Respiration-independent Binding of Sr\(^{2+}\) to Bean Mitochondria

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ABSTRACT

Binding of Sr\(^{2+}\) to bean mitochondria (Phaseolus vulgaris) shows a dissociation constant of 25 \( \times \) 10\(^{-4}\) and results in 40 to 50 nmoles of Sr\(^{2+}\) bound per mg protein. The binding is partially inhibited by valinomycin plus K\(^+\), 2,4-dinitrophenol, as well as ruthenium red at a level of the 120 nmoles per mg protein. These compounds also partially inhibit active uptake of Sr\(^{2+}\). Calcium and Mg\(^{2+}\) also partially inhibit binding in the same magnitude as previously reported for inhibition of transport. Phosphate which is required for divalent cation transport is without effect on the binding of Sr\(^{2+}\). The possible role of the observed binding sites in divalent cation transport is discussed.

Mitochondria isolated from both plant and animal sources are capable of actively transporting divalent cations (1, 5, 9, 10). The accumulation of Ca\(^{2+}\) in rat liver mitochondria results in a transient stimulation of respiration accompanied by the ejection of H\(^+\) (9, 10, 12). Recently, Carafoli and Lehninger (2) have shown that the mitochondria from a number of vertebrate animal tissues have a high affinity respiration-independent binding site for Ca\(^{2+}\) which has been implicated as associated with the Ca\(^{2+}\) transport process.

In comparison with animal mitochondria, active transport of Ca\(^{2+}\) by corn shoot mitochondria (5) and of Sr\(^{2+}\) by bean hypocotyl mitochondria (7) lacks a stimulation of respiration and ejection of H\(^+\) ions (7) and has an absolute requirement for Pi. Since no studies have been reported on the binding of divalent cations to higher plant mitochondria, this study examines bean mitochondria for the presence of respiration-independent Sr\(^{2+}\) binding sites. In addition, the effect of a number of compounds on Sr\(^{2+}\) binding has been observed including ruthenium red, a specific inhibitor of Ca\(^{2+}\) transport and binding in rat liver mitochondria (11, 17).

MATERIALS AND METHODS

Mitochondria were isolated from bean hypocotyl (Phaseolus vulgaris cv. Kentucky Wonder Pole) by differential centrifugation as previously described (7) in a medium containing: 0.4 M mannitol, 1 mg/ml bovine serum albumin 50 mM Tricine buffer (pH 7.5), and 2.0 mM Na-EDTA. The mitochondria were washed in a medium free of bovine serum albumin and Na-EDTA during the final centrifugation. Respiration-independent binding of Sr\(^{2+}\) was measured in a reaction mixture of 3.2 ml containing: 0.4 M mannitol, 50 mM Tricine buffer (pH 7.5), 5 \( \mu \)M rotenone, 2.4 \( \mu \)M antimycin A, and concentrations of Sr\(^{2+}\), as indicated in the legends. Under these conditions no respiration could be detected. After incubation for 90 sec, the mitochondria were harvested by centrifugation in the cold at 27,000g for 5 min, and aliquots of the supernatant were analyzed for Sr\(^{2+}\) by atomic absorption spectroscopy. The procedure for determining the active uptake of Sr\(^{2+}\) involved comparing the net uptake in the presence and absence of substrate, as has previously been described (7).

RESULTS

The binding of small molecules to macromolecules can formally be described by equations analogous to those used to describe enzyme-substrate interactions (4). In plotting binding data Edsall and Wyman (4) have discussed the advantages of a Scatchard plot which formally coincides with the Eadie plot used in enzyme kinetics (3, 16) and is based on the equation:

\[
\frac{P}{(A)} = K(n - \bar{V})
\]

Where \( \bar{V} \) is the probability that a macromolecule chosen at random from a solution will have a molecule \( A \) attached to it, and \( K \) is the association constant for the interaction of the macromolecule \( P \) and \( A \) as follows:

\[
P + A \rightarrow PA
\]

\[
K = \frac{[PA]}{[P][A]}
\]

The symbol \( n \) is the number of binding sites at each macromolecule (4). In a plot of \( P/A \) against \( V \) when \( P/A = 0 \) then \( n = \bar{V} \), and the extrapolated intercept on the abscissa gives \( n \), the maximum number of groups associated with binding. When \( P = 0 \) the intercept on the ordinate gives \( Kn \) from which \( K \) may be derived. The plot is particularly useful when more than one class of binding site may be involved (4).

Respiration-independent Sr\(^{2+}\) binding to isolated bean hypocotyl mitochondria is summarized in the form of a Scatchard plot in Figure 1. While a biphasic curve for Sr\(^{2+}\) binding is observed, the binding sites do not correspond to the high and low affinity binding sites reported for rat liver and other vertebrate mitochondria (13). The higher affinity binding site in Figure 1 has a dissociation constant of 25 \( \times \) 10\(^{-4}\) and binds...
**DISCUSSION**

Bean mitochondria lack a high affinity site for binding Sr²⁺ equivalent to that of vertebrate mitochondria (2, 13). The lack of a high affinity binding site in bean mitochondria leaves open the possible involvement of the binding site in Figure 1 at low Sr²⁺ concentrations in active transport. Support for this possibility comes from the similarity in the dissociation constant for Sr²⁺ binding which is 25 µM and the Km for Sr²⁺ transport which is 30 µM under similar conditions (6). Further, the partial inhibition of both Sr²⁺ binding and transport by DNP and valinomycin plus K⁺ may reflect a similarity in both binding and transport sites, although with these compounds as well as with ruthenium red the inhibition of active transport was greater than the inhibition of binding (6). The reason for the greater inhibition of transport compared with binding with these compounds is not clear but may be related to effects on the energy requirement for transport which is not reflected in the binding. Finally, the similarity in the order and magnitude of inhibition of Sr²⁺ transport and binding by Ca²⁺ and Mg²⁺ is consistent with the involvement of the binding sites in transport. Thus, it is appealing to suggest that the binding sites seen at low Sr²⁺ levels may be involved in the divalent cation and Pi transport system.

