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INTERACTION OF CHEMICAL PROBES WITH SARCOPLASMIC RETICULUM MEMBRANES

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Summary

The labelling of the sarcoplasmic reticulum membranes by the chemical probes, trinitrobenzenesulfonate (TNBS) and fluorodinitrobenzene (FDNB) has been investigated. The incorporation of TNBS, but not of FDNB, depends on the binding of Ca^{2+} or Mg^{2+} to the membranes. The labelling of lipids and of the various reticulum proteins by TNBS is increased by those agents, but the effect is not uniform for all membrane proteins. The Ca^{2+}-ATPase contributes only 2.2% for the total labelling of the sarcoplasmic reticulum proteins, whereas the proteins of molecular weight 90 000 and 30 000 contribute about 34 and 56%, respectively. However, the Ca^{2+}-ATPase isolated from the membrane reacts with an amount of TNBS 5-fold higher than that which reacts with the enzyme in situ.

Both probes, TNBS and FDNB, inhibit the Ca^{2+}-ATPase activity and the Ca^{2+} uptake by sarcoplasmic reticulum, whereas the Mg^{2+}-ATPase remains unaffected.

The results indicate that FDNB is maximally incorporated into the sarcoplasmic reticulum membrane, whereas only some of the membrane amino groups are accessible to TNBS in the absence of Ca^{2+}, Mg^{2+} or ATP which, when present, make additional amino groups available to TNBS. The highest degree of TNBS incorporation takes place into proteins, other than the ATPase, but sufficient reaction occurs with the enzyme to inhibit its activity.

Abbreviations: FDNB, 1-fluoro-2,4-dinitrobenzene; TNBS, 2,4,6-trinitrobenzenesulfonate.

Introduction

Trinitrobenzenesulfonate and fluorodinitrobenzene, which react with primary amino groups of lipids and proteins [1—4] have been utilized as chemical probes to label amino compounds in biological membranes [5—8].

Earlier studies [9,10] on the mechanism of the active Ca^{2+} transport by sarcoplasmic reticulum showed that Ca^{2+}, Mg^{2+} and ATP increase the TNBS incorporation by the membranes, and it was assumed that the Ca^{2+}-ATPase was responsible for the TNBS binding. These results were interpreted in favour of rotatory movements of the ATPase molecule during Ca^{2+} transport [9,10].

On the other hand, exhaustive studies by Godin et al. [11—13] suggested that inorganic cations increase the incorporation of TNBS into erythrocyte membranes probably by inducing structural perturbations which alter the accessibility and/or the reactivity of membrane protein and phospholipid amino groups. In another work (Vale, M.G.P. (1980), unpublished data), we also found that several types of cations increase the TNBS incorporation by sarcoplasmic reticulum and that this effect depends on the amount of cations bound to the membrane.

In this work we studied which components of the reticulum membrane show increased trinitrophenylation in the presence of Ca^{2+}, Mg^{2+} or ATP and we investigated to what extent the interaction with the probe affects the activity of the sarcoplasmic reticulum-Ca^{2+} pump. We also studied the interaction of the reticulum vesicles with the probe FDNB, which in contrast to TNBS, easily penetrates biological membranes [6,7,14].

Materials and Methods

Isolation of sarcoplasmic reticulum and purification of Ca^{2+}-ATPase. Sarcoplasmic reticulum was isolated from rabbit white skeletal muscle as previously described [15]. The Ca^{2+}-ATPase was purified by centrifugation in a sucrose gradient after solubilization of the membranes with deoxycholate according to the method of Warren et al. [16].

Assay of ATPase activity. The ATPase activity was determined from the H^+ production associated with the ATP hydrolysis [17—19]. The reaction medium contained 5 mM Tris-HCl, 5 mM $MgCl_2$, 50 mM KCl, 5 mM potassium oxalate, 1 mM Mg-ATP and 1 mg of sarcoplasmic reticulum protein in a total vol. of 5 ml, at pH 7.0. The Mg^{2+}-ATPase activity was initiated by addition of ATP, and that of the Ca^{2+}-stimulated ATPase by the addition of 500 nmol $CaCl_2$, at pH 7.0. At this pH the hydrolysis of 1 mol ATP corresponds to the liberation of 0.75 mol of H^+. The membranes were pre-incubated for various periods with different concentrations of TNBS or FDNB, as indicated in the figure legends.

