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OXALATE DEPENDENCE OF CALCIUM UPTAKE KINETICS OF RABBIT SKELETAL MUSCLE MICROSOMES (FRAGMENTED SARCOPLASMIC RETICULUM)

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SUMMARY

The initial rate of oxalate-facilitated Ca^{2+} uptake by skeletal microsomes depends on both Ca^{2+} and oxalate concentrations in the medium. The apparent K_m for Ca^{2+} increases with increasing oxalate concentration, indicating that Ca^{2+} uptake can involve a carrier-mediated transport system.

INTRODUCTION

Muscle microsomes, which contain membrane fragments derived from the sarcoplasmic reticulum, can remove Ca^{2+} from solution in the presence of Mg^{2+} and ATP [1–3]. The uptake of Ca^{2+} is markedly increased when a calcium-precipitating agent such as oxalate is present in addition to Mg^{2+} and ATP. We have previously reported that oxalate-facilitated Ca^{2+} uptake differs from Ca^{2+} transport in the absence of calcium-precipitating agents in its dependence on the level of ionized Ca^{2+} in the medium. In the range of Ca^{2+} concentration between 0.1 and 3.0 μM, the steady-state level of Ca^{2+} transport in the absence of oxalate exhibits saturation kinetics, whereas the initial rate of Ca^{2+} uptake, when measured at a constant high level of oxalate (5 mM) gives no evidence of saturability at Ca^{2+} concentrations below 3 μM [4]. In these studies of Ca^{2+} uptake, experiments could not be extended to higher Ca^{2+} concentrations because of inhibitory effects seen at Ca^{2+} concentrations above 3 μM. These and similar data for cardiac microsomes [5], were interpreted to indicate the microsomal Ca^{2+} uptake could represent a non-facilitated diffusion through the membrane, and thus suggested that this translocation of Ca^{2+} did not involve a Ca^{2+} carrier.

In the present communication, we report the effect of varying oxalate concentration on the Ca^{2+} dependence of the initial rate of Ca^{2+} uptake by skeletal muscle

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microsomes. These data show that the non-saturable kinetic characteristics of Ca^{2+} uptake described in the previous paper [4] occur only at high concentrations of oxalate, and that the apparent K_m for Ca^{2+} is directly proportional to oxalate concentration. The present kinetic data are therefore consistent with the view that oxalate-facilitated Ca^{2+} uptake can be mediated by a specific Ca^{2+} carrier system.

METHODS

Microsomes prepared from rabbit white skeletal muscle as described previously [4] by a slightly modified method of Harigaya and Schwartz [6] were studied either on the day of preparation or on the following morning; there being no significant loss of activity when microsomes were kept on ice. Protein concentration was determined by the biuret method with bovine serum albumin as a standard.

Ca^{2+} uptake was measured at 35 °C in reaction mixtures containing 0.12 M KCl, 40 mM histidine pH 6.8, 5 mM MgATP, and $^{45}CaCl_2$–EGTA buffers with 10 μg microsomal protein per ml in the presence of varying concentrations of Tris–oxalate. A total $^{45}CaCl_2$ concentration of 25 μM was used in all experiments; EGTA concentration was varied [4] to achieve the desired Ca^{2+} concentration. Samples of the Ca^{2+} uptake reaction mixtures were taken at various time intervals after the addition of Ca^{2+} and filtered through Type HA (0.45 μm pore size) Millipore filters mounted in Swinny Adapters. A Micromedic automatic pipette permitted accurate delivery of 50-μl aliquots of the filtrate into a polyethylene vial along with 5 ml Bray's solution for counting in a Packard liquid scintillation spectrometer.

All reagents used were reagents grade. ATP, purchased as the disodium salt from Sigma Chemical Co., was deionized with Dowex 50W-X8 and neutralized with $MgCl_2$ and Tris. Distilled water was deionized and redistilled from glass prior to use.

All lines for kinetic plots were computed by the method of least squares with a PDP-8e computer.