It is of interest that Pi which is required for divalent cation transport in bean mitochondria does not appear to influence the binding of Sr²⁺. Plant mitochondria show no detectable H⁺ movement during divalent cation and Pi uptake, suggesting that transport occurs independently of the need for the opposing movement of H⁺ as either a driving force or as a means of maintaining charge balance across the inner membrane. There may be a specific carrier containing independent sites.

**Table II. Effect of Ruthenium Red on the Binding and Uptake of Sr²⁺**

<table>
<thead>
<tr>
<th>Ruthenium Red</th>
<th>Sr²⁺ Added</th>
<th>Sr²⁺ Bound</th>
<th>Inhibition</th>
<th>Sr²⁺ Uptake</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmoles/mg protein</td>
<td>%</td>
<td>nmoles/mg protein</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>96</td>
<td>25</td>
<td>306</td>
<td>125</td>
<td>96</td>
</tr>
<tr>
<td>250</td>
<td>96</td>
<td>17</td>
<td>32</td>
<td>84</td>
<td>73</td>
</tr>
</tbody>
</table>

**Fig. 1.** Scatchard plot of Sr²⁺ binding to bean mitochondria. The conditions are given under "Materials and Methods." Total protein was 0.9 mg. The concentration of DNP was 100 µM.

Table I. Effect of a Number of Additives on Respiration-independent Binding

<table>
<thead>
<tr>
<th>Additive</th>
<th>Sr²⁺ Bound</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoles/mg protein</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>20.1</td>
<td>49</td>
</tr>
<tr>
<td>2 µg Valinomycin plus 2 mm K⁺</td>
<td>10.2</td>
<td>49</td>
</tr>
<tr>
<td>0.1 mm DNP</td>
<td>13.4</td>
<td>32</td>
</tr>
<tr>
<td>50 µM CaCl₂</td>
<td>12.7</td>
<td>37</td>
</tr>
<tr>
<td>50 µM MgCl₂</td>
<td>16.3</td>
<td>19</td>
</tr>
<tr>
<td>100 µM K₂PO₄</td>
<td>19.8</td>
<td>1</td>
</tr>
</tbody>
</table>

between 40 to 50 nmoles Sr²⁺ per mg protein, which is within the range reported for the low affinity binding site of mitochondria from several animal sources (2). For example, the low affinity binding sites in rat liver mitochondria have a dissociation constant between 38 to 52 × 10⁻⁴ and bind between 20 to 28 nmoles/mg protein. The results with bean mitochondria show the lack of a high affinity binding site with a dissociation constant within the range of approximately 1 × 10⁻⁴ found with mitochondria isolated from tissues of a number of vertebrate animals (2, 13). In this respect, bean mitochondria resemble yeast, Neurospora, and blowfly muscle mitochondria which also lack a high affinity binding site. The binding at still higher Sr²⁺ concentrations in bean mitochondria may reflect nonspecific binding at various sites.

In bean mitochondria DNP inhibits Sr²⁺ transport approximately 85 to 90%, as well as promoting the discharge of previously accumulated Sr²⁺ (7, 8). However, DNP diminishes only partially the Sr²⁺ binding at all concentrations (Fig. 1), in contrast with rat liver mitochondria where it completely inhibits binding at the high affinity site (13).

Table I summarizes the effect of a series of compounds on the binding of Sr²⁺ in the concentration range of the higher affinity binding site in bean mitochondria. Valinomycin in the presence of KCl resulted in the greatest decrease with a nearly 50% reduction in binding. Of the inorganic ions examined Ca²⁺ at 50 µM inhibited the Sr²⁺ binding 37%, whereas Mg²⁺ inhibited binding just under 20% (Table I). The same concentrations of Ca²⁺ inhibited Sr²⁺ transport 42%, and the same concentrations of Mg²⁺ inhibited Sr²⁺ uptake 22% (7). Phosphate, which is required for divalent cation uptake with bean mitochondria, has no effect on the respiration-independent binding of Sr²⁺.

Recent studies with rat liver mitochondria have shown that low concentrations of ruthenium red specifically inhibit both Ca²⁺ binding and transport (11, 17), probably by binding to the Ca²⁺ carrier (15). With bean mitochondria, low concentrations of ruthenium red (20 nmoles/mg protein or less) had no effect on the uptake or binding of Sr²⁺ (data not shown), but higher concentrations inhibited Sr²⁺ binding about 30% (Table II). The transport of Sr²⁺ at high concentrations of ruthenium red was also partially inhibited, but proportionally more than the binding.

*Abbreviations: DNP: 2,4-dinitrophenol.*
for binding both a divalent cation Pi, and the transport occurs as a neutral salt when both sites are loaded.

The lack of high affinity divalent cation binding sites in bean mitochondria equivalent to that observed in mitochondria from vertebrates may account for the low priority that the divalent cation and Pi transport system assumes among the energy-utilizing events of electron transport in bean mitochondria, as well as the lack of a divalent cation stimulation of respiration (7, 8). These results contrast with those for rat liver mitochondria where Ca²⁺-induced respiration is greater than the ADP-induced stimulation of respiration and thus assumes a higher priority in the hierarchy of energy-utilizing processes in mitochondria (2). Such differences in plant and vertebrate animal mitochondria may reflect basically different metabolic roles of the divalent cation transport system in these organisms.

LITERATURE CITED