Active uptake of Ca^{2+} by sarcoplasmic reticulum vesicles. The uptake of Ca^{2+} induced by ATP in sarcoplasmic reticulum was carried out in a medium of the same composition as that utilized for the ATPase assay. Aliquots of the suspension medium containing 0.2 mg protein were filtered by the Millipore filtration technique [20] at several reaction times.

Passive binding of Ca^{2+} or Mg^{2+} by sarcoplasmic reticulum membranes. Sarcoplasmic reticulum vesicles (0.4 mg/ml) were incubated for 20 min in 4.0 ml

of a medium containing 20 mM Tris-maleate, at pH 7.0, variable concentrations of $CaCl_2$ or $MgCl_2$ and TNBS or FDNB, as described in the figure legends. After the incubation period, 0.4 mg of the sarcoplasmic reticulum vesicles were removed from the medium by the filtration method [20].

The filters (Millipore HA 0.45 μm) were washed twice by each 1.0 ml of 0.25 M sucrose. The filters were finally immersed in 2.5 ml of a solution containing 2% trichloroacetic acid and 0.5% La^{3+} and, after vigorous agitation, Ca^{2+} or Mg^{2+} analyses were performed in this solution by atomic absorption spectroscopy in a Perkin-Elmer spectrophotometer, Model 305.

Incorporation of TNBS or FDNB by sarcoplasmic reticulum membranes. The incorporation of the probes TNBS or FDNB in the membranes was determined after incubation as described above. The absorbance of the reaction mixtures was measured after stopping the reaction by adding 1.0 ml of a solution containing 5% SDS and 0.5 M HCl [11]. The measurements were performed in a Bausch and Lomb Spectrophotometer at 335 nm for TNBS and at 345 nm for FDNB. The results are expressed as A units/mg protein. In the case of TNBS, the amount of the probe incorporated by the reticulum proteins was estimated according to the expression:

$$(A_{335nm}/\epsilon) \cdot \text{vol} \cdot (\text{wt. (mg of SR proteins/unit vol.)})^{-1}$$

and is presented as mol TNBS/mol protein. For these calculations we utilized the following molecular weights for the different proteins (Fig. 3): Band I, 100 000; Band II, 90 000; Band III, 60 000; Band IV, 50 000 and Band V, 30 000. The value of ϵ for the TNP-proteins was taken as $10^4\ M^{-1} \cdot cm^{-1}$ [21].

Chromatography separation of the molecular components of sarcoplasmic reticulum membranes. Separation of phospholipids. After 20 min incubation in a medium containing 0.25 M sucrose, 20 mM Tris-maleate at pH 7.0, 1 mM TNBS, 4 mM $CaCl_2$, $MgCl_2$ or ATP and 20 mg of protein in a total volume of 20 ml, the suspensions were centrifuged 30 min at 40 000 $\times g$. Then, the phospholipids of the membranes were extracted in $CHCl_3/CH_3OH$ (1 : 1, v/v) mixtures [22] and separated by TLC (silica gel G, type 60, Merck) using the solvent $CHCl_3/CH_3OH/NH_4OH/H_2O$ (70 : 30 : 4 : 1, v/v). The lipids which reacted with TNBS were identified by their yellow colour and the free lipids were located using iodine vapors. The quantitative analysis of the trinitrophenylated phosphatidylethanolamine was determined by measuring the amount of P_i by the method of Bartlett [23] in the scraped spots previously digested in 70% $HClO_4$ at 190°C [24]. In control experiments, the membranes were incubated as described above, but in the absence of Ca^{2+}, Mg^{2+} and ATP.

Separation of proteins. The labelled proteins were separated by gel filtration. The membranes were solubilized in 1.0 ml solution of 1% SDS/1% β-mercaptoethanol and were heated in a boiling-water bath for 5 min. The samples were applied to a Sephadex G-100 column (2 $\times$ 100 cm) and were eluted with 1% SDS/0.05 M $NaHCO_3$, pH 7.0. Each fraction (2.0 ml) was analysed for absorbance at 335 nm as a measure of the TNBS incorporation.

Isolated Ca^{2+}-ATPase [16] was incubated with TNBS as described above and, subsequently, it was passed through a Sephadex G-100 column and was analysed for incorporation of the probe.

All assays were carried out at about 22°C. The protein concentration was

measured by the biuret method [25] using bovine serum albumin as standard.

Reagents. All reagents were analytical grade. TNBS and FDNB were utilized as aqueous solutions at the pH value of 7.0.

