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ANION EFFLUX AND CALCIUM TRANSPORT

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## DIDS INHIBITION OF SARCOPLASMIC RETICULUM ANION EFFLUX AND CALCIUM TRANSPORT

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Sarcoplasmic reticulum vesicles have been shown to take up large amounts of calcium when the incubation medium is supplemented with calcium-precipitating anions such as oxalate or phosphate.<sup>1</sup> Calcium is taken up in approximately stoichiometric amounts with oxalate or phosphate, although calcium uptake always exceeds anion uptake by a small amount.<sup>1</sup> The passive anion permeability of the sarcoplasmic reticulum is very high,<sup>2</sup> but the mechanism and the pathway of anion transport is not known.

The disulfonic stilbenes are potent and specific inhibitors of anion transport in red blood cells and several other cellular systems, but they have little or no effect on cation permeability.<sup>3</sup> 4,4'-Diisothiocyano-2,2'-stilbene disulfonic acid (DIDS), an irreversible inhibitor of anion transport in red blood cells, has been used to identify Band 3 as the anion transport protein.<sup>3</sup> Recently, Kasai and Kometani<sup>4</sup> have used light scattering to show that 4-acetoamido-4'-isothiocyano-stilbene-2,2'-disulfonate (SITS) inhibits anion permeability in sarcoplasmic reticulum vesicles.

DIDS was found to inhibit [<sup>32</sup>P]phosphate and [<sup>14</sup>C]oxalate efflux from isolated sarcoplasmic reticulum vesicles with no effect on calcium efflux (FIGURE 1). Half-maximal inhibition of phosphate efflux occurred at  $\approx 3 \mu\text{M}$  DIDS. Complete inhibition of phosphate or oxalate efflux required approximately  $50 \mu\text{M}$  DIDS.

DIDS was also found to be a potent inhibitor of calcium transport in sarcoplasmic reticulum vesicles (FIGURE 2). Half-maximal inhibition of  $\text{Ca}^{2+}$  transport in the presence of oxalate or phosphate occurred at  $\approx 4 \mu\text{M}$  DIDS and  $\text{Ca}^{2+}$  transport was completely inhibited by  $\approx 12 \mu\text{M}$  DIDS. Inorganic phosphate competed with DIDS and reduced the inhibition of  $\text{Ca}^{2+}$  transport due to low concentrations of DIDS ( $\approx 2 \mu\text{M}$ ). Comparison of DIDS inhibition of  $\text{Ca}^{2+}$  transport in the presence of various anions showed that DIDS inhibition of  $\text{Ca}^{2+}$  transport was dependent on the permeability of the anion in the medium. At  $4 \mu\text{M}$  DIDS (where phosphate and oxalate efflux were inhibited by 50%),  $\text{Ca}^{2+}$  transport in the presence of oxalate or phosphate was inhibited by  $\approx 50\%$ ; in the presence of  $\text{Cl}^-$ , an anion of high permeability, DIDS inhibited  $\text{Ca}^{2+}$  transport by  $\approx 15\%$ ; and in the presence of  $\text{SCN}^-$ , a lipid-permeable anion, DIDS only inhibited by 5%. This implies that DIDS inhibition of  $\text{Ca}^{2+}$  transport is through its inhibition of anion transport. DIDS also inhibited  $\text{Ca}^{2+}$  uptake by 50% at a concentration of  $12 \mu\text{M}$  in vesicles reconstituted with purified  $\text{Ca}^{2+}$ -ATPase. Inhibition occurred in spite of high internal oxalate so that anion transport was not essential for  $\text{Ca}^{2+}$  precipitation within the vesicles. At pH 6.8, DIDS inhibition of  $\text{Ca}^{2+}$  transport was independent of the time of preincubation of the vesicles with DIDS and could be reversed by the addition

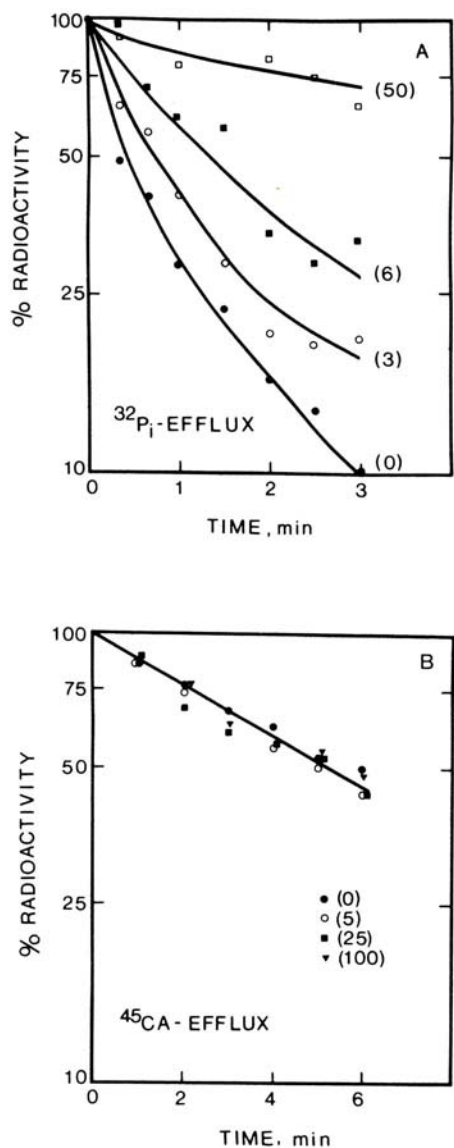


FIGURE 1. Effect of DIDS on ion efflux from sarcoplasmic reticulum vesicles. Vesicles were equilibrated for 40 hr at 0°C with (A) 1 mM  $^{32}\text{P}_i$  or (B) 0.5 mM  $^{45}\text{Ca}$  in the presence of 100 mM KCl, 5 mM  $\text{MgCl}_2$ , 20 mM histidine (pH 6.8), 0.5 mM  $\text{CaCl}_2$ , and 0.5 mM EGTA [ethylene glycol bis ( $\beta$ -aminoethylether)- $N,N,N',N'$ -tetraacetic acid]. They were then diluted 400-fold into a medium of identical composition but containing no radioisotopes. Exchange rates were determined at 23 °C by Millipore filtration. Percent radioactivity remaining in the vesicles was plotted against time for various concentrations of DIDS in the dilution medium. DIDS concentration ( $\mu\text{M}$ ) is indicated in parenthesis.

