CALCIUM CARRIER AND THE "HIGH AFFINITY CALCIUM BINDING SITE" IN MITOCHONDRIA

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Several different approaches are available for measuring the affinity of mitochondria for calcium and other divalent cations such as manganese and strontium.

Firstly, intrinsic indicators of calcium uptake can be used. Whereas the overall uptake of Ca^{++} by mitochondria reaches a plateau at relatively high Ca^{++} concentrations (1), the responses of cytochrome b and pyridine nucleotides are much more sensitive. In pigeon heart mitochondria in the absence of permeant anions the maximal cytochrome b response is reached at 7 nmoles of Ca^{++} per mg protein, and the maximal pyridine nucleotide response at 25 nmoles Ca^{++} per mg protein (2). In rat liver mitochondria in the presence of inorganic phosphate the responses of cytochrome b and pyridine nucleotides to an addition of 18 nmoles Ca^{++} per mg protein are clearly measurable (1).

Secondly, external Ca^{++} indicators, such as murexide (ammonium purpurate) show Ca^{++} uptake down to 5 μM (3), and when the jellyfish protein aequorin is used, the external Ca^{++} concentration can be followed down to 10^{-8} M (4).

Thirdly, a sensitive method for determining the Ca^{++} affinity of

mitochondria is afforded by the lanthanides which specifically titrate out the Ca^{++} uptake activity at remarkably low concentrations. Experimental evidence has been presented that at concentrations of about 0.07 - 0.1 nmoles lanthanides per mg protein inhibit the accumulation of Ca^{++} in rat liver mitochondria as measured by murexide (3 - 7), or as shown by the inhibition of the other mitochondrial reactions induced by the energy-dependent Ca^{++} accumulation, namely the pH changes in the membrane and the redox changes of the respiratory chain components (5 - 8).

The fourth approach to the study of the reaction of Ca^{++} with the membrane is to employ ^{45}Ca, and measure the incorporation of Ca^{++} into the membrane - a technique which fails to distinguish between binding and exchange in the membranes, a particular difficulty in rat liver mitochondria which contain as much as 5 nmoles endogenous Ca^{++} per mg of mitochondrial protein (9). In spite of this difficulty Lehninger and Carafoli (10) and Reynafarje and Lehninger (11) using this method as a tool report "high affinity Ca^{++} binding sites" in rat liver and kidney mitochondria, which sites bind in an energy-independent reaction 1.2 nmoles Ca^{++} or 1.0 nmole Mn^{++} per mg of mitochondrial protein.

Reynafarje and Lehninger (11) postulate that the "high affinity Ca^{++} binding site" is "a membrane carrier molecule or permease" which is specific for Ca^{++}, Mn^{++}, and Sr^{++} in spite of the fact that their experimental method does not give evidence for any functional activity of the "high affinity Ca^{++} binding site" in energy-dependent Ca^{++} accumulation. Our results on Ca^{++} uptake differ: the data on the lanthanide inhibition of the Ca^{++} accumulation show that only less than 0.1 nmole of lanthanides per mg protein is needed to inhibit the Ca^{++} accumulation, and thus clearly indicate the active function of the lanthanide sensitive site. We have postulated that this site is a specific divalent cation carrier in the mitochondrial membrane (4 - 7). It has been shown that this site is active in Mn^{++} as

well as Ca^{++} accumulation (6 - 8). However, this site is activated more readily by Ca^{++} than by Mn^{++} ions. In the presence of a low concentration of Ca^{++}, Mn^{++} uptake is considerably accelerated (3, 12), which finding also has been reported by Ernster and Nordenbrand (13).

A second difference lies in the biological distribution of "the high affinity Ca^{++} binding site" and the active Ca^{++} carrier. Lehninger and Carafoli (10) have found the site only in rat liver and kidney, but not in heart mitochondria. We have tested rat liver and kidney and pigeon heart mitochondria and found that the lanthanide sensitive Ca^{++} carrier exists in mitochondria from all of these tissues. The data showing the inhibition of the energy-dependent Ca^{++} accumulation in liver, kidney and heart mitochondria by the lanthanides holmium (Ho^{+++}) and praseodymium (Pr^{+++}) are presented in Figure 1.

These data indicate that the lanthanide sensitive site, which is functionally active as a cation carrier, exists as well in heart as in liver and kidney mitochondria at concentration levels lower than the

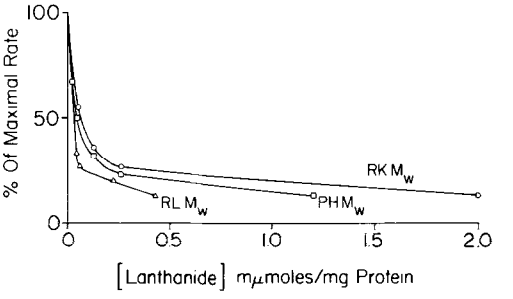

Figure 1. Inhibition of the initial rate of Ca^{++} uptake by lanthanides. Extramitochondrial Ca^{++} was measured by murexide at 540-510 nm in a dual wavelength spectrophotometer. Mitochondria at a concentration of 3.0 mg per ml were suspended in medium consisting of 0.225 M mannitol, 0.075 M sucrose, 20 mM Tris-Cl, pH 7.4, 5 mM succinate and 30 μM murexide. In case of rat liver mitochondria (RLM_w) 380 μM Ca^{++} was added, and praseodymium (Pr^{+++}) was used as inhibitor; in pigeon heart mitochondria (PHM_w) 155 μM Ca^{+++} was added, and holmium (Ho^{+++}) was used as inhibitor; in rat kidney mitochondria (RKM_w) 230 μM Ca^{+++} was added, and Ho^{+++} was used as inhibitor. The initial rate of Ca^{++} accumulation in the presence of lanthanides as percentage of the control rate (-lanthanides) is plotted against the lanthanide concentration.

cytochrome c content of these mitochondria. ^{45}Ca method seems to have failed to detect the binding of Ca^{++} to its carrier at less than 0.1 nmole per mg protein in liver and kidney mitochondria, and has failed to detect it altogether in heart mitochondria. Thus, the work of Lehninger and co-workers does not seem to provide evidence for their suggestion that the "high affinity Ca^{++} binding site" would be a specific membrane carrier, similar to the lanthanide sensitive, functionally active divalent cation carrier in the mitochondrial membrane.

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