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THE INFLUENCE OF SODIUM TRICHLOROACETATE ON THE TRYPTOPHAN FLUORESCENCE OF SARCOPLASMIC RETICULUM ATPase

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The chaotropic anion trichloroacetate quenches the tryptophan fluorescence of the sarcoplasmic reticulum calcium transport ATPase. Half-maximum quenching was observed at 50 mM trichloroacetate. In contrast to native preparations, in trichloroacetate-treated sarcoplasmic reticulum vesicles a decrease of the tryptophan fluorescence is observed on addition of millimolar concentrations of calcium. It is concluded that trichloroacetate renders the tryptophan fluorescence of the ATPase sensitive to the occupancy of its low-affinity sites.

It has been shown by Hatefi and Hanstein [1] that chaotropic agents such as trichloroacetate or perchlorate affect the structure and lipophilicity of water and destabilize biological membranes. On the other hand, The and Hasselbach [2] reported that in sarcoplasmic reticulum membranes of fast skeletal muscle chaotropic anions did not solubilize either membrane proteins or membrane lipids, even at concentrations which maximally inhibit enzymatic activities (i.e., calcium transport, ATPase activity, phosphoprotein formation, calcium efflux and ATP binding). It was concluded that the interference between the binding of ATP and chaotropic anions is the mechanism of their action. Among the anions tested, trichloroacetate was shown to be the most effective. As inferred from the results of The and Hasselbach, trichloroacetate does not affect the interaction of calcium with its high-affinity binding sites. As in native vesicles, in the absence of chaotropic anions, the occupancy of the high-affinity calcium binding sites leads to an enhancement of the tryptophan fluorescence. However, in the presence of trichloroacetate, the saturation of calcium binding sites with an affinity of 10^{-3} M to 10^{-2} M results in a decrease of the tryptophan fluorescence. Thus it is possible to monitor both high- and low-affinity binding sites using tryptophan fluorescence in trichloroacetate-containing sarcoplasmic reticulum suspensions.

Sarcoplasmic reticulum vesicles were prepared according to Hasselbach and Makinose [3] as modified by De Meis and Hasselbach [4]. Protein concentration was determined by the biuret method with Kjeldahl calibrated standards. Purified ATPase preparations were prepared according to Hasselbach and Koenig [5]. Fluorescence measurements were carried out with a SLM 4800/A spectrofluorometer (SLM Instruments Inc. Urbana, IL, U.S.A.). Excitation wavelength was 285 nm $\pm$ 0.5 nm and emission was detected using a WG 320 cut-off filter (Schott, Mainz, F.R.G.). Unless otherwise stated, the vigorously stirred reaction mixture contained 50 mM 3-(*N*-morpholine)propanesulfonic acid (pH 7.0), 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.45 mM EGTA, 0.1 mg $\cdot$ ml^{-1} vesicular protein and the given amounts of sodium trichloroacetate. $CaCl_2$ and EGTA were added as indicated in the figures from a 1 M or 0.1 M stock solution, respectively. Binding of calcium to the vesicles was measured by millipore filtration technique as described elsewhere [6,7]. The solu-

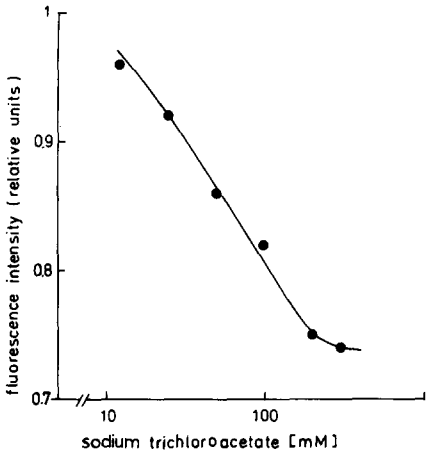

Fig. 1. Quenching of tryptophan fluorescence by trichloroacetate. The tryptophan fluorescence was measured as described, after the addition of increasing amounts of sodium trichloroacetate. Fluorescence decrease due to the absorption of sodium trichloroacetate at 285 nm was taken into account, but was significant only at concentrations higher than 0.1 M.

tions contained: 20 mM imidazole (pH 7.0), 40 mM KCl, 10 mM $MgCl_2$, 0.27 mg · ml^{-1}, 0.2 M NaCl or 0.2 M sodium trichloroacetate, respectively. All chemicals were p.a. grade and either from Serva, Heidelberg (F.R.G.), C. Roth, Karlsruhe (F.R.G.) or E. Merck, Darmstad, (F.R.G.). ^{45}Ca was from Amersham Buchler, Braunschweig (F.R.G.).