RESULTS AND DISCUSSION

The rate of Ca^{2+} uptake was directly dependent on oxalate concentration at both high and low levels of Ca^{2+} (Fig. 1), in accord with previous reports [7, 8]. Analysis of the initial rates of Ca^{2+} uptake, measured over a range of both Ca^{2+} and oxalate concentrations, was carried out using double reciprocal plots of $1/v$ against $1/[Ca^{2+}]$. A typical experiment, shown in Fig. 2, demonstrates that the apparent K_m for Ca^{2+} is variable, increasing as oxalate concentration is increased. In view of evidence that, under conditions, Ca^{2+} transport by skeletal muscle sarcoplasmic reticulum may show evidence of cooperativity [9], the possibility that the points shown in Fig. 2 could be better fitted by lines other than those based on a linear fit was examined. The results from two preparations of microsomes, in which initial Ca^{2+} uptake velocities in the presence of 0.5 mM oxalate were calculated at eleven Ca^{2+} concentrations (Fig. 3) showed no evidence for upwards concavity, a finding that agrees with our earlier observations in the presence of 5 mM oxalate [4] and those of Yamamoto and Tonomura [10] who examined the Ca^{2+} dependence of ATPase activity. A Hill Plot constructed for these data in Fig. 3 had a slope of 1.02.

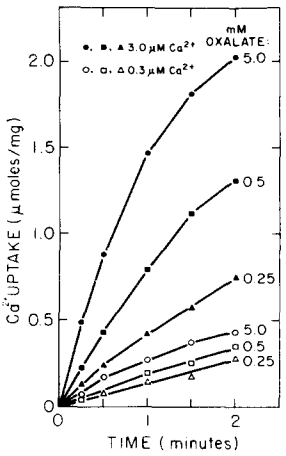

Fig. 1. Dependence of Ca^{2+} uptake on oxalate concentration. Ca^{2+} uptake was carried out as described in Methods with 9.7 μg microsomal protein per ml, 3 μM Ca^{2+} (closed symbols), 0.3 μM Ca^{2+} (open symbols), at 5 mM (●, ○), 0.5 mM (■, □) and 0.25 mM (▲, △) Tris–oxalate.

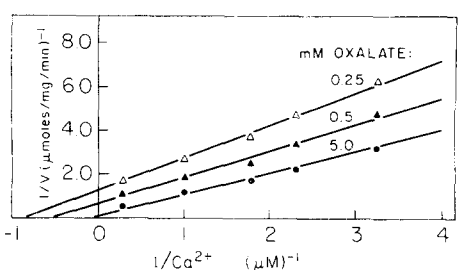

Fig. 2. Double reciprocal plots of Ca^{2+} dependence of Ca^{2+} uptake velocity. Data were derived from the rates of Ca^{2+} uptake at Ca^{2+} concentrations of 0.3 μM, 0.42 μM, 0.56 μM, 1 μM and 3 μM in 0.25 mM oxalate (△), 0.5 mM oxalate (▲) and 5.0 mM oxalate (●).

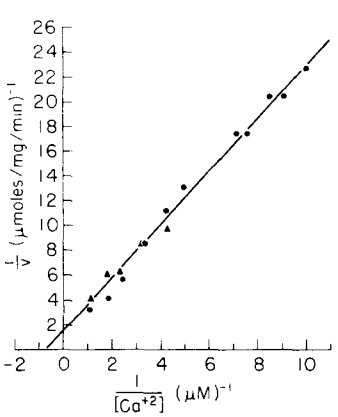

Fig. 3. Double reciprocal plot of Ca^{2+} dependence of Ca^{2+} uptake velocity in 0.5 mM oxalate. Reactions were carried out as described in Methods with two different microsome preparations (○, ●) at a protein concentration of 20 μg per ml.

If a carrier-mediated transport process is involved in Ca^{2+} uptake this latter process may be described by the following reactions:

$$Ca^{2+}{}_0 + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} CaE \tag{1}$$

$$CaE \underset{k_4}{\overset{k_3}{\rightleftharpoons}} CaE' \tag{2}$$

$$CaE' + Ox^{2-} \xrightarrow{k_5} CaOx + E' \tag{3}$$

where E is the concentration of a membrane carrier for $Ca^{2+}{}_0$ on the outside of the microsomal vesicle and CaE is the concentration of the Ca^{2+} carrier complex at the membrane. Eqn 2 describes the translocation of the Ca^{2+} carrier complex at the outside of the membrane (CaE) to the inside of the membrane (CaE'). In Eqn 3, Ox^{2-} is the concentration of oxalate inside the vesicles (which is the same as the outside oxalate concentration), CaOx is the calcium oxalate precipitate within the vesicle and E' is the modified carrier that is released at the interior of the microsomal vesicle. It is assumed that during measurement of the initial rate of Ca^{2+} uptake, the reverse reaction of Eqn 3 is negligible. This formulation, which indicates that oxalate reacts with Ca^{2+} that is bound to the membrane rather than with free Ca^{2+} released within the microsomal vesicle, follows from the nonsaturable kinetics of oxalate supported Ca^{2+} uptake at high oxalated concentration (refs 7 and 8, Fig. 2). Similar observations regarding the role of acceptor substances have been made in a classical study of the interaction between various oxygen acceptors and the peroxidase–H_2O_2 complex [11].