Results

Effects of Ca^{2+} and Mg^{2+} on the incorporation of TNBS and FDNB by sarcoplasmic reticulum membranes

In the absence of cations, the incorporation of TNBS, expressed as the absorbance at 335 nm/mg protein (Fig. 1A), increases as the probe concentration increases in the medium up to about 0.5 mM. Between 0.5 and 1.0 mM TNBS, the incorporation level remains constant and then it increases again at higher concentrations. At these high concentrations of TNBS the membrane is disrupted and therefore, it is permeable to the probe [7]. If the binding sites of sarcoplasmic reticulum are saturated with Ca^{2+} or Mg^{2+}, the absorbance is higher by about 0.1 or 0.05 units/mg protein, respectively, as compared to that in the absence of cations, and these increments are nearly the same at all concentrations used. The reticulum membranes also incorporate FDNB, but the binding of Ca^{2+} or Mg^{2+} does not increase the incorporation of the probe (Fig. 1B). The absorbance at 345 nm increases as the concentration of FDNB increases in the medium up to 1.5—2.0 mM and similar values were obtained either in the absence or in the presence of Ca^{2+} or Mg^{2+}. In both cases, the incorporation of the probes does not affect the passive binding of Ca^{2+} or Mg^{2+}

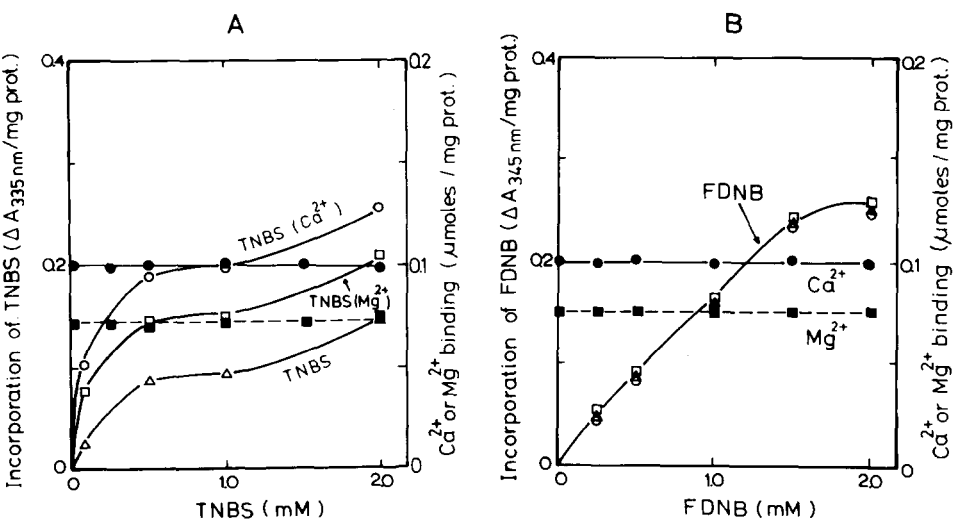


Fig. 1. Incorporation of TNBS and FDNB by sarcoplasmic reticulum membranes in the presence and absence of Ca^{2+} or Mg^{2+}. Sarcoplasmic reticulum was incubated in a medium containing 20 mM Tris-maleate (pH 7.0) and 2.0 mM $CaCl_2$ or $MgCl_2$ in the presence of various concentrations of TNBS (A) or FDNB (B). After 20 min of reaction, 1.0 ml aliquots (0.4 mg protein) were filtered through Millipore filters (0.45 μm). The TNBS or FDNB incorporated by the membranes was determined as described in the text. ●———●, Ca^{2+} binding; ■———■, Mg^{2+} binding. ○———○, incorporation of TNBS (A) or FDNB (B) in the presence of Ca^{2+}; □———□, incorporation of TNBS (A) or FDNB (B) in the presence of Mg^{2+}. △———△, incorporation of TNBS (A) or FDNB (B) in the absence of Ca^{2+} and Mg^{2+}.

to the membranes. About 100 nmol Ca^{2+} or 75 nmol Mg^{2+} per mg of protein were retained at the various concentrations of TNBS or FDNB used.

The effect of the passive binding of Ca^{2+} or Mg^{2+} on the incorporation of TNBS and FDNB into sarcoplasmic reticulum membranes is also shown in Fig. 2. At the constant value of 1.0 mM TNBS, its incorporation depends on the amount of Ca^{2+} or Mg^{2+} bound to the membranes. In contrast, the incorporation of FDNB is not altered by the cation binding (Fig. 2).

