Fluorescence Energy Transfer between ATPase Monomers in Sarcoplasmic Reticulum Reconstituted Vesicles, in the Presence of Low Concentrations of a Nonionic Detergent

Philippe Champeil, Jean-Louis Rigaud, and Marcel Pierre Gingold

Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette, France

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We measured fluorescence energy transfer between ATPase monomers labeled with either a donor or an acceptor fluorophore, in order to test the hypothesis that the presumably oligomeric sarcoplasmic reticulum ATPase would dissociate into monomers within the membrane on the addition of low nonsolubilizing amounts of a nonionic detergent, $C_{12}E_{18}$. We found that at low nonsolubilizing detergent concentrations below the critical micellar concentration (c.m.c.), there was indeed direct interaction between the detergent and the protein; however, only solubilizing detergent concentrations reduced fluorescence transfer to a minimum. Considering the current literature, fluorescence energy transfer proved insufficient, at the present time, to confirm or reject the hypothesis.

Introduction

The quaternary structure of the ATPase protein responsible for calcium transport across sarcoplasmic reticulum membranes has aroused much interest and controversy. Several experimental approaches, including electron microscopy, cross-linking experiments, stoechiometry, activity measurements after detergent solubilization, and a variety of biophysical techniques have aroused speculation regarding the oligomeric structure of the calcium pump (for a review, see ref. [1]). One of the latter techniques is particularly attractive: the fluorescence energy transfer between ATPase monomers labeled with either a donor on an acceptor fluorophore [2].

In addition, the results of spin label experiments performed in the presence of $C_{12}E_8$ were considered consistent with the hypothesis that oligomeric ATPase would dissociate into monomers within the membrane on the addition of low nonsolubilizing amounts of detergent [3]. Furthermore, concentrations of $C_{12}E_8$ below the critical micellar concentration were found to affect the ATPase activity of nonsolubilized SR membranes (Andersen and Møller, in preparation, see also ref. [4]).

Abbreviations: ATPase, adenosine triphosphatase; SR, Sarcoplasmic Reticulum; DOC, potassium deoxycholate; $C_{12}E_8$, dodecyloctaoxyethylene glycol monoether; c.m.c., critical micellar concentration.

Reprint requests to Philippe Champeil.

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We therefore tested the effect of low $C_{12}E_8$ concentrations by fluorescence transfer and other simple techniques. We reached two conclusions:

(i) At low nonsolubilizing detergent concentrations below the c.m.c., there is indeed direct interaction between the detergent and the protein.

(ii) Only solubilizing detergent concentrations reduce fluorescence transfer to a minimum. This result points to the necessity of reevaluating the significance of fluorescence transfer experiments.

Materials and Methods

SR vesicles [5] were labeled with either the donor (1,5-IAEDANS) or the acceptor (IAF) fluorophore, essentially as in [2]: 3 mg SR protein per ml in a medium containing 1 M KCl, 0.25 M sucrose and 50 mM tricine, pH 8, was incubated overnight at 5 °C with 0.16 mM final dye concentration (ATPase: dye mole ratio of 1:8). As in [2], covalently bound donor and acceptor dyes amounted to about 3 and 1 mol/mol of ATPase respectively, and the ATPase activities of labeled preparations were within 20% of the unlabeled control.

In the energy transfer experiments, donor-labeled vesicles, acceptor-labeled vesicles, or a mixture of equal amounts of both were solubilized with deoxycholate and slowly diluted with 0.1 M KCl, 0.1 mM $CaCl_2$ and 10 mM Hepes (pH 7.5) at room temperature: upon slow dilution, vesicles re-formed

(see results). Fluorescence spectra and light scattering were recorded on a Perkin Elmer MPF-44A fluorimeter. Data were recorded and processed with a Hewlett-Packard 9825A desk-top computer.

$C_{12}E_8$ was obtained from Nikko Chemicals Co., Tokyo.