Given that the velocity constant for the precipitation of large quantities of Ca^{2+} within the microsomal vesicles as calcium oxalate is k_5, the measured Ca^{2+} uptake velocity, v, would be equal to k_5 [CaE'] [Ox^{2-}]. If the concentrations of CaE and CaE' remain at a steady state during measurement of initial Ca^{2+} uptake velocity, a rate equation can be derived to describe the Ca^{2+} uptake process in the presence of oxalate:

$$\frac{E_t}{v} = \frac{1}{k_3} + \frac{(k_3 + k_4)}{k_3 k_4} \frac{1}{(Ox^{2-})} + \frac{(k_2 + k_3)}{k_1 k_3} \frac{1}{(Ca^{2+}{}_0)} + \frac{k_2 k_4}{k_1 k_3 k_5 (Ca^{2+}{}_0)(Ox^{2-})} \tag{4}$$

where v is the rate of Ca^{2+} uptake and E_T is the total membrane carrier ($E + CaE + CaE'$) available to Ca^{2+} at the outside of the membrane vesicle. This formulation predicts that the rate of Ca^{2+} uptake will depend not only on Ca^{2+} concentration but also on the concentration of oxalate. If $k_3 \gg 1$ and if the rate constants for the reverse reactions, k_2 and k_4, are much smaller than k_3, the term $1/k_3$ becomes very small and thus negligible and the other terms in Eqn 4 can be rewritten:

$$\frac{E_t}{v} = \frac{1}{k_5(Ox^{2-})} + \frac{1}{k_1(Ca^{2+})} + \frac{k_2 k_4}{k_1 k_3 k_5 (Ca^{2+})(Ox^{2-})} \tag{5}$$

When the initial rate of Ca^{2+} uptake is measured under conditions in which high concentrations of oxalate are maintained (e.g. 5 mM) while Ca^{2+} is varied at low concentrations, (e.g. 0.3–3 μM), the terms containing oxalate in the denominator of Eqn 5 will become small and hence negligible as a factor in the initial rate equation.

Under conditions where oxalate concentration is high, therefore, Eqn 5 can be reduced to:

$$v = k_1(E_t)(Ca^{2+}) \quad (6)$$

Eqn 6 indicates that the rate of Ca^{2+} uptake at high oxalate and low Ca^{2+} concentrations can be a linear function of Ca^{2+} concentration even when a saturable carrier is involved, so that the apparent non-saturable nature of kinetic data obtained in our previous studies of Ca^{2+} uptake by skeletal [4] and cardiac [5] microsomes does exclude the participation of a carrier-mediated transport mechanism.

From Eqn 5, one would predict that when the concentrations of Ca^{2+} and oxalate are varied, direct plots of the initial rate of Ca-uptake versus Ca^{2+} concentration at various oxalate concentration would give rectangular hyperbolic curves at low concentrations of oxalate and straight lines when oxalate concentration becomes very high. In other words, the apparent K_m for Ca^{2+} in the Ca^{2+} uptake process should increase with increasing oxalate concentration. As shown in Fig. 2, this prediction is borne out from experiments in which Ca^{2+} concentration is varied in the range between 0.3 and 3 μM and oxalate concentration is varied in the range between 0.25 mM and 5 mM. Double reciprocal plots of the Ca^{2+} dependence of Ca^{2+} uptake at varying oxalate concentrations give values for the apparent K_m of Ca^{2+} of 1.2, 1.8 and 13.3 μM at oxalate concentrations of 0.25, 0.5 and 5 mM, respectively, as predicted by Eqn 5 (Fig. 2). Thus, the kinetic characteristics of Ca^{2+} uptake by skeletal microsomes in the presence of oxalate can be reconciled with a carrier-mediated mechanism as described in Eqns 1–3 in which oxalate reacts with a Ca^{2+} carrier complex to precipitate the Ca^{2+} as calcium oxalate inside the membrane vesicle.

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