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INHIBITION OF ANION PERMEABILITY OF SARCOPLASMIC RETICULUM VESICLES BY 4-ACETOAMIDO-4′-ISOTHIOCYANO-STILBENE-2,2′-DISULFONATE

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Summary

The permeabilities of sarcoplasmic reticulum vesicle membrane for various ions and neutral molecules were measured by following the change in light scattering intensity due to the osmotic volume change of the vesicles. 4-Acetoamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS), which is a potent inhibitor for the anion permeability of red blood cells membrane, inhibited the permeability of sarcoplasmic reticulum for anions such as Cl^-, P_i and methanesulfonate, while it slightly increased that for cations and neutral molecules such as Na^+, K^+, choline and glycerol. Binding of 5 μmol SITS/g protein was necessary for the inhibition of anion permeability. These results suggest the existence of a similar anion transport system in sarcoplasmic reticulum membrane as revealed in red blood cell membrane.

Introduction

The permeabilities of sarcoplasmic reticulum vesicles for ions such as K^+, Na^+, and Cl^- are so high that it was difficult to follow their permeation by usual tracer methods. In a previous paper [1], we measured the passive permeabilities of sarcoplasmic reticulum vesicles for various ions by following their volume change through the light scattering method. As a result, it was found that the permeability for Cl^- is about 50 times higher than that for K^+. This result suggests the existence of an anion transport system in sarcoplasmic reticulum membrane. Such a high anion permeability has been found in red blood cell membrane [2,3], and the concept of the special transport system has

Abbreviation: SITS, 4-acetoamido-4′-isothiocyanostilbene-2,2′-disulfonate.

been established from the experiments done using specific inhibitors.

In this paper, the effect of inhibitors was studied to elucidate the similarity between these two anion transport systems. It was found that the anion permeability of sarcoplasmic reticulum membrane was also inhibited by 4-acetoamido-4′-isothiocyanostilebene-2,2′-disulfonate (SITS) which is potent inhibitor of anion transport in red blood cell membrane [2,3].

Materials and Methods

Sarcoplasmic reticulum vesicles were isolated from rabbit dorsal and hind leg muscle as a microsomal fraction by the method of Weber et al. [4] with modification [5]. SITS was purchased from Nakarai Chemical Co. Japan, and phloridzin and valinomycin were from Sigma Chemical Co., U.S.A. Other reagents were commercial products of analytical grade.

Sarcoplasmic reticulum vesicles were treated with various concentrations of SITS in 0.1 M KCl, 2 mM Tris-maleate (pH 6.5) and 20 mg protein/ml for 10 min at 37° C in experiments to examine the effect of SITS, unless specified otherwise. The sarcoplasmic reticulum vesicles were then diluted 40 times with 5 mM Tris-maleate (pH 6.5).

Ionic permeabilities were determined through the osmotic volume change by the method described earlier [1]. The osmotic volume change of sarcoplasmic reticulum vesicles was monitored with scattered intensities of light (400 nm) at right angles to the incident beam using a stopped flow spectrophotometer (UNION RA-401 and RA-450) at 23° C. In most experiments, sarcoplasmic reticulum vesicles were preincubated in 2 mM KCl and 5 mM Tris-maleate (pH 6.5), 0.5 mg protein/ml for about 3 h at room temperature. This incubated suspension was mixed with an equal volume containing 200 mosmol of salts or neutral molecules in addition to 5 mM Tris-maleate buffer, and the change of the scattered intensity was followed. By this method net movement of ions or molecules can be followed. When valinomycin was used, it was added to the incubated suspension about 30 min before the mixing.

Fluorescence of SITS was determined by a fluorescence spectrophotometer (UNION FS-501, Japan).

The protein concentration was estimated by the biuret method.

Results

Effect of SITS on the permeability of ions and neutral molecules

When sarcoplasmic reticulum vesicles incubated in low salt concentration were mixed with a solution of 100 mM KCl, the scattered intensity of light changed as shown in Fig. 1. According to the previous paper [1], the initial increase in the intensity is due to the water efflux from the vesicles and the later decrease to the influx of salt ions accompanying water. The permeabilities for salt ions could be determined from the latter rate. They depended on the movement of the slower ions. The permeation rate corresponds to the permeability for K^+ in the absence of valinomycin (curves A and B), because the permeability for Cl^- is much higher than that for K^+. In the presence of valinomycin (curves C and D), the permeation rate corresponds to the Cl^- permeabil-