Incorporation of TNBS by lipids and proteins of sarcoplasmic reticulum

Since Ca^{2+} and Mg^{2+} facilitate the incorporation of TNBS by sarcoplasmic reticulum (Figs. 1 and 2), we studied which components of the membrane show increased incorporation in the presence of these reagents.

Analysis of lipids. The membranes were previously labelled with TNBS and, after phospholipid extraction, we determined the extent of trinitrophenylation of the phosphatidylethanolamine, which is the major amino phospholipid component of the membrane.

Table I shows that 65% of the phosphatidylethanolamine is labelled when intact sarcoplasmic reticulum reacts with TNBS in the absence of cations or ATP, and the labelling increases to about 94, 85 and 78% in the presence of Ca^{2+}, Mg^{2+} or ATP, respectively. The effect of the cations or ATP is lower in membranes disrupted by treatment with deoxycholate, and it is not observed

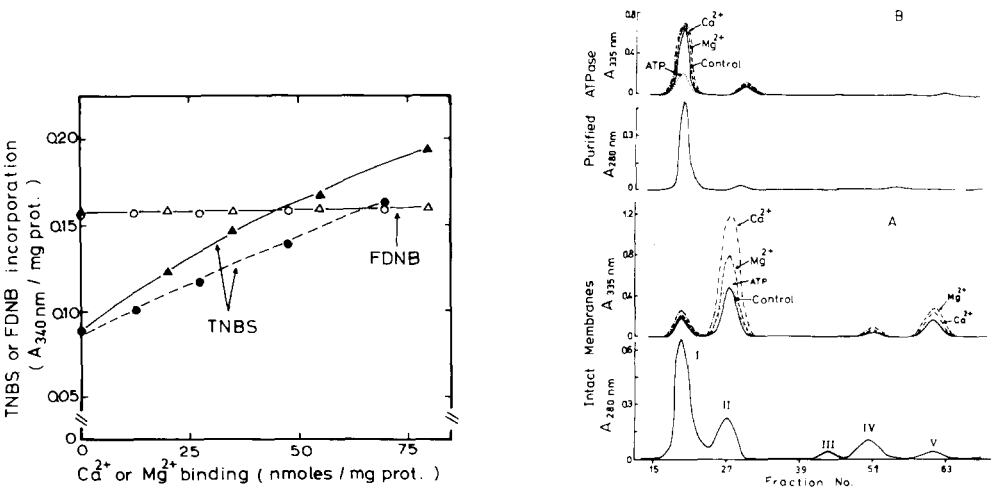


Fig. 2. Effect of Ca^{2+} or Mg^{2+} binding on the incorporation of TNBS and FDNB by sarcoplasmic reticulum membranes. Sarcoplasmic reticulum vesicles were incubated in a medium containing 20 mM Tris-maleate (pH 7.0); 1.0 mM TNBS or FDNB and various concentrations of Ca^{2+} or Mg^{2+}. At 20 min of reaction time, aliquots were withdrawn and filtered as described in Fig. 1. Incorporation of TNBS: (▲——▲); (●——●), in the presence of Ca^{2+} or Mg^{2+}, respectively. Incorporation of FDNB: (△——△); (○——○), in presence of Ca^{2+} or Mg^{2+}, respectively.

Fig. 3. TNBS-labelling patterns of the sarcoplasmic reticulum proteins in the presence and absence of Ca^{2+}, Mg^{2+} or ATP. After incubating the membranes or the purified ATPase with 1.0 mM TNBS in the absence or presence of 4.0 mM $CaCl_2$, $MgCl_2$ or ATP, the proteins were separated in a Sephadex G-100 column as described in Materials and Methods. Elution was performed with 1% SDS/0.05 M $NaHCO_3$ (pH 7.0) and the TNP-proteins were identified by measuring the absorbance at 335 nm. The ATPase was purified in a sucrose density gradient according the method of Warren et al. [16].

TABLE I

TNBS LABELLING OF SARCOPLASMIC RETICULUM PHOSPHATIDYLETHANOLAMINE IN THE PRESENCE AND ABSENCE OF Ca^{2+}, Mg^{2+} OR ATP

After labelling intact or leaky membranes (0.2% deoxycholate) with 1.0 mM TNBS and 4.0 mM $CaCl_2$, $MgCl_2$ or ATP, the lipids were extracted and chromatographically separated using the solvent $CHCl_3$/CH_3OH/NH_4OH/H_2O (70:30:4:1, v/v). In some experiments, extracted lipids were further incubated with the probe. The TNP-phosphatidylethanolamine was measured as described in the text. Control experiments were carried out in the absence of cations and ATP.

