PROTEIN TRANSPORT PROCESSES IN THE WATER-WATER INTERFACE IN INCOMPATIBLE TWO PHASE SYSTEMS

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Proteins, such as ovalbumin, albumin, cytochrome C or a chromobacter lipase were added to either the upper or lower phase of a PEG 6000, Dextran T500 system, and the phases placed carefully in contact. Diffusion processes and interfacial accumulation were then observed by using the optical scanning system of an MSE Centriscan ultracentrifuge run at low speeds. The overall process was one of simple diffusion, though in such complex systems movement up the protein concentration gradient can occur and was observed. Interfacial accumulation roughly in accord with expectation based on a simple interfacial tension theory was also seen.

INTRODUCTION

The phenomenon of incompatible phase separation is known and largely understood. Tompa for example gave a full theoretical description in 1956 [1].

Since then Ogston and coworkers over a long period applied the concepts of coexclusion to biological macromolecules, particularly hyaluronic acid and related carbohydrates. This resulted in theoretical developments [2,3] and an awareness of the role that coexclusion can play in the functional properties of biological macromolecules [e.g. 4].

More recently it led to an interesting insight into the way in which coexclusion can affect the diffusion of macromolecules in matrices [5,6]. Rather unexpectedly diffusion can be greater in the presence of a matrix than in its absence in properly chosen conditions [7].

In a distinct line of investigation, Albertsson and coworkers [8] used multiple aqueous phase systems for the purification and isolation of both macromolecules and cell fragments.

Despite all this there has been almost no work on the events at the water-water interface. This is surprising since it would appear to offer excellent opportunities to model biologically significant systems.

While it is unlikely that any biological situation occurs where two phases are produced by coexclusion (with the possible exception of mobilised seed protein bodies[9]), membranes almost always separate two aqueous phases. Thus the water-water interface provides a locus, at thermodynamic equilibrium, in which synthetic membranes could be examined.

Numerous processes of biotechnological interest involve the movement of proteins towards, into and through matrices. The production of immobilised enzymes, biosensors, and the use of multiple phase separation itself all involve a diffusion step. Moreover, there is often a matrix, or at least a concentrated solution to be penetrated.

There are few previous studies bearing on this. England and Nazarian [10] measured the diffusion rate of ribonuclease and concluded that the interface was freely penetrated near the critical point composition.

Shanbhag [11] made some transfer rate measurement on proteins in PEG-dextran systems in stirred cells, and found a relationship between partition coefficients and transfer rate. Meares [12] has recently analysed interphase diffusion in terms of stagnant layers. Wells [6] and Laurent et al [7] have developed ideas about diffusion to account for events at unstable layers, while Linde [13] has considered two immiscible fluids.

EXPERIMENTAL

We measured the concentration profile by using the scanning optical system of an MSE Centriscan ultracentrifuge. We loaded 0.22 ml of lower phase followed by the same volume of top phase into the 10 mm cell. At the position of the interface the cross sectional area was 25.9 mm^2. The rotor was run at 4000 rpm at 25° which should produce no significant sedimentation. Appropriate blanks were included, mainly the two phases with no added protein.

Most experiments were done in the two phases produced by mixing 5 g of PEG 6000 (BDH Biochemical grade, with low 280 nm absorbance) 5.3 g of Dextran T500 (Pharmacia) with 100 ml of 50 mm sodium phosphate pH 7. After equilibration phases were separated and protein added to the appropriate phase. Proteins were obtained from Sigma, FITC derivatives from Molecular Probes Ltd, except lipases and FITC lipases, which were prepared as described elsewhere [16].

Surface tension measurement (we thank Dr J. Mead) by the spinning drop method was $0.09 \pm 0.03 \times \Delta\rho$ (about 0.03) so that the interfacial energy was 0.0027 - 0.0036 erg. cm^{-2}.

RESULTS

Transient water fluxes

Wells [6] has recently proposed a theory to describe disequilibria due to diffusion in moderately concentrated polymer solutions. Although this was intended for layered solutions, it can also be applied to quaternary two-phase systems.

Considerable transient water flux would be expected and, as is shown in Fig. 1, complex steps in the concentration gradients formed. These gradually

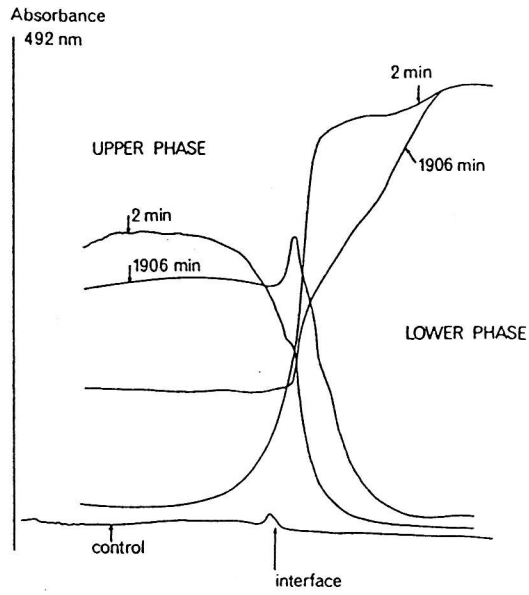

Fig. 1 *Concentration profiles measured in three cells run simultaneously. The lowest, control trace, contained no protein. The others are for fluorescein labelled bovine serum albumin at 1 mg/ml in either the upper or lower phases*

disappeared, and after about 3 days equilibrium was reached, and it was possible to assess the magnitude of the interfacial accumulation.

The initial transient fluxes were asymmetric, and were greater when protein was initially in the lower phase than when it was in the upper phase. Predialysis almost completely eliminated them: in this, both phases, containing protein were inside the sac, and both phases with no protein were outside the sac. After dialysis to equilibrium, which would result in a small hydrostatic pressure difference, the protein-containing phases from inside the sac were placed in contact with the corresponding phase from outside.

Simplification of the interfacial accumulation theory described by Albertsson [8] leads to a relationship

$$\frac{C_i}{C_1} = e^{6.8\,\tau_{12}}$$

where C_i is the interfacial concentration, C_1 the bulk phase concentration at equilibrium, and τ_{12} the interfacial tension in the absence of protein.

This predicts values of 3-4% for the interfacial excess concentration, as found for albumin and ovalbumin. Cytochrome C and the lipase gave higher values, but of the correct order of magnitude.

CONCLUSIONS

Interfacial turbulence and accumulation are important in the practical application of phase separation, particularly where it is hoped to separate the phases without the use of centrifuges. These results show that very complex initial transient fluxes occur and have implication for the correct choice of phase and systems.

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