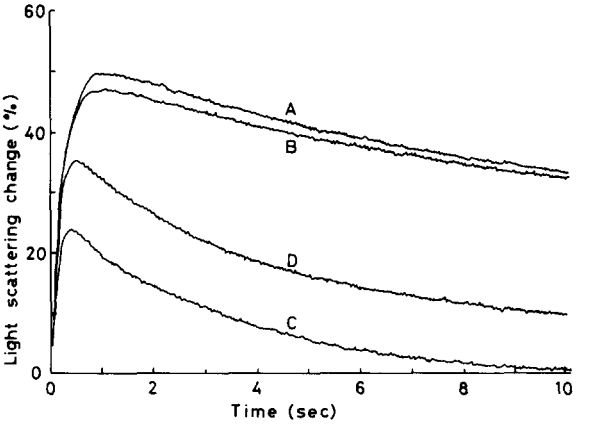


Fig. 1. Change in the light scattering intensity of sarcoplasmic reticulum vesicles caused by the volume change. Sarcoplasmic reticulum vesicles incubated in 2 mM KCl, 5 mM Tris-maleate (pH 6.5) and 0.5 mg protein/ml was mixed with an equal volume containing 100 mM KCl and 5 mM Tris-maleate using a stopped-flow apparatus. The ordinate shows the percentage change of the light scattering intensity, where the maximal change caused by a slowly penetrating solute such as glucose was taken as 100%. (A) No valinomycin and no SITS treatment; (B) no valinomycin and treated with 1 mM SITS: (C) $1 \cdot 10^{-6}$ g valinomycin/ml and no SITS treatment; (D) $1 \cdot 10^{-6}$ g valinomycin/ml and treated with 1 mM SITS.

ity because the permeability for K^+ exceeds that for Cl^-. Results suggested that two kinds of transport system exist in sarcoplasmic reticulum membrane, i.e., for cations and for anions. Sarcoplasmic reticulum vesicles were treated with SITS, which is a potent inhibitor for the anion transport system of red blood cell membrane [2,3], in order to elucidate the characteristics of these transport systems. As clearly seen in Fig. 1, SITS inhibited the permeability for Cl^- significantly (compare curves C and D), while it increased that for K^+ slightly (compare curves A and B).

Fig. 2 shows the concentration dependence of SITS on the Cl^- permeability. When the concentration of SITS at the treatment increased, the Cl^- permeabil-

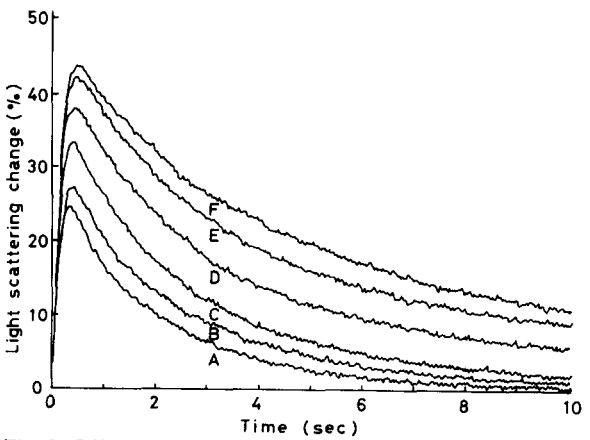
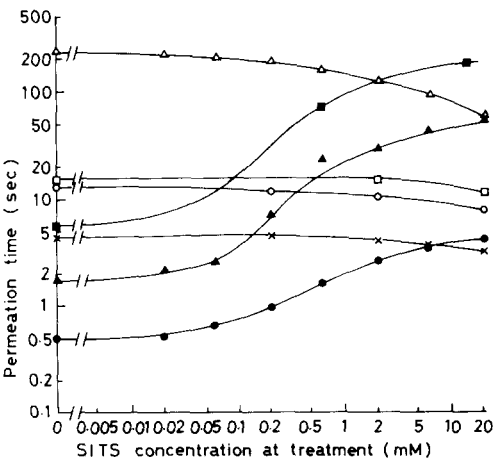


Fig. 2. Effect of SITS on the Cl^- permeability. Experiment similar to that reported in Fig. 1 in the presence of $1 \cdot 10^{-6}$ g valinomycin/ml was carried out. Concentration of SITS at the treatment was changed as follows: (A) 0, (B) 0.2 mM; (C) 0.6 mM; (D) 2 mM; (E) 6 mM; (F) 20 mM.

ity decreased. Effect of SITS on the permeability for other ions and neutral molecules was also examined by the same method. The permeation time, τ, was defined to evaluate the effect of SITS quantitatively as the time when the intensity reached a half value of the maximal change [1]. This value is considered to be inversely proportional to the permeability coefficient. In Fig. 3, the permeation times were plotted against the concentration of SITS at the treatment. The permeabilities for anions such as methanesulfonate and phosphate were also inhibited in the same concentration range of SITS as in the case of Cl^-. The permeabilities for Na^+, choline and glycerol increased slightly as observed in the case of K^+. This acceleration effect is small and might be nonspecific.