Results

In order to study fluorescence energy transfer between ATPase monomers in a reconstituted system, we used a reconstitution procedure involving dilution of deoxycholate-solubilized sarcoplasmic reticulum ATPase [2]. However, such procedure had been reported to cause formation of 100–200 Å particles rather than of vesicular structures [6, 7]. We therefore first investigated the morphology of reconstituted samples, which was found critically dependent on the rate of dilution of the solubilized enzyme: fairly large vesicles (500–1000 Å) could be obtained by controlled dilution (3 ml dilution buffer were added within 1–2 h to 150 µl of solubilized material, using a peristaltic pump; see [15] for similar results with Triton X-100).

Donor-labeled vesicles and acceptor-labeled vesicles were mixed together in the absence of deoxycholate, and then diluted in detergent-free buffer. As expected, the fluorescence spectrum recorded was the sum of the spectra of the two separately measured components. On the other hand, when the donor and acceptor-labeled vesicles were mixed in the presence of deoxycholate before slow dilution, vesicles containing both fluorophores were reconstituted. Under the latter conditions (Fig. 1 a), interaction between donor and acceptor fluorophores in the reconstituted sample was revealed by significant quenching of donor fluorescence and significant enhancement of acceptor fluorescence (full line) in relation to the theoretical contribution to the spectrum of donor and acceptor fluorophores (dotted line, obtained by numerical summation). This is a typical fluorescence transfer experiment [2].

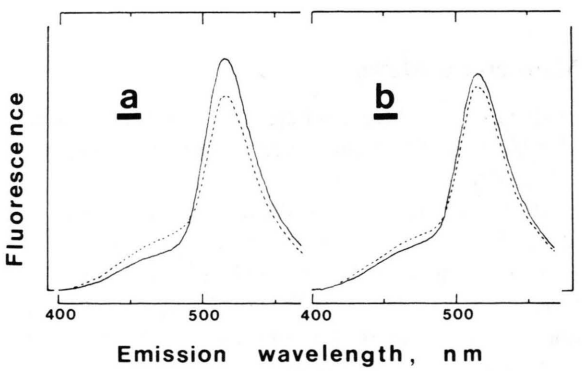

Fig. 1. Emission spectra of labeled vesicles. Excitation wavelength, 320 nm. Full lines: 75 µg of donor-labeled vesicles + 75 µg of acceptor-labeled vesicles were solubilized with 0.15 mg DOC in a final volume of 150 µl, and slowly diluted to 3 ml. These mixed reconstituted vesicles were then diluted with an equal volume of buffer without $C_{12}E_8$ (a) or with $C_{12}E_8$ at a final concentration of 100 µg/ml (b). Dotted lines: Numerical sum of the spectra recorded after separate reconstitution of donor and acceptor-labeled vesicles and dilution as in (a) or (b) above. For all spectra, the baseline was computer-subtracted.

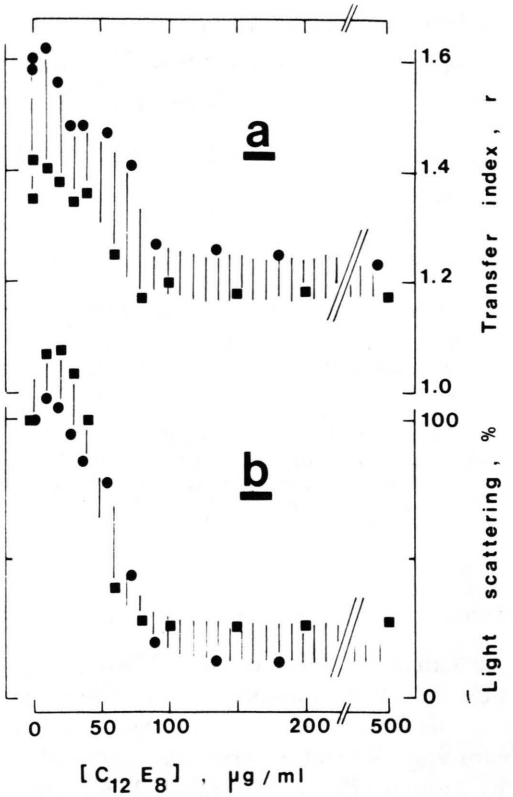

Fig. 2. Effect of the $C_{12}E_8$ concentration on transfer efficiency and light scattering. Vesicles were reconstituted as described in Fig. 1 and diluted with various concentrations of detergent. Note that the protein concentration was low (25 µg/ml), so that the free $C_{12}E_8$ concentration was close to the total concentration below the c.m.c. (50 µg/ml). **a)** Transfer efficiency. See text for definition of the "*r*" index. **b)** Light scattering at 320 nm. ● and ■ refer to two different experiments.