The addition of sodium trichloroacetate linearly decreases the tryptophan fluorescence emission intensity of sarcoplasmic reticulum vesicles up to a concentration of 0.2 M (Fig. 1). This quench increases considerably when the vesicles are solubilized. For instance after addition of myristoylglycerophosphocholine (2 mg/mg protein) the fluorescence response to 50 mM sodium trichloroacetate rises from 14% to 20%. The effects of trichloroacetate were also observed in purified ATPase preparations and in buffer solutions containing 25 μM tryptophan, excluding the consideration that accessory proteins or light scattering might have caused the decrease in tryptophan fluorescence. It is known that the tryptophan fluorescence intensity of the sarcoplasmic reticulum vesicles increases when the calcium concentration is raised from 10^{-7} M to 10^{-5} M [8,9]. As shown in Fig. 2 this was also observed in vesicles treated with 50 mM sodium trichloroacetate. In native vesicles, tryptophan fluorescence remains constant up to a calcium concentration of 20 mM. In contrast, in trichloroacetate-treated vesicles the tryptophan fluorescence continuously decreased when the calcium concentration was increased from 1 mM to 20 mM (Fig. 2). The half-maximal effective calcium concentration was about 5 mM, which is in agreement with the K_m values of 3–7 mM reported by Hasselbach and Koenig [5] for the low-affinity calcium binding sites of different ATPase preparations. The decrease is reversible, since after the removal of calcium by the addition

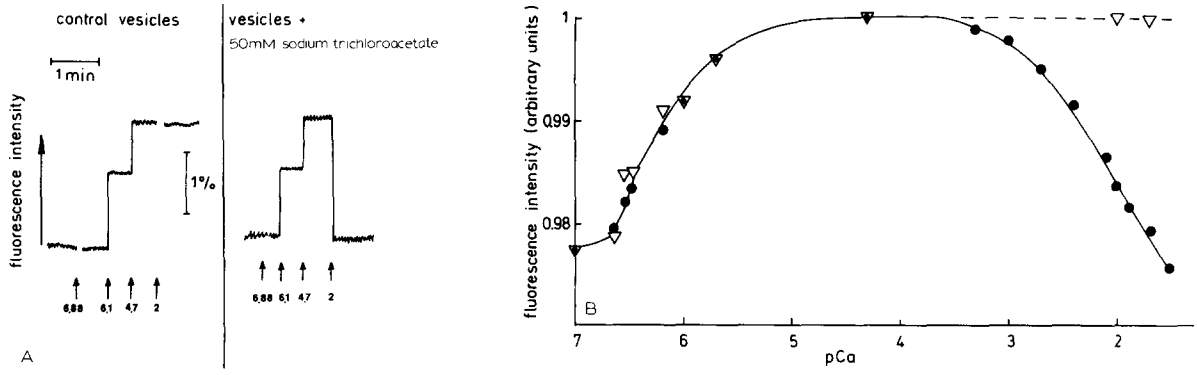

Fig. 2. Calcium-dependence of tryptophan fluorescence. A. Original traces of changes in tryptophan fluorescence after the addition of calcium to a vesicle suspension (0.1 mg · ml^{-1}) containing 1 mM EGTA. At the arrows, $CaCl_2$ from a 1 M stock solution was added to give the final calcium concentration indicated in the figure as p*Ca*. B. Increasing amounts of calcium or EGTA were added to a vesicle suspension (0.1 mg · ml^{-1}) in 50 μM free calcium containing no or 50 mM sodium trichloroacetate to give the final calcium concentration indicated on the abscissa. ▽–▽, native vesicles; ●–●, vesicles in 50 mM sodium trichloroacetate.