	Trinitrophenylation of phosphatidylethanolamine (%)		
	Intact membranes	Leaky membranes	Extracted lipids
Control	65	77	90
$CaCl_2$	94	94	91
$MgCl_2$	85	87	90
ATP	78	81	90

when lipids extracted from the membrane are incubated with the probe (Table I). Under these conditions, about 90% of the extracted phosphatidylethanolamine is labelled either in the presence or in the absence of cations and ATP

Analysis of proteins. The labelled membranes were dissolved by SDS and the proteins were chromatographically separated by a Sephadex G-100 column. Fig. 3 shows the TNBS-labelling patterns of the sarcoplasmic reticulum proteins obtained at 335 nm, and the relative amount of each component as revealed by the absorbance records obtained at 280 nm. In Fig. 3A, it can be seen that two proteins, Band II (M_r 90 000) and Band V (M_r 30 000), are predominantly labelled by TNBS and the labelling is significantly increased by the presence of Ca^{2+}, Mg^{2+} or ATP. The Ca^{2+}-ATPase (Band I) is slightly labelled by the probe, whereas no reaction was detected for the protein identified as the high affinity Ca^{2+}-binding protein (Band III). Table II summarizes the distribution of TNBS incorporated into the proteins in the presence or absence of Ca^{2+}, Mg^{2+} or ATP. The highest incorporation of TNBS (46 mol TNBS/mol protein) takes place into the protein of Band V, and the incorporation increases to about 85 and 75 mol TNBS/mol protein in the presence of $MgCl_2$ or $CaCl_2$, respectively. In contrast, Ca^{2+} is more effective than Mg^{2+} in increasing the labelling of the Band II protein (M_r 90 000). For this protein, we found that Ca^{2+} increases the incorporation to 30 mol TNBS/mol protein, whereas Mg^{2+} increases it to about 20 mol TNBS/mol of protein. The labelling of the Ca^{2+}-ATPase was also increased by Ca^{2+} and Mg^{2+}, but the level of trinitrophenylation is low (0.15—0.2 mol TNBS/mol protein) as compared to that of the others proteins. When Ca^{2+}-ATPase is isolated and made to react with TNBS, the incorporation of the probe increases by about 5-fold to a value of 0.75 mol TNBS/mol protein, and no significant stimulation by Ca^{2+} or Mg^{2+} is observed (Fig. 3B and Table II). Surprisingly, marked reduction in the labelling of isolated ATPase with TNBS was observed in the presence of ATP (0.2 mol TNBS/mol protein).

A general conclusion is that Ca^{2+}-ATPase contributes only 2.2% for the in situ labelling by TNBS of the protein moiety of the membrane, whereas calsequestrin and the proteins of molecular weight 90 000 and 30 000 contribute

TABLE II

INCORPORATION OF TNBS BY THE SEVERAL PROTEINS OF SARCOPLASMIC RETICULUM AND BY THE PURIFIED ATPase IN THE PRESENCE AND ABSENCE OF Ca^{2+}, Mg^{2+} OR ATP

The experiments were carried out as described in Fig. 3. The Ca^{2+}-ATPase was purified by the method of Warren et al. [16]. The amounts of TNBS incorporated, expressed as mol TNBS/mol protein were estimated as indicated in Materials and Methods. The results represent the mean values obtained in three different experiments.

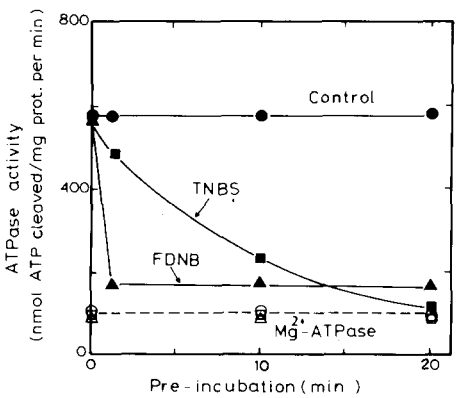
	Incorporation of TNBS (mol TNBS/mol protein)					
	Intact membranes					Purified ATPase Band I
	Band I	Band II	Band III *	Band IV *	Band V	
Control	0.15	10	0	8	46	0.75
$CaCl_2$	0.20	30	12		75	0.80
$MgCl_2$	0.18	20	11		85	0.78
ATP	0.14	15	9		50	0.20

* In the presence of cations or ATP, Band III and Band IV were not completely resolved through the Sephadex G-100 column.

about 8, 34 and 56%, respectively. However, other amino groups do exist in the Ca^{2+}-ATPase which can react with TNBS, but only after isolation of the enzyme from the membrane.

