Reconstitution of membrane proteins: A selected bibliography from Biophysical Society workshop on membrane protein reconstitution, 2 March 1988

Dear Sir:

A workshop on the reconstitution of membrane proteins was held as part of the recent Biophysical Society meeting in Phoenix, Arizona. Drs. Terry Allen (University of Alberta), Dov Lichtenberg (Tel Aviv University), Burton Litman (University of Virginia), Michael Raftery (University of Minnesota), Anthony Scotto (Cornell Medical School), and Anne Walter (Wright State University) discussed both general considerations and specific methods that have proven useful in the reconstitution of a variety of membrane proteins, including viral coat proteins, ion pumps and channels, receptor proteins, and membrane enzymes. The speakers have provided the following bibliography for the material that was discussed, in the hope that it may help other workers to gain entrée to the field or to formulate new approaches to a technology that has often seemed more like a black art than a science.

Not surprisingly, it was evident from the presentations at the workshop, and from the general discussion that followed, that no one strategy is likely to serve equally well for the reconstitution of all membrane proteins. As the bibliography below will illustrate, the speakers described how a variety of detergents (and in some cases, no detergents) have been employed, under a variety of experimental conditions, in efforts to reconstitute active and well-defined preparations of membrane proteins. In spite of this diversity in the approaches applied to the problem of reconstitution, some useful general principles emerged as recurrent themes in the workshop presentations.

One of the most important considerations in the design of any reconstitution strategy stems from the fact that the reconstitution of a membrane protein with lipid typically occurs through coalescence of protein/lipid/detergent complexes with lipid or lipid/detergent complexes, usually under nonequilibrium conditions. It is thus important to consider the compositions, structures, and stabilities of both types of complexes and how these may change during the overall reconstitution procedure. At a fundamental level, the workshop speakers stressed the importance of knowing the compositions and the relative proportions of the different moecular aggregates present in a given protein/lipid/detergent sample (vesicles, mixed micelles, etc.), and how these quantities depend on such variables as the overall sample composition and the experimental temperature. This question is particularly acute when detergents are employed, since detergent molecules partition freely between molecular aggregates and the aqueous phase. Thermodynamic approaches were described to quantitate and, for new applications, to predict the compositions of the vesicles and/or mixed micelles that form in protein/lipid/detergent mixtures of varying compositions. The importance of this question was illustrated by observations that the sizes and the stabilities of vesicles produced by detergent removal from lipid/detergent mixed micelles can depend strongly on the residual content of detergent in the vesicles.

Kinetic as well as thermodynamic factors can be of key importance in the design of a successful reconstitution procedure. This point was illustrated by descriptions of the mechanisms of vesicle formation and growth when micellar detergent/lipid dispersions are diluted or dialyzed. Rapid dilution of cholate/phosphatidylcholine or octyl glucoside/phosphatidylcholine samples, for example, leads initially to the formation of small vesicles, which must be subsequently be equilibrated to generate the larger vesicles that are most useful in many reconstitution studies. Such equilibration proceeds slowly (in hours to days) when cholate is used but rapidly (in minutes) when octyl glucoside is used in such reconstitutions. Interestingly, for the reconstitution of rhodopsin with lipid using octyl glucoside, rapid dilution of micellar protein/lipid/detergent samples provides more compositionally homogenous preparations than does the slow removal of detergent by dialysis. The explanation for this somewhat suprising result lies in the fact that lipid/detergent micelles and protein/lipid/detergent micelles revert to vesicles at distinct octyl glucoside concentrations when the detergent is removed slowly; rapid dilution of the micellar sample circumvents this problem. Successful reconstitution strategies may thus sometimes require the avoidance, rather than the achievement, of true equilibrium during the removal of detergent from protein/lipid/detergent micelles. Practical methods were discussed to optimize the convenience and the reproducibility of reconstitution methods that demand either very rapid or very gradual treatments to achieve a homogenous association of membrane proteins with excess lipid.

A final general lesson that was clear from all of the speakers' presentations was the necessity for thorough characterization of the composition, the homogeneity, and the functionality of a reconstituted membrane protein preparation. Such groundwork is important, not only to demonstrate that the properties of a reconstituted protein truly reflect its properties in the native membrane, but also to assess how well a given approach can produce biochemically or pharmacologically active samples with a homogenous and controllable composition. It is only by the gradual accumulation and evaluation of such information that the "science" of membrane protein reconstitution will be placed on the systematic footing that will make it most useful to membrane workers generally.

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