LETTER TO THE EDITOR

Reply to Lüdi and Hasselbach

Dear Sir:

I believe the points raised by Drs. Lüdi and Hasselbach do not account for the differences between their report and ours (Papp et al., 1986). Lüdi and Hasselbach (1982, 1983) interpreted the excimer fluorescence observed in pyrene maleimide–labeled sarcoplasmic reticulum in terms of ATPase oligomers. This interpretation rested upon two unsupported assumptions (*a*) Pyrene maleimide was assumed to react selectively with a single SH group in the ATPase molecule, and (*b*) the decrease in excimer fluorescence caused by detergents was assumed to reflect the dissociation of putative ATPase oligomers.

No attempt was made by Lüdi and Hasselbach (1983) to document the validity of either of these assumptions. In reinvestigating these postulates, Papp et al. (1986) observed that pyrene maleimide reacts with several SH groups in the Ca^{2+}-ATPase, raising the possibility of intramolecular excimer formation; furthermore, several detergents, including dodecyl octaoxyethyleneglycol monoether ($C_{12}E_8$), that are known to dissociate ATPase oligomers, did not diminish the excimer fluorescence.

These differences cannot be attributed to different pyrene maleimide isomers used by the two groups. As noted by Haugland (1985), *N*-(3-pyrene)maleimide is an incorrect name for *N*-(1-pyrene)maleimide. The *N*-(1-pyrene)maleimide designation used by us is based on the Chemical Abstracts numbering system for pyrene. Therefore we believe that the same pyrene maleimide isomer was used in both laboratories under different names.

Lüdi and Hasselbach state that the assumed selectivity of pyrene maleimide reaction with a single SH group was based on the observations of Miki et al. (1981). In fact, Miki et al. (1981) described the fast reaction of a class of SH groups with *N*(1-anilinonaphthyl-4)maleimide (ANM), rather than with pyrene maleimide; furthermore, beyond simple kinetic measurements, no direct chemical evidence was presented by Miki et al. for preferential reaction of ANM with a "fast reacting thiol group."

Using peptide mapping we found that pyrene maleimide reacts nonselectively with several SH groups (see Fig. 4 in Papp et al., 1986), contrary to the claim by Lüdi and Hasselbach, peptides 6 and 7 did not react selectively, since their disappearance was accompanied by large decrease in the labeling of peptides 1, 2, and 3 as well, even at pyrene maleimide concentrations as low as 10 nmol/mg protein.

We are pleased that Lüdi and Hasselbach now revised their observations on $C_{12}E_8$ bringing it closer in line with ours. However, if $C_{12}E_8$ (and a number of other detergents tested in our work) do not abolish excimer fluorescence while they dissociate ATPase oligomers, this further undermines the validity of the interpretation of excimer fluorescence as arising from oligomer formation.

The original report of Lüdi and Hasselbach (1983) does not state that the reaction of sarcoplasmic reticulum vesicles with pyrene maleimide was carried out at room temperature. Exposure of sarcoplasmic reticulum vesicles for 1 h to room temperature significantly inhibits their Ca^{2+} transport activity. This may explain the low level of Ca^{2+} accumulation reported by Lüdi and Hasselbach (1983). To avoid this problem, we performed the labeling at 2°C in a medium of identical composition to that used by Lüdi and Hasselbach (1983). The somewhat lower level of excimer fluorescence reported by Papp et al. (1986) may indicate a smaller amount of denatured Ca-ATPase, but clearly there was significant excimer fluorescence.

In summary I quote one of the closing sentences of the report of Papp et al. (1986) to restate our position: "While the observations of this report do not exclude contributions by ATPase–ATPase interactions to the excimer fluorescence, they suggest that such contributions may not be sufficiently exclusive or dominant to consider the excimer fluorescence a reliable and established indicator of ATPase–ATPase interactions."

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