Effect of TNBS and FDNB on the Ca^{2+}-ATPase activity and Ca^{2+} transport of sarcoplasmic reticulum

As reported before [26], TNBS inhibits the Ca^{2+}-ATPase activity of the sar-



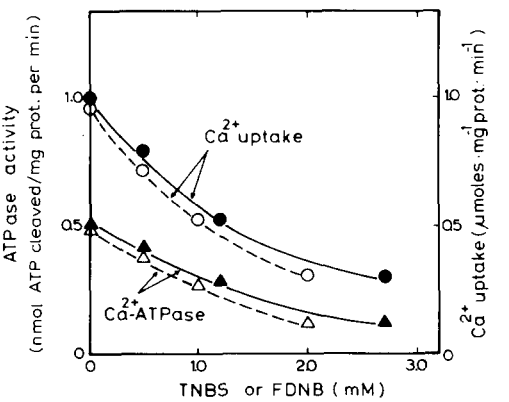


Fig. 4. Effect of TNBS and FDNB on the ATPase activity of sarcoplasmic reticulum membranes. The ATPase activity was assayed as described in the text. TNBS or FDNB (1.0 mM) reacted with the membranes for periods up to 20 min before the ATPase assay. Ca^{2+}-ATPase: ●———●, control; ■———■, TNBS-treated vesicles; ▲———▲, FDNB-treated vesicles. Mg^{2+}-ATPase: ○———○, control; □———□, TNBS-treated vesicles; △———△, FDNB-treated vesicles.

Fig. 5. Effect of TNBS and FDNB on the active Ca^{2+} uptake by sarcoplasmic reticulum. The ATPase activity and Ca^{2+} uptake were assayed as described in the text. The reticulum membranes were pre-incubated for 20 min with concentrations of TNBS or FDNB up to 2.6 mM. ●———●; ○———○, rates of Ca^{2+} uptake in the presence of FDNB or TNBS, respectively. ▲———▲; △———△, rates of ATP hydrolysis in the presence of FDNB or TNBS, respectively.

coplasmic reticulum, whereas the Mg^{2+}-ATPase activity remains unaffected. Fig. 4 shows that for 1 min of pre-incubation, TNBS (1.0 mM) inhibits the Ca^{2+}-ATPase activity from a value of 550 to 470 nmol ATP cleaved per mg protein per min, while after 20 min of pre-incubation, the Ca^{2+}-ATPase was reduced to 100 nmol ATP cleaved per mg protein per min. In preliminary experiments we observed that the time course of the inhibition of ATPase by TNBS corresponds to the time course of the TNBS incorporation. In contrast, at 1 min of pre-incubation with FDNB (1.0 mM), the Ca^{2+}-ATPase activity was already drastically inhibited from 550 to 175 nmol ATP cleaved per mg protein per min, and this value was maintained for longer periods of pre-incubation with the probe (Fig. 4).

The activity of the Mg^{2+}-ATPase (100 nmol ATP cleaved per mg protein per min), was not affected by the presence of either TNBS or FDNB (Fig. 4).

The inhibition of ATP hydrolysis by TNBS or FDNB is reflected in the accumulation of Ca^{2+} by sarcoplasmic reticulum. Fig. 5 shows that, as the concentration of the probes increases in the medium, the rate of Ca^{2+} uptake is reduced as is the Ca^{2+}-ATPase activity. However, the ratio of the Ca^{2+} taken up to the ATP hydrolysed is maintained at a value of about 2 at the various concentrations of the probes used.

Discussion

In this work we observed that the binding of Ca^{2+} or Mg^{2+} significantly increases the incorporation of TNBS, but not FDNB, into sarcoplasmic reticulum membranes, (Figs. 1 and 2). FDNB easily penetrates membranes [6,7,14] so that, probably, it has ready access to all the amino groups of the membrane, while TNBS penetrates biological membranes much less readily [5,7].

