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Letter to the Editor

Quenched Fluorescence of Chlorophyll Bilayer Lipid Membranes

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In a recent article, Steinemann *et al.* (A. Steinemann, N. Alamuti, W. Brodmann, O. Marschall & P. Läuger, 1971. *J. Membrane Biol.* **4**:284) illustrate the effectiveness of 2.5 mM potassium peroxodisulfate ($K_2S_2O_8$) in quenching the fluorescence of chlorophyll containing bilayer lipid membranes (chl-BLM). Exactly half of the fluorescence was quenched by adding the $K_2S_2O_8$ to the solution on each side of the membrane, so that no fluorescence remained after adding the chemical to both sides. The article further states (p. 293), "if $K_2S_2O_8$ is added to a methanolic solution of chlorophyll *a*, the green color slowly disappears," and one may then conclude that the $K_2S_2O_8$ discolors the chlorophyll in the biface, thus accounting for the fluorescence loss. We report data here which disagrees with this conclusion, but which, nevertheless, is in agreement with the Steinemann *et al.* data.

In our experiments we have used spinach chloroplast extracts prepared as previously described (J. S. Huebner & H. T. Tien, 1971. *Biochim. Biophys. Acta* **256**:300). The optical absorption of aqueous suspensions of submicron particles of these extracts indicate the predominant pigment is chlorophyll. Such aqueous suspensions are a better model of chl-BLM than the methanolic solutions of chlorophyll used by Steinemann *et al.* We have found that the green color of these suspensions is not lost even after several days in 2.5 mM $K_2S_2O_8$ solutions, provided the pH remains above 5. If, however, the pH is less than 4, the green color disappears even in the absence of $K_2S_2O_8$. Since the addition of $K_2S_2O_8$ produces a decrease in pH of unbuffered solutions, the color loss observed by Steinemann *et al.* may be caused by the low buffering capacity of their methanolic solutions. Further, if 2.5 mM $K_2S_2O_8$ is added to either or both membrane bathing solutions, the chl-BLM photoelectric effects are seen to be enhanced from 2 to 10

times in about 20 min. This is the time required for the fluorescence quenching observed by Steinemann *et al.* to occur.

We thus conclude: (1) that $K_2S_2O_8$ does not discolor chlorophyll in a lipid biface, a result which optical absorption measurements on chl-BLM should verify; (2) that $K_2S_2O_8$ does quench the chlorophyll fluorescence in a process which also enhances the electrical charge transport when a suitable asymmetrical condition exists across a chl-BLM (a 2.5 mM $K_2S_2O_8$ gradient in a sufficient asymmetry to induce chl-BLM photovoltages of a few millivolts). This second point is quite reasonable; energy lost to fluorescence is obviously not available for promoting electrical charge transport. We note that these results are still in agreement with the previously proposed position of chlorophyll in a lipid biface (H. P. Ting, W. A. Huemoeller, S. Lalitha, A. L. Diana & H. T. Tien, 1968. *Biochim. Biophys. Acta* **163**:439) as pointed out by Steinemann *et al.*

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