# CHANGES IN THE MEAN DISTANCE BETWEEN TRYPTOPHAN AND FLUORESCENCE PROBE IN THE LABELED SARCOPLASMIC RETICULUM MEMBRANES INDUCED BY DETERGENTS

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## 1. Introduction

The sarcoplasmic reticulum membranes mainly consist of $Ca^{2+}$, $Mg^{2+}$-ATPase [1,2] and are a good preparation for the study of conformational changes in the membrane protein [3,4].

Solubilization of the membranes with detergents has been done by several investigators to study the properties of this membrane system [5,6], and it is thought that the solubilization induces a conformational change of the ATPase molecule. In the previous work, one of the authors reported on the properties of the membranes labeled with 1-dimethylaminonaphthalene-5-sulfonyl chloride (DnsCl) [7]; most of the dye was associated with ATPase and phosphatidyl-ethanolamine in the membranes and there was energy transfer from tryptophan to the bound dye.

In the present study, therefore, we intended to monitor the conformational change of ATPase molecule during solubilization by estimating the mean distance between tryptophan and the bound dye, using the efficiency of energy transfer between the two.

## 2. Experimental

Sarcoplasmic reticulum membranes were prepared from rabbit skeletal muscle and labeled with DnsCl as described earlier [7]. Total lipids were extracted from the dansylated membranes as described by Forti et al. [8], and the residual lipid-free proteins were further washed twice with chloroform/methanol (2:1, v/v). Hydrolyses of the protein and separation of the resulting amino acids by two dimensional polyamide thin-layer chromatography were performed according to the method of Woods and Wang [9] with a slight modification as follows [10]: the first solvent system of thin-layer chromatography being ammonia water (28%)/water (1/100) and the second being formic acid/*n*-butanol/heptane (1/10/10). Corrected fluorescence spectra were measured at 20°C with Shimadzu corrected recording spectrofluorometer RF-502.

## 3. Results and discussion

As previously described [7], the molar ratio of ATPase to the bound dye was about one. This one-to-one binding led us to consider that there might be a specific amino acid residue associated with the dye. Consequently, the protein of the dansylated membranes was hydrolysed and the amino acid analysis was performed. As shown in fig.1, most of the dye was associated with lysine, though a trace amount of it was also found with tyrosine. A small amount of DnsOH always appeared upon the hydrolization of labeled proteins. These results indicate that most DnsCl bound to the protein under our experimental conditions is associated with lysine.

It is reasonably assumed that the energy transfer mainly occurs from tryptophan to the dye bound to ATPase. Thus, the mean distance between tryptophan and the dye bound to lysine could be estimated from

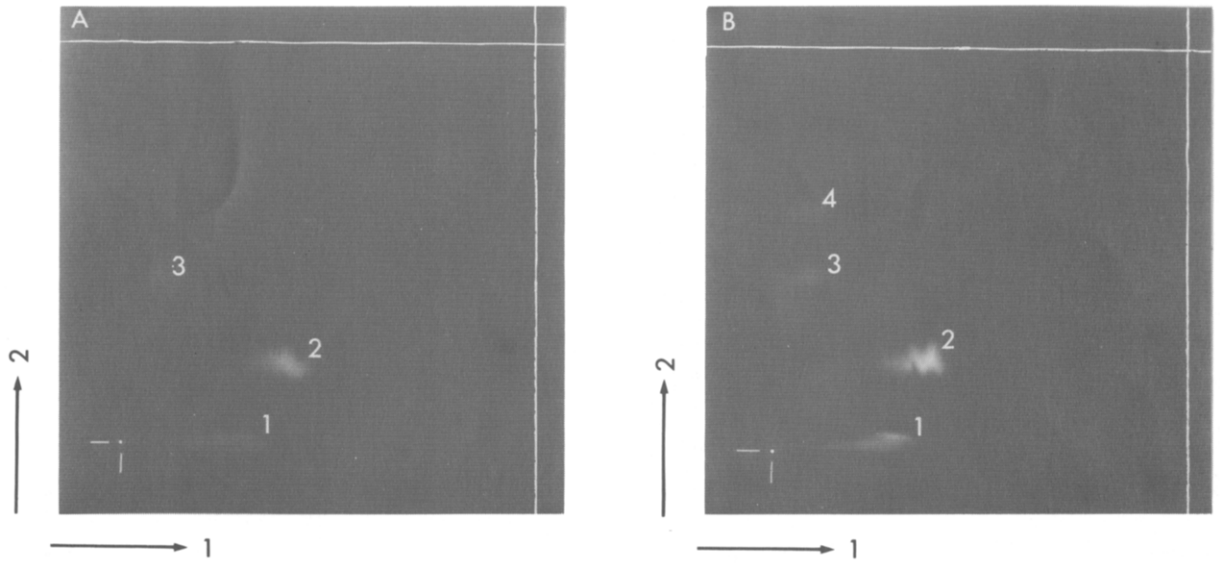
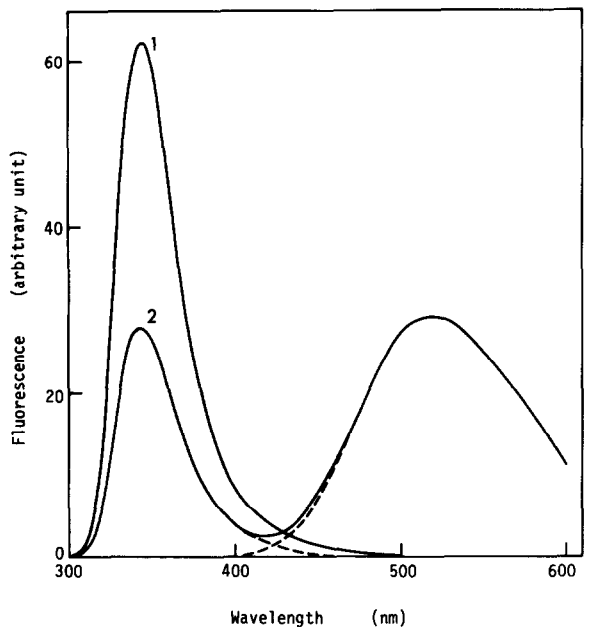



