Structural comparisons of native and reaggregated membranes

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The problem of obtaining detailed information about the organization of membrane components would be simplified if the membranes could be properly reconstituted from the separated membrane lipids and proteins. This would allow specific labelling of each membrane component with appropriate probe groups to report on the interactions of the components in the reassembled structure. It would also provide more detailed information about the interaction of anaesthetics and so on with the membrane.

There are several reports in the literature that membranes which have been substantially separated into their lipid and protein components can reaggregate under appropriate conditions to structures which are similar to the native membranes. The most extensively studied reaggregates are of *Mycoplasma laidlawii* membranes, obtained by dissolving the membranes in 10 mM sodium dodecyl sulphate (SDS) and allowing the soluble lipo-protein complex to reaggregate by dialysing against a buffer containing Mg^{2+} ions (Engelman, 1968). The reaggregated structures resemble the intact membranes in composition and buoyant density; in electron micrographs the reaggregates also show the characteristic triple layered structure of the original membranes.

We have extended these criteria to more stringent tests of structural organization based on probe techniques using nuclear magnetic resonance (Metcalfe, Seeman & Burgen, 1968) and sensitized fluorescence measurements. By these methods it is clear that the reaggregates differ substantially from the original membranes in their interactions with probe molecules including benzyl alcohol and ANS (8-anilino-1-naphthalene sulphonic acid). The reaggregates have binding properties for the probes intermediate between the intact membrane and the fully separated components. The most important difference is that the reaggregates have many protein binding sites which are not available in the intact membrane structure, suggesting that some of the membrane proteins have not been properly reconstituted. There is evidence that a lipid bilayer is reformed in the reaggregates, and that it has the same thermal transition as the original membrane. The intermediate nature of the reaggregate structures probably accounts for their similarities to the original membranes by the gross structural criteria previously applied.

The probe experiments show that the reaggregates cannot be used to achieve the specific labelling of membrane components required for detailed structural studies. On the other hand we have established a sensitive set of criteria for intact membrane structure and particularly for the conformation of membrane proteins which we consider to be essential for the integrity of the membrane.

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