We attempted to characterize the magnitude of energy transfer through a quantitative index, *r*. For this purpose, we divided the ratio of acceptor over donor fluorescence intensities in the mixed reconstituted spectrum (full line, Fig. 1 a) by the ratio of acceptor over donor fluorescence intensities in the interaction-free, theoretical emission spectrum (dotted line in Fig. 1 a).

$$r = \frac{(Fluo_{515nm}/Fluo_{475nm})\ \text{mixed vesicles spectrum}}{(Fluo_{515nm}/Fluo_{475nm})\ \text{numerical sum spectrum}}.$$

In the experiment shown in Fig. 1 a, *r* was about 1.5.

Fig. 1 b shows that after addition of 100 µg $C_{12}E_8$/ml, fluorescence transfer was significantly reduced, although not abolished. We tested various concentrations of detergent, and Fig. 2 a confirms that the transfer efficiency parameter, *r*, did not fall to 1 even for very high $C_{12}E_8$ levels, although it dropped significantly from about 1.5 to about 1.2 for detergent concentrations between 0 and 80–90 µg/ml. In Fig. 2 b, light scattering by reconstituted vesicles after addition of detergent was taken as a convenient solubilization index; minimal light scattering values were attained around 80–100 µg/ml.

We verified that light scattering was a reasonable index of SR membrane solubilization by $C_{12}E_8$ by comparing it with the amount of SR proteins which could not be spun down during high speed centrifugation. The experiment in Fig. 3 was performed with native vesicles using various $C_{12}E_8$ levels. The protein concentration in the supernatant, measured by tryptophane fluorescence (○), was in fact reasonably correlated with the final drop in light scattering (+), although for very low detergent concentrations light scattering might be sensitive to changes in the refractive index of the medium.

In addition, Fig. 3 shows that the intrinsic fluorescence level (●) in the total sample, *i.e.* before centrifugation, was slightly but reproducibly enhanced for concentrations of 0 to 20 µg detergent/ml.

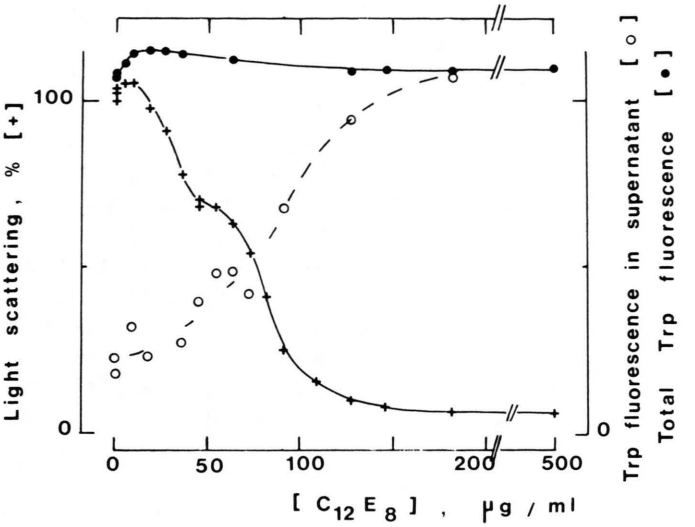

Fig. 3. Interaction of $C_{12}E_8$ with native SR vesicles (25 µg/ml). (+) Light scattering. Note a slight enhancement (*cf.* [12]) for very low concentrations, as in Fig. 2 b, and a plateau-like curve around the c.m.c. (50 µg/ml); this plateau aspect was more apparent at higher protein concentrations. At present, we have no completely satisfactory explanation for these two features. (●) The same samples were examined for tryptophane fluorescence ($\lambda_{ex} = 290$ nm and $\lambda_{em} = 330$ nm). (○) 3 ml of these samples were then centrifuged (45000 rpm, 20 min at 20° in the 50 Ti rotor) and tryptophane fluorescence in the supernatant was measured.

