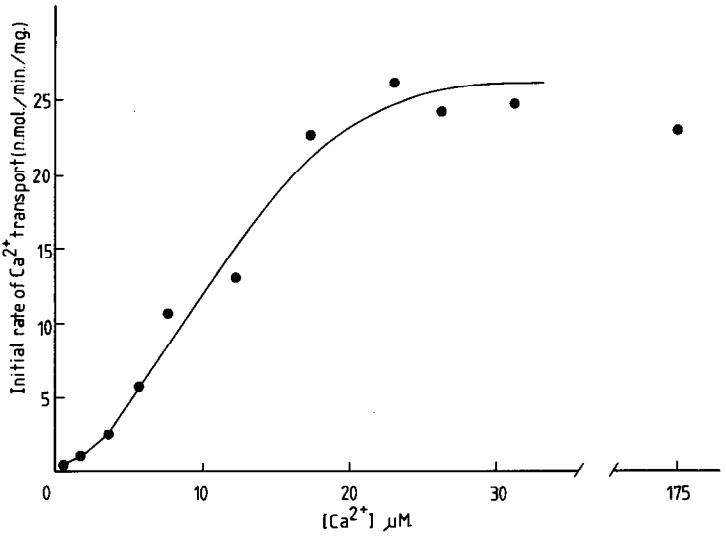

Figure 1. Typical plot of initial rate of respiration supported calcium accumulation by rabbit cardiac mitochondria against free calcium concentration at 10°C, pH 7.4.

investigated. A typical plot of initial rate of calcium transport against free calcium concentration is shown in figure 2 and the data is replotted in figure 3 in the form of a Hill plot. At 25°C the relationship between the initial rate of calcium transport and the free calcium concentration was found to be considerably less sigmoidal than at 10°C. Transport reached a mean maximal rate of 90.2 $\pm$ 8.3 n moles calcium/minute/mg protein (S.E.M., n = 4). The mean Km value is 18.5 $\pm$ 1.1 μ molar calcium and the data provides a mean Hill coefficient of 1.26 $\pm$ 0.04.

The results presented in this study suggest that temperature influences the relationship between the initial rates of calcium transport by rabbit cardiac mitochondria and calcium concentration. These findings may help to explain the apparent differences between data obtained by different groups who have determined the initial rates of mitochondrial calcium transport at different temperatures (2, 4). Recent work by Crompton et al. (4) suggests

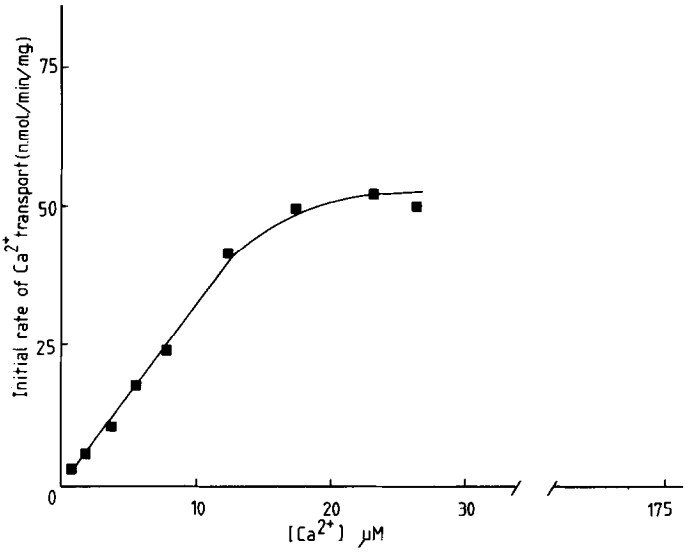

Figure 2. Typical plot of initial rate of respiration supported calcium accumulation by rabbit cardiac mitochondria against free calcium concentration at 25°C, pH 7.4.

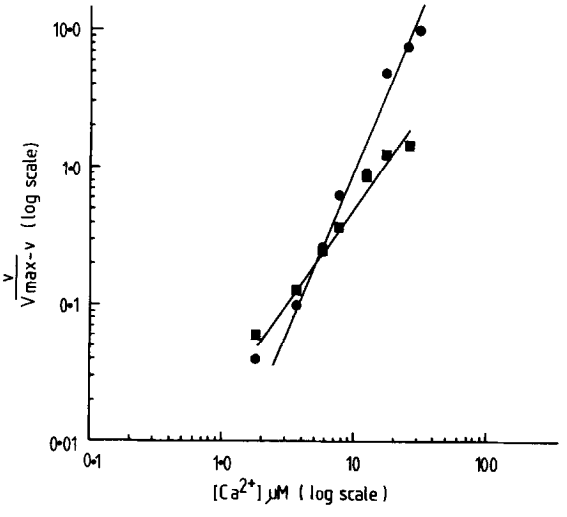

Figure 3. Hill plots of initial rates of respiration supported calcium accumulation at 10°C (●——●) and 25°C (■——■).

that the relationship between the initial rate of calcium transport by cardiac mitochondria and calcium concentration may also be altered by magnesium. The

presence of 1 mM magnesium was found to produce similar alterations to those demonstrated in this study by lowering temperature, transforming a hyperbolic to a sigmoidal relationship. These authors suggested that magnesium may influence the relationship by binding to sites on the mitochondrial inner membrane other than those responsible for calcium binding.

At 10°C the calcium transporting system of rabbit cardiac mitochondria appears to behave allosterically, showing positive cooperativity between at least two interacting binding sites (Hill coefficient approximately 1.9, fig 3). At 25°C this cooperativity is reduced, the velocity of calcium transport becoming a less sigmoidal function of calcium concentration. It would appear, therefore, that in the presence of magnesium or at low temperatures, that is under conditions in which the velocity of mitochondrial calcium transport is reduced, positive cooperativity exists between binding sites of the mitochondrial inner membrane. This cooperativity is reduced or abolished when such constraints are removed.

ACKNOWLEDGEMENT

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