Table 1. Effect of increase in total initial concentration \((C^0)\) on monomer, dimer and total species concentrations \((c_1, c_2, \text{and } \Sigma c_i)\) of an isodesmically associating system at sedimentation equilibrium

<table>
<thead>
<tr>
<th>System: (M_i = 5 \times 10^4; \bar{c}_i = 0.63 \text{ ml/g}; \rho = 1.0 \text{ g/ml}; T = 25^\circ \text{C}; a = 6.8 \text{ cm}; b = 7.1 \text{ cm}; \text{speed} = 3000 \text{ rev/min}; k = 1.5 \text{ (units of (C^{-1})).}</th>
<th>(C^0 = 1)</th>
<th>(C^0 = 2)</th>
<th>(C^0 = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r)</td>
<td>(c_1)</td>
<td>(c_2)</td>
<td>(\Sigma c_1)</td>
</tr>
<tr>
<td>6.80</td>
<td>0.102</td>
<td>0.031</td>
<td>0.134</td>
</tr>
<tr>
<td>6.95</td>
<td>0.218</td>
<td>0.143</td>
<td>0.484</td>
</tr>
<tr>
<td>7.05</td>
<td>0.366</td>
<td>0.402</td>
<td>1.80</td>
</tr>
<tr>
<td>7.10</td>
<td>0.475</td>
<td>0.677</td>
<td>5.74</td>
</tr>
</tbody>
</table>

\(10^{-4} M_1^2 = 1.82 \text{ (0.92\*)}\) \(2.94 \text{ (1.01\*)}\) \(4.12 \text{ (0.99\*)}\)

* Values of \(M_1^2\) in parentheses are those obtained from eqn. (6) if \(c(h)\) had been taken as the value at \(r = 7.075 \text{ cm}\) rather than \(7.10 \text{ cm}\).

molecular weight. Combination of heterogeneity and non-ideality functions has proved a very recalcitrant problem (Williams, 1972).

Although no easy solution to this problem emerges, the values of \(M\) and \(\sigma\) (log \(M\)), always regarded as underestimates, do have comparative value. There is, however, an alternative representation of the heterogeneity; in terms of an isodesmic association (Kim et al., 1977). Although the concentration-dependence of \(s\) or \(M\) for these materials does not immediately indicate such an association, the extreme curvature near the base in sedimentation equilibrium (Creeth & Knight, 1968), together with the absence of a plateau in sedimentation velocity (Creeth & Knight, 1967), are suggestive features, and make the possibility worth examining.

A striking property of isodesmic association in the ultracentrifuge (in certain concentration ranges) is that, as the total concentration rises, the distribution of monomer and low polymers may be little affected, whereas the concentration of the higher polymers, detectable only near the cell-base, is greatly increased (Table 1). Clearly, if observation is restricted to, say, 95% of the total column length, the very steep rise near the base would be undetected, and the characteristic increase in \(M_1^2\) could well remain undetected.

Experimental verification of the association has so far been obtained only with two samples, but this is sufficient [together with the results reported by Hill et al. (1977) for a glycoprotein of simpler structure] to suggest that the gel-forming properties of these materials are a simple consequence of well-defined thermodynamic properties observable at all concentrations. Such a conclusion would have important physiological implications, for these glycoproteins are intimately concerned with protection of all mucous membranes.


Nichol, L. W., Jeffrey, P. D. & Milthorpe, B. K. (1976) Biophys. Chem. 4, 259-267


Ultra-centrifuge studies of interactions and equilibria: impact of interactive computer modelling

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Important aspects of the behaviour of proteins including co-operativity, allostery and structural stability reflect the presence of specific molecular interactions located at interfaces between subunits or whole molecules (Klotz et al., 1975). Much effort has therefore been devoted to quantifying the energies of these interactions with the hope that in time it will be possible to relate them to features of the amino acid composition of the contact interfaces. It has, however, turned out to be exceedingly
difficult to make measurements of the necessary precision, owing partly to limitations, partly to difficulties arising from the substances themselves. Occasionally even the chotioio-
metry and scope of the reactions involved can remain in doubt. In such circumstances, recourse is needed to a wide range of methods, the results of which must be consistent for acceptance. Two methods that have been used extensively since the inception of such studies are equilibrium ultracentrifugation and velocity
ultracentrifugation. Their application has been reviewed by
Adams (1967), Aune (1978), Creeth & Pain (1967), Eisenberg
(1976), Fujita (1975), Roark & Yphantis (1969) and Van Holde
(1975) among others.

It will be noticed from these reviews that equilibrium ultracentrifugation has been the more favoured of the two
methods, particularly because it provides a firm thermodynamic
basis from which to interpret results. Very pertinent, however, is
the remark by Van Holde (1975), who says concerning this
method ‘Unfortunately, it is much more difficult than it sounds
to obtain a unique set of parameters and show that it is unique’. Contributing to this difficulty is the relative lack of resolution of
the equilibrium method, as has been demonstrated numerically
by Aune (1978).

On the other hand velocity ultracentrifugation suffers from
the disadvantages that attach to non-equilibrium transport
methods, but to balance these it has greater resolving power. In
one respect too, it is a true equilibrium method since, as shown
by Goldberg (1953), the movement of the equivalent sharp
boundary (Longsworth, 1943) between plateau region and
solvent depends only upon the properties of the molecules within
the plateau region, and is completely independent of re-
equilibrium reactions occurring within the boundary. Even so,
current interest is in the actual shape of the boundary, and in
finding out how to extract the information that it carries about
the mass and reactions of the molecular species present.

