LETTER TO THE EDITOR

Excimer Formation in Pyrenemaleimide-labeled Sarcoplasmic Reticulum ATPase

Dear Sir:

Recently, Papp et al. (1986) described repetition and extension of our experiments with *N*-(3-pyrene)maleimide-labeled sarcoplasmic reticulum ATPase from fast skeletal muscle (Lüdi and Hasselbach, 1983) using *N*-(1-pyrene)maleimide-labeled ATPase. We would like to add some clarifications and additions to our work and reply to some of the comments made in the paper by Papp et al. We hope to resolve the conflicting results in the two publications.

It is obvious that one difference in the publications is the nomenclature of the pyrene derivative. Whereas Papp et al. (1986) used *N*-(1-pyrene)maleimide we used *N*-(3-pyrene)maleimide. Due to a different numbering of the C-atoms of the pyrene ring used in the literature and probably also by the manufacturers, it is not clear if indeed different isomers were used by the two groups. Yet the following statements apply irrespective of the pyrene isomer used by us and Papp et al.

Our interpretation of the excimer peak in *N*-(3-pyrene)maleimide-labeled ATPase was based on the following observations: (*a*) *N*-(3-pyrene)maleimide binds preferentially to the fast reacting thiol group described by Miki et al. (1981), and (*b*) the excimer peak was decreased after the addition of nonsolubilizing amounts of detergent, such as myristoylglycerophosphocholine or $C_{12}E_8$. Both arguments were considered as nonvalid due to the results of Papp et al (1986).

Regarding *a*, we claimed that *N*-(3-pyrene)maleimide "preferentially binds to a thiol group, which is in close contact to an adjacent thiol group but attached at a neighboring ATPase molecule." This does not mean that at *N*-(3-pyrene)maleimide ratios larger than ~ 0.5 mol/mol ATPase other slow reacting thiol groups are not labeled. Therefore double labeling of one ATPase molecule certainly occurs and we believe that there is no discrepancy between the results obtained by the two groups. But as mentioned in Lüdi and Hasselbach (1983), "if one assumes four to six equivalent thiol groups/ATPase molecule to which pyrene maleimide could be bound, only a negligible amount of excimers is expected at a labeling degree of 1 mol label/mol ATPase (3–10%)."

In addition, close inspection of Fig. 4, *A–C*, in Papp et al. (1986) demonstrates that, under the conditions we have used (i.e, in the presence of Ca^{++} ions) an approximate 50% decrease in the ^{14}C-*N*-ethylmaleimide (NEM)-labeled peptides bands numbered 1–4 is observed if sarcoplasmic reticulum ATPase was pretreated with 1 mol pyrene maleimide per mol ATPase. But contrary to the authors' claim, bands 6 and 7 of the NEM-labeled peptides selectively disappeared. This was not observed if labeling was carried out in the presence of EGTA (Fig. 4, *E–F*, in Papp et al., 1986).

With regard to *b*, we have demonstrated that nonsolubilizing amounts of myristoylglycerophosphocholine decrease the amount of excimers in *N*-(3-pyrene)-maleimide-labeled ATPase (Fig. 2 in Lüdi and Hasselbach, 1983) and stated that the same results were obtained with $C_{12}E_8$. Since then we have realized that due to photo-damage the excimers also decreased if the intensity of the exciting light is above a critical value. This can be shown by repetitively recording an emission spectrum of a vigorously stirred solution of labeled sarcoplasmic reticulum vesicles. We have therefore repeated the experiments in which we used detergents and applied a very narrow slit for the exciting light beam (0.5 or 1 nm). Under these conditions, five to six consecutive emission spectra gave the same amount of excimers (i.e., the same D/M-value was obtained). Whereas the results now obtained with myristoylglycerophosphocholine are very similar to the results shown in Lüdi and Hasselbach (1983), it could be demonstrated that $C_{12}E_8$ was not able to completely abolish the