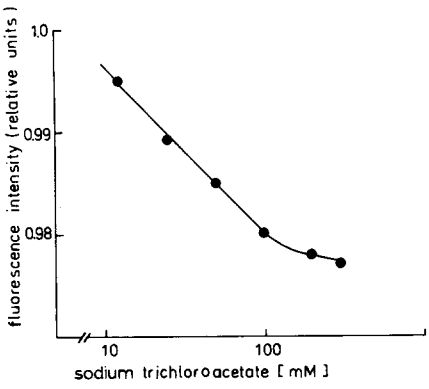

Fig. 3. Trichloroacetate-dependence of the fluorescence decrease at high calcium concentrations. 10 mM calcium was added to a vesicular suspension (0.1 mg·ml^{-1}) incubated in 50 μM free calcium and increasing amounts of Na^+-trichloroacetate. The decrease in tryptophan fluorescence was monitored.

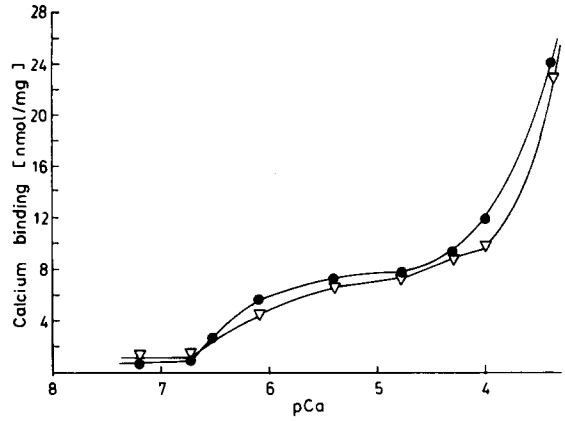

Fig. 4. Binding of calcium to sodium trichloroacetate-treated vesicles. Calcium binding was determined at increasing concentrations of calcium. ▽–▽, native vesicles in 0.2 M NaCl; ●–●, vesicles incubated in 0.2 M sodium trichloroacetate.

of citrate to a vesicular solution containing 10 mM calcium and 50 mM sodium trichloroacetate the fluorescence again increased to 100%. Fig. 3 demonstrates the sodium trichloroacetate dependence of the fluorescence increment resulting from an increase in the calcium concentration from 0.05 mM to 10 mM. Half-maximal effect was observed at 30 mM sodium trichloroacetate. To examine the relation between calcium binding and trichloroacetate binding to sarcoplasmic reticulum vesicles, the dependence of calcium binding on sodium trichloroacetate was investigated. As shown in Fig. 4, binding of calcium in the range of $5 \cdot 10^{-8}$ M to $3 \cdot 10^{-4}$ M is not affected by 0.2 M sodium trichloroacetate.

As shown by The and Hasselbach [2], trichloroacetate interferes with the ATP-binding to the ATPase protein of the sarcoplasmic reticulum. Since ATP (5 mM) did not reduce the trichloroacetate induced decrement of tryptophan fluorescence (trichloroacetate quench), there seems to be no direct competition between ATP and trichloroacetate for a single binding site. The fact that in the solubilized state of the enzyme the trichloroacetate quench is increased indicates that additional tryptophan residues became exposed. Solubilization increases the trichloroacetate quench of native vesicles as well as of ATPase preparations, suggesting that the additional quench occurs at the hydrophobic section of the enzyme. Trichloroacetate might interact with the 'non-annular' lipid binding sites described by Simmonds et al. [10]. The calcium-induced changes in the tryptophan fluorescence intensity of the ATPase protein in the absence and presence of trichloroacetate are interpreted as follows.

Fluorescence increment parallels the high-affinity calcium binding, both of which prove to be unaffected by trichloroacetate (Figs. 2 and 4; Refs. 8,9). The decrement occurring only in the presence of trichloroacetate coincides with the saturation of the low-affinity binding sites titrated in different sarcoplasmic reticulum preparations [5]. It is concluded that trichloroacetate does not affect calcium binding, but merely renders the tryptophan fluorescence emission intensity sensitive to low-affinity calcium binding.

It should be noted that the results shown in Figs. 1–4 are rarely observed with other chaotropic anions such as thiocyanate or perchlorate, and were therefore considered to be specific for trichloroacetate, which of all tested chaotropic anions exerted the strongest effects on ATPase activities [2].

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