Fig.1. Two-dimensional separation of dansylated amino acids on polyamide sheets. (A) Hydrolysate of dansylated membrane protein; (B) A + 4 standard dansylated amino acids consisting of Dns-OH (1), ε-Dns-lysine (2), *O*-Dns-tyrosine (3) and Dns-$NH_2$ (4). Solvent 1, ammonia water (28%)/water (1/100): solvent 2, formic acid/*n*-butanol/heptane (1/10/10).




the efficiency of energy transfer between the two fluorophores. Figure 2 shows the emission spectra of the dansylated and non-dansylated membranes excited at 290 nm at the same protein concentration.

The efficiency of energy transfer ($E$) was calculated to be 54–56% with several preparations from the following equation:

$$E = \frac{F_0 - F}{F_0}$$

where $F_0$ and $F$ indicate the fluorescence intensities of non-dansylated and dansylated membrane proteins at the same protein concentration. The mean distance

Fig.2. Corrected fluorescence spectra of the dansylated and non-dansylated membranes. Measurements were performed in 3 ml of a medium consisting of 0.15 mg protein of the membranes, 0.3 M sucrose and 2 mM Tris–HCl (pH 7.4) at 20°C. Excitation was made at 290 nm. Band widths were 10 nm for excitation and 3 nm for emission. (1) Non-dansylated membranes; (2) dansylated membranes.

of two fluorophores ($R$) was calculated using the efficiency of energy transfer from the following equation:

$$\frac{R}{R_0} = \sqrt[6]{\frac{1}{E} - 1}$$

In this equation, $R_0$ means a critical transfer distance and is represented as follows according to Förster [11]:

$$R_0 = \sqrt[6]{8.79 \times 10^{-25} \cdot \eta \cdot k^2 \cdot n^{-4} J_{\bar{\nu}}}$$

where $\eta, k, J_{\bar{\nu}}$ and $n$ indicate the quantum yield of the donor emission in the absence of the acceptor, the orientation factor, the overlap integral between the donor emission and the acceptor absorption on wave number scale, and the refractive index of the medium, respectively.

The quantum yield of membrane tryptophan was calculated to be 0.21 from that of L-tryptophan, 0.30, by comparing their fluorescence intensities at the same optical densities. For $k^2$, 2/3 is reasonably adopted in case of randomly oriented pairs of the donor and the acceptor [12]. $J_{\bar{\nu}}$ was calculated according to the formula modified by Yagi et al. [13].

Table 1 summarizes an example of the effect of deoxycholate and sodium dodecyl sulfate on energy transfer and the mean distance between tryptophan and the bound dye. These values were reproducible with several preparations. Deoxycholate did not alter the $R$ value significantly, while sodium dodecyl sulfate elongate it by 6 Å. It should be noticed that deoxycholate as well as sodium dodecyl sulfate caused delipidation of the membrane protein [14]. Therefore, the dye bound to phosphatidylethanolamine does not seem to be involved in the energy transfer.

Table 1
Effect of two detergents on the mean distance between tryptophan and the bound dye ($R$) in dansylated membranes

| Addition | $E$ (%) | $R/R_0$ | $R$ (Å) |
|---|---|---|---|
| None | 55 | 0.967 | 20.4 |
| DOC 0.5% | 45 | 1.034 | 20.1 |
| SDS 0.5% | 10 | 1.456 | 26.1 |

Fluorescence measurements were made as described in Legend for fig.2. $E$, $R$ and $R_0$ represents the efficiency of energy transfer, the mean distance between tryptophan and the bound dye and the critical transfer distance, respectively

Schechter et al. reported that sodium dodecyl sulfate caused a gross comformational change of a membrane protein, spectrin, while deoxycholate did not, by monitoring circular dichroism spectra [15]. Although there remains a possibility that sodium dodecyl sulfate only alters the rotation of the dansylated lysine side chain, it is reasonably considered that the detergent also induced a gross conformational change of the ATPase molecule, which brought the elongation of the interchromophoric distance.

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