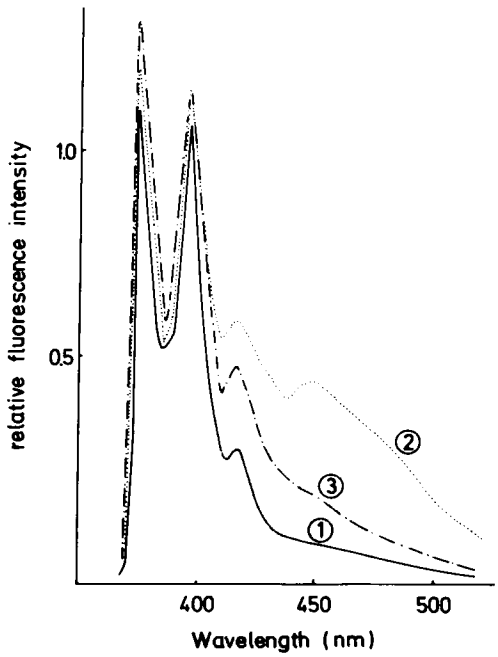

FIGURE 1 Superposition of emission spectra of pyrenemaleimide-labeled sarcoplasmic reticulum vesicles. The spectra were redrawn from the publications of Papp et al. (1986) and Lüdi and Hasselbach (1983). (*1*) Spectrum obtained by Papp et al. (1986) (Fig. 1) with *N*-(1-pyrene) maleimide at a labeling ratio of 1:1 (mol label/mol ATPase). The labeling was carried out at 2°C for 3 h in the presence of Ca^{++} ions. Similar spectra were shown throughout the paper, even for the dithiothreitol adduct of pyrenemaleimide. (*2*) Spectrum obtained by Lüdi and Hasselbach (1983) (Figs. 1 and 2). The labeling was carried out at room temperature for 1 h in the presence of 50 μM Ca^{++}. This spectrum can be compared with the spectrum of 1,3-di(1-pyrene)propane (Dangeau et al., 1982; Fig. 1) or with the spectrum shown by Galla and Sackmann (1974) for pyrene in a dipalmitoyllecithin bilayer. All these spectra contain a significant excimer peak at around 450 nm. (*3*) Spectrum obtained after the addition of 5 mg myristoylglycerophosphocholine/mg protein to the labeled sarcoplasmic reticulum vesicles (Lüdi and Hasselbach, 1983; Fig. 2). The same spectrum as in *3* is obtained, for example, if the vesicles were labeled at a ratio of 1:20 (mol label/mol ATPase or with *N*-(3-pyrene)maleimide-labeled monomeric (or polymerized actin (Lüdi, H., and Ritter, K., unpublished results).

excimer peak. This is in agreement with Papp et al. (1986) and with previous results obtained in our laboratory (Lüdi and Hasselbach, 1984). But the main problem concerning the influence of detergents on excimer fluorescence arises from the fact that we do not think that in any of the spectra shown in Papp et al. (1986) where the labeling was done in the presence of Ca^{++} ions there exists an excimer peak. Some excimers might exist if the labeling was carried out by incubating the sarcoplasmic reticulum vesicles with EGTA for 30 min. But the influence of the detergents in the excimer fluorescence of sarcoplasmic reticulum ATPase labeled under these conditions was not investigated. In addition, even for the spectra of the pyrene maleimide adduct of dithiothreitol (Fig. 10 in Papp et al., 1986) there exists no excimer peak. This is in contrast to several publications using the same approach (e.g., Dangreau et al., 1982; and Ishi and Lehrer, 1986). This is illustrated in Fig. 1, where we superimpose the spectra obtained with the *N*-(3-pyrene)maleimide-labeled ATPase (reproduced from Lüdi and Hasselbach, 1983) and the spectra of Papp et al. (1986) where the ATPase was labeled with *N*-(1-pyrene)maleimide. The reason for the nonexistence of excimers in the preparation of Papp et al. (1986) might be due to a different labeling procedure used. In Lüdi and Hasselbach (1983), freshly prepared sarcoplasmic reticulum vesicles were labeled at ph 7.0 and at room temperature for 1 h. After the labeling the vesicles were stored in ice. Papp et al. (1986) used a sarcoplasmic reticulum vesicle preparation that was stored at −70°C in 0.3 M sucrose and the labeling was carried out at 2°C at ph 7.0 for up to 19 h.

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