Fractionation of the sarcoplasmic reticulum membranes showed that TNBS labels both lipids and proteins. The labelling of both moieties in the intact membranes is increased by Ca^{2+}, Mg^{2+} or ATP, but the amount of TNBS incorporated varies for the different proteins of the membrane. The highest degree of TNBS incorporation took place into the protein component of the Band V (M_r 30 000) in agreement with the result of Hidalgo and Ikemoto [27] who found that a protein of molecular weight 30 000 is strongly labelled by cycloheptaamylose-fluorescamine complex, which also reacts with membrane amino groups. This protein probably corresponds to the glycoprotein previously isolated by Ikemoto et al. [28]. However, in our experiments, Band V may include more than one protein [29], which probably accounts for the high label incorporation observed in this fraction. The other protein with high-TNBS incorporation is the protein of Band II (M_r 90 000) which migrates in the Sephadex column slightly behind the Ca^{2+}-ATPase (Band I). Since disruption of the membrane increases its labelling by TNBS [29], it appears that it is a real component of the membrane rather than a contaminating product of the sarcoplasmic reticulum preparation. Furthermore, this protein has also been detected electrophoretically by Yu et al. [30] and recently by Carley and Racker [31] who suggested that it functions as a phosphate carrier in the sarcoplasmic reticulum membrane.

The TNBS labelling of Bands II and V is significantly increased by Ca^{2+},

Mg^{2+} or ATP, but Ca^{2+} is more effective than Mg^{2+} in increasing the labelling of Band II, whereas the opposite is observed for Band V. This difference is probably related to the different localization of the proteins in the membrane and to the different permeability of sarcoplasmic reticulum to Ca^{2+} and Mg^{2+}. The labelling of the Ca^{2+}-ATPase (Band I) and of the calsequestrin (Band IV) was only slightly increased by cations. However, the amount of TNBS incorporated by the Ca^{2+}-ATPase after its isolation from the membrane is increased 5-fold over the value obtained for the enzyme in intact membranes, but the effect of Ca^{2+} or Mg^{2+} on TNBS incorporation of the isolated enzyme is negligible. It is evident that the Ca^{2+}-ATPase has more amino groups which can react with the membrane probe than those accessible when the enzyme is in situ and, by and large, the amino groups are accessible to TNBS when the enzyme is isolated, but not when it is buried in the structure of the membrane. Only a few of these amino groups can be made accessible by divalent cations which significantly increase the exposure of the amino groups from the other proteins of the sarcoplasmic reticulum membrane (Fig. 3).

The labelling of the amino phospholipids from the membrane is also stimulated by Ca^{2+}, Mg^{2+} or ATP, but no effect of these agents was detected when isolated lipids were reacted with the probe. It appears, therefore, that cations and ATP act at the level of the membrane in facilitating the accessibility of the amino groups to TNBS. Godin et al. [13] suggested that structural perturbations induced by divalent cations in membranes of erythrocytes increase their incorporation of TNBS.

The divalent ions have no effect on the TNBS incorporation by the isolated Ca^{2+}-ATPase and ATP actually suppresses the TNBS incorporation. This suppression has also been observed for the TNBS incorporation by the $-NH_2$ groups of myosin [32—34] which apparently are protected by ATP against trinitrophenylation.

The probes TNBS and FDNB inhibit the Ca^{2+}-ATPase and the Ca^{2+} uptake activities of the sarcoplasmic reticulum (Figs. 4 and 5). However, the inhibition by TNBS depends on the pre-incubation time with the probe, whereas FDNB rapidly inhibits the Ca^{2+} pump activity. This difference may be due to the size and neutral charge of the FDNB molecule which penetrates the membrane rapidly while TNBS does not easily penetrate biological membranes [5—7].

Earlier results obtained by using TNBS as a tool to study the mechanism of Ca^{2+} transport by sarcoplasmic reticulum [9,10] have been interpreted in favour of rotatory movements of the ATPase molecule during Ca^{2+} transport. However, it was assumed in those studies, that the Ca^{2+}-ATPase was responsible for most of the TNBS binding since the others proteins are minor components of the membrane. Our study shows that the highest degree of TNBS incorporation takes place into proteins other than the Ca^{2+}-ATPase and that their labelling is also increased by Ca^{2+}, Mg^{2+} or ATP. The interaction of these agents with the sarcoplasmic reticulum probably facilitates the exposure of the protein amino groups to the probe TNBS.

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