Discussion

We observed the fluorescence transfer between ATPase monomers originally described by Vanderkooi *et al.* [2], in reconstituted systems whose morphological integrity has been checked by electron microscopy. We then tested the effect of various $C_{12}E_8$ concentrations on the efficiency of this energy transfer.

At high detergent concentrations, residual energy transfer was still observed. This might be due to incomplete monomerization of some possibly denatured protein aggregates [8]. In the following, we focus on that part of the energy transfer which is abolished by the detergent: we found that energy transfer efficiency was reduced to its minimal value only for solubilizing detergent concentrations (Fig. 2).

Considering that energy transfer only reflects the presence of ATPase oligomers, our data would suggest that these oligomers are only dissociated by detergent concentrations solubilizing the membrane. Alternately, a biphasic decreasing curve might perhaps be drawn through our experimental points, with a kind of plateau around 40–50 µg/ml $C_{12}E_8$, the significance of which would be: (i) fluorescence transfer initially drops due to oligomer dissociation for very low detergent concentrations before solubilization, and (ii) there is some residual transfer between individual monomers in the membrane;

this residual transfer would drop in parallel with solubilization.

Because of the scatter in our experimental points, no discrimination can be made between these two possibilities. Anyhow this points to the necessary re-evaluating of the significance of energy transfer. According to Estep and Thompson, energy transfer in reconstituted SR can in fact be simply accounted for by a dense but random distribution of monomers in the absence of stable complexes [9], and the same conclusion can be deduced from the calculations of two other groups [10, 11]. More experiments are therefore needed before energy transfer can serve as a clearcut criterion for the aggregation or disaggregation of ATPase monomers [13].

Our intrinsic fluorescence data in Fig. 3 (●) at least show that direct interaction between detergent and protein takes place at very low detergent concentrations, below the critical micellar concentration, in accordance with previous results showing that the fluorescence changes experienced by the pump upon calcium addition vanish for the same very low $C_{12}E_8$ concentrations ([4, 14], and Andersen & Møller, in preparation).

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[1] J. V. Møller, J. P. Andersen, and M. le Maire, Mol. Cell. Biochem., in press.
[2] J. M. Vanderkooi, A. Ierokomas, H. Nakamura, and A. Martonosi, Biochemistry **16,** 1262–1267 (1977).
[3] P. Fellmann, J. Andersen, P. F. Devaux, M. le Maire, and A. Bienvenue, Biochem. Biophys. Res. Comm. **95,** 289–295 (1980).
[4] S. Verjovski-Almeida and J. L. Silva, J. Biol. Chem. **256,** 2940–2944 (1981).
[5] P. Champeil, S. Buschlen-Boucly, F. Bastide, and C. M. Gary-Bobo, J. Biol. Chem. **253,** 1179–1186 (1978).
[6] K. E. Jørgensen, K. E. Lind, H. Røigaard-Petersen, and J. V. Møller, Biochem. J. **169,** 489–498 (1978).
[7] Z. Selinger, M. Klein, and A. Amsterdam, Biochim. Biophys. Acta **183,** 19–26 (1969).
[8] J. V. Møller, K. E. Lind, and J. P. Andersen, J. Biol. Chem. **255,** 1912–1920 (1980).
[9] T. N. Estep and T. E. Thompson, Biophys. J. **26,** 195–208 (1979).
[10] P. K. Wolber and B. S. Hudson, Biophys. J. **28,** 197–210 (1979).
[11] T. G. Dewey and G. G. Hammes, Biophys. J. **32,** 1023–1036 (1980).
[12] L. D. Powell and L. C. Cantley, Biochim. Biophys. Acta **599,** 436–447 (1980).
[13] M. P. Gingold, J. L. Rigaud, and P. Champeil, Biochimie **63,** 923–925 (1981).
[14] Y. Dupont and M. le Maire, FEBS Lett. **115,** 247–252 (1980).
[15] J. M. Wolosin, Biochem. J. **189,** 35–44 (1980).