Relation between SITS binding and inhibition of anion permeability

The amount of SITS bound to sarcoplasmic reticulum vesicles was determined in order to elucidate how much SITS was needed to block the anion permeability. After the reaction with SITS, the suspensions were diluted by a buffer solution and ultracentrifuged at 150 000 $\times g$ for 30 min. Fluorescence intensity of the supernatant was measured at 450 nm, excited at 350 nm. In Fig. 4, the inhibition of Cl^- permeability was plotted against the amount of SITS bound to sarcoplasmic reticulum vesicles. From the initial gradient, binding of 5 μmol SITS/g protein was necessary to inhibit the Cl^- permeability. In higher concentration of SITS, however, no more than 80% inhibition could be achieved.



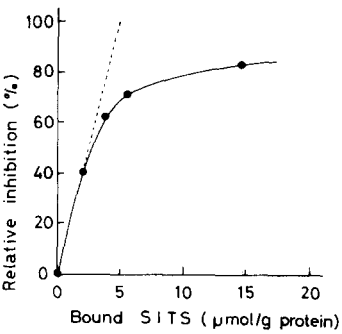


Fig. 3. Changes in the permeation times for various ions and neutral molecules as a function of the concentration of SITS in the treatment. Experiments similar to those shown in Fig. 2. were carried out using various kinds of ions and neutral molecules. The permeation times were defined as the time when the light scattering intensity reached a half of the maximal change. ●, Cl^-; ▲, methanesulfonate; ■, phosphate, ○, K^+; □, Na^+; △, choline; X, glycerol.

Fig. 4. Inhibition of the Cl^- permeability by SITS. Sarcoplasmic reticulum vesicles were treated in various concentrations of SITS. The suspensions were diluted 40 times by 5 mM Tris-maleate and aliquots were centrifuged. The amounts of SITS bound to the vesicles and the Cl^- permeability were assayed as described in the text. As a measure of the inhibition of the permeability, $(1 - \tau_0/\tau)$ was used, where τ_0 is the permeation time of Cl^- not treated with SITS and τ that treated with SITS.

As another inhibitor of the anion permeability in red blood cell [6], the effect of phloridzin was also studied. Phloridzin inhibited all permeabilities for cations, anions and neutral molecules slightly in the millimolar range. This effect might be nonspecific.

Discussion

The high anion permeability of sarcoplasmic reticulum membrane suggested the existence of an anion transport system. In this paper it was shown that this anion transport was blocked by the same inhibitor of the anion transport in red blood cell membrane. In higher concentration of SITS, however, no more than 80% inhibition could be achieved. In this paper, we measured the net movement of salt ions and not the exchange of one anion against another. This may be the reason why more inhibition could not be achieved. In the case of red blood cell, it was also reported that SITS is less effective for the inhibition of the net movement of Cl^- than that of the exchange [3]. The amount of SITS necessary to inhibit the anion transport was estimated to be about 5 μmol/g protein from Fig. 4. This value coincides with the number of the active site of the calcium pump protein, since its molecular weight is 100 000 and its content is about 50% in our preparation [7]. As a preliminary experiment, sarcoplasmic reticulum vesicles were studied by the electrophoresis in the presence of sodium dodecyl sulfate after the SITS treatment. Localization of SITS was assayed by fluorescence measurement. A large peak appeared at the position of the pump protein. At present, however, the molecular characteristics of this site are not clear. In the case of the red blood cell the site is connected with the band 3 protein and the molecular weight is about 100 000. Moreover, the physiological meaning of the anion transport system of red blood cell is clear [2], but that of sarcoplasmic reticulum is unknown. It is probable that this site plays some role in the 'depolarization'-induced, or Cl^--induced, Ca^{2+} release mechanism in sarcoplasmic reticulum [5,7—9].

Acknowledgements

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References

1 Kometani, T. and Kasai, M. (1978) J. Membrane Biol. 41, 295—308
2 Cabantchik, Z.I., Knauf, P.A. and Rothstein, A. (1978) Biochim. Biophys. Acta 515, 239—302
3 Knauf, P.A., Fuhrmann, G.F., Rothstein, S. and Rothstein, A. (1977) J. Gen. Physiol. 69, 363—386
4 Weber, A., Herz, R. and Reiss, I. (1966) Biochem. Z. 345, 329—369
5 Kasai, M. and Miyamoto, H. (1976) J. Biochem. (Tokyo) 79, 1053—1066
6 Kaplan, J.H. and Passow, H. (1974) J. Membrane Biol. 19, 179—194
7 Miyamoto, H. and Kasai, M. (1979) J. Biochem. (Tokyo) 85, 765—773
8 Kasai, M. and Miyamoto, H. (1976) J. Biochem. (Tokyo) 79, 1067—1076
9 Stephenson, E.W. (1978) J. Gen. Physiol. 71, 411—430