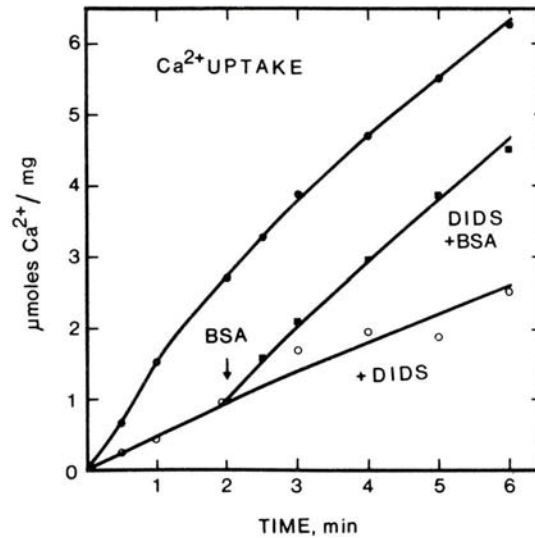


FIGURE 2. Reversible inhibition of sarcoplasmic reticulum calcium transport by DIDS as pH 6.8.  $\text{Ca}^{2+}$  uptake was measured at 20 Mg/ml in the presence of 100 mM KCl, 5 mM  $\text{MgCl}_2$ , 20 mM histidine (pH 6.8), 50 mM  $\text{PO}_4^{2-}$ , 0.5 mM  $^{45}\text{CaCl}_2$ , 0.5 mM EGTA, and 5 mM ATP, using Millipore filtration. DIDS (6  $\mu\text{M}$ ) was added to the reaction medium just prior to the addition of sarcoplasmic reticulum vesicles. DIDS inhibition of calcium uptake was reversed by the addition of 5 mg/ml BSA (fatty-acid-free) at 1.5 min.

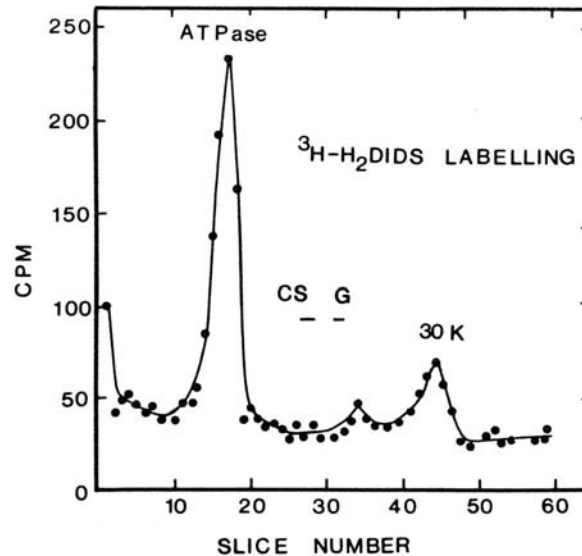


FIGURE 3.  $^3\text{H}$ - $\text{H}_2\text{DIDS}$  labeling of sarcoplasmic reticulum vesicles. Vesicles were labeled with 10  $\mu\text{M}$   $^3\text{H}$ - $\text{H}_2\text{DIDS}$  at pH 8.0 for 20 min at 37°C. They were subsequently washed three times with BSA and proteins were separated by SDS gel electrophoresis.  $^3\text{H}$ - $\text{H}_2\text{DIDS}$ -labeled proteins were identified following gel slicing and counting. ATPase, CS (calsequestrin), G (intrinsic glyeoprotein), and the 30 K region are indicated. The rate of  $\text{Ca}^{2+}$  uptake in the  $^3\text{H}$ - $\text{H}_2\text{DIDS}$ -labeled vesicles was inhibited by 35% when compared to control vesicles.

of fatty-acid-free BSA (5 mg/ml) (FIGURE 2). At pH 8.0, DIDS bound irreversibly to the vesicles and inhibited  $\text{Ca}^{2+}$  transport. Upon analysis of [ $^3\text{H}$ ]H<sub>2</sub>-DIDS-labeled vesicles by SDS gel electrophoresis, radioactivity was found to be associated predominantly with the  $\text{Ca}^{2+}$ -ATPase (FIGURE 3).

DIDS inhibition of calcium transport in sarcoplasmic reticulum vesicles could occur through inhibition of the active site of the  $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase or through inhibition of an anion transport component in the sarcoplasmic reticulum. Our results indicate that DIDS inhibits the anion permeability of sarcoplasmic reticulum vesicles at very low concentrations (2–6  $\mu\text{M}$ ) and that this inhibition is probably responsible for inhibition of calcium uptake when phosphate or oxalate are required for calcium transport. At higher concentrations of DIDS (8–12  $\mu\text{M}$ ) calcium transport is also inhibited when anion transport is not necessarily involved in calcium transport. DIDS could be acting on the ATPase active site in this concentration range or the ATPase might be tightly coupled to anion transport, so that inhibition of anion transport inhibits the  $\text{Ca}^{2+}$  transport indirectly.

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