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THE 'ENERGISED STATE' IN BACTERIORHODOPSIN SHEETS

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It has recently been proposed /1/ that the 'energised state' of energy-transducing biomembranes may perhaps best be viewed as a charging of the interfacial membrane/solution capacitances, and that the protic current flow coupling such processes as electron transport and ATP synthesis may be largely confined to the interfacial regions /1,2/ in vivo. Such coupling protons would not be osmotically active. Whilst the uptake of lipophilic substances has been widely used as a probe of energisation in biological membrane systems /3/, the interpretation of such data is complicated by the fact that, for thermodynamic reasons, most bioenergetic systems form vesicles upon isolation, and it is unclear to what extent the probe molecules are binding to membranes or are osmotically active. Recent experiments using resonance Raman spectroscopy indicate that a great deal of binding may in fact occur /4/. However, purple membrane sheets /5/, isolated from Halobacterium halobium do not form vesicles and the effect of illumination of this system on its ability (or otherwise) to interact with 'probe' molecules should provide much information on the requirement or otherwise of an intact vesicular structure for membrane energisation. The purpose of the present communication is to describe a novel polarographic assay for commonly-used 'probes' of energisation, and to report that the energised state is readily observed in bacteriorhodopsin sheets by this method.

In a typical experiment differential pulse polarograms or linear sweep voltammograms were performed on a solution of the probe of choice, in the dark and under illumination, and then an appropriate concentration of bacteriorhodopsin sheets were added, polarography being again performed in the dark and in the light. Of those probes currently used to monitor membrane energisation we have tested safranine, 9-amino acridine (9AA), 1-anilinonaphthalene-8-sulphonate (ANS) and Tl^+, since all are electroactive at the mercury electrode. The electrochemical apparatus has been described elsewhere /6/.

Figure 1 shows linear sweep voltammograms at the hanging mercury-drop electrode of a safranine solution (dark or light) in the absence (trace a) or presence (traces b, c) of bacteriorhodopsin sheets. Of the 4 peaks given by the native solution, two are not observed in the presence of bacteriorhodopsin, and the major peaks (II and III) are reduced, implying that some of the safranine is bound by the sheets. This binding is increased upon illumination. In the case of the negatively-charged ANS, illumination causes a desorption of probe molecules bound in the dark (data not shown).

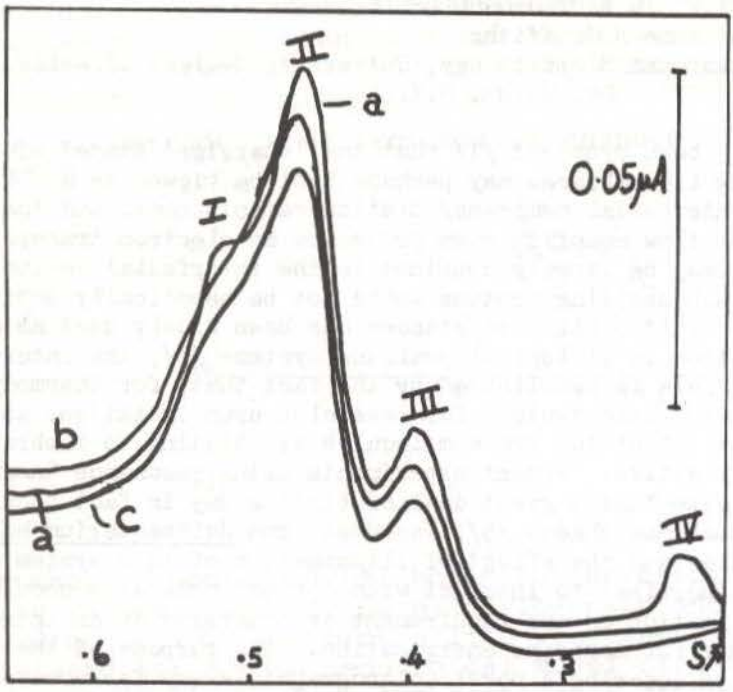

Fig.1. Binding of safranine by illuminated bacteriorhodopsin sheets

Single sweep voltammograms (20 mV/s) were taken of safranine solutions (33 μM) in 0.1 M Tris-Cl pH 7.0. In traces b (dark) and c (illuminated with orange light from a 250W projector lamp) bacteriorhodopsin sheets (90 μM) were also present. All scans were taken with a fresh Hg drop from the point marked S.

In conclusion, we feel that care should be exercised in the interpretation of experiments using lipophilic probe molecules, and that the bacteriorhodopsin sheet system provides a useful control for the energy-dependent binding of such molecules to biomembranes.

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