Algebraic analysis of the exact shape of a boundary in a
sector-shaped cell, taking into account time, composition and other
parameters, is so difficult that it has been almost entirely
superseded by computer modelling based on the numerical
solution of the transport equations. This has been achieved in
different ways, described in original papers by Bethune &
Kegeles (1961), Cann & Goad (1965), Goad & Cann (1969),
Cox (1965, 1971) and Dilson et al. (1966). The work of these
authors and others is reviewed in several places, e.g. by Cann
and Claverie (1976) have lately made effective use of the method of
finite elements. However, as Cox (1978) points out, there has
not yet been a comparative test of the efficacy of the different
methods, and it would be valuable to have one done that uses the
same test systems for each of the procedures in turn. Most
published simulations have aimed to show what might be
expected in general from interacting systems during sedi-
mentation rather than to evaluate the parameters of actual
systems. The latter aspect is what we discuss now.

At least three papers have compared computer-simulated
tscherien patterns with experimental patterns. Our own (Gilbert
& Gilbert, 1973) used the program given by Cox (1971, 1978)
to calculate schlieren patterns of β-lactoglobulin A under
polymerizing conditions for comparison with the experimental
data of Armstrong & McKenzie (1967). Conclusions were
reached in agreement with those of Townend & Timasheff
(1960), but quite at variance with those of Adams & Lewis
(1968), who studied the same system by equilibrium centri-
fugation. Frigon & Timasheff (1975) successfully simulated
their experimental schlieren patterns of tubulin, and Inners et al.
(1978) simulated the concentration profile of α-chymotrypsino-
gen A (see also Trautman et al., 1969). In all cases the program
of Cox was used.

Imitating a schlieren pattern faithfully is, of course, a
guarantee that the parameters used are valid, but failure
certainly casts doubt on them. It may well be that discrim-
inution between alternatives thrown up by other methods will be
one of the principal uses of the technique. Our own experience
with β-lactoglobulin A emphasized the need for fast interactive
computing that we lacked at that time. Without it the different
parameters cannot be adjusted iteratively in a reasonable time.
In addition, the final comparison between calculated and
experimental schlieren patterns requires a fast accurate plotter
and a projection system for superimposing plot and photog-
graphic image. An essential intermediate step is interactive
comparison of measured schlieren pattern with computed
pattern both numerically and visually on a visual-display-unit
screen.

A thorough test of the validity of simulation procedures must
include the analysis of simple systems as well as interacting
systems. For this purpose we have analysed data in the form of
schlieren plates kindly provided by Drs. K. A. Cammack and Dr.
D. S. Miller for the clinically important enzyme Erwina corallo-
ora L-asparaginase (EC 3.5.1.1), which they have already thoroughly characterised (Cammack et al., 1972). In
Fig. 1 we show a simulated schlieren pattern superimposed on
a photographic projection of an experimental pattern. The
simulation was carried out by using the program given by Cox
(1978) modified to keep the co-ordinate system centred on the
moving boundary. The close correspondence between the two
patterns has been achieved by using in the program a diffusion
coefficient (assumed independent of concentration) appropriate
to a mol.wt. of 140000. This value lies within the range of values
(134000–144000) found by Marlborough et al. (1975).

Brief summary of simulation procedure

(1) The parameters, g, s, v and p (see the legend to Fig. 1) are
determined in advance.

(2) From a given run, select two schlieren patterns, one at
time t, just clear of the meniscus, the other at time t, still clear
of the cell bottom, and measure their co-ordinates with a
projection microcomparator. Measure the solvent base-line for

(3) Subtract base-line from schlieren by computer, allowing
for any difference in meniscus position, to give corrected

Fig. 1. Computer simulation of UC schlieren pattern of L-asparaginase superimposed upon experimental pattern

Experiment: Cammack et al. (1972) and unpublished results
from Drs. K. A. Cammack and D. S. Miller. Schlieren pattern
(121 min frame) for L-asparaginase, 7.79 mg/ml pH 7.4, 0.11
NaCl, 0.11 NaH2PO4/NaHPO4. Beckman Model E, 44770 rev/
min, 25°C. Sedimentation to right. Simulation: Computer
program adapted from Cox (1978). Input 41 min frame,
simulation period 80 min. Parameters: S20, w, solvent = 7.60S,
mol wt. = 140000, hydrodynamic coefficient g = 0.003 ml/g, 
diffusion coefficient D20, w, solvenr = 5.26 x 10-11 m2/S, 
v = 0.739 ml/g, p = 1.007 g/ml.
ordinates $Y$ versus radial distances $r$. Smooth $Y$ by using orthogonal polynomials and integrate to obtain area. Equate total area to plateau concentration to give the scaling factor for converting area into concentration $w$ for each value of $r$. Enter these values of $w$, $r$ for time $t_1$ and the parameters in (1) above into the program given by Cox (1978).

(4) Begin the iteration by choosing a reasonable starting value for molecular weight $M$ and hence diffusion coefficient $D$ (assumed to be independent of concentration) and integrate for a time $t_2 - t_1$.

(5) Compare for time $t_2$ the resulting pattern with the experimental file for $Y$ versus $r$. Vary $M$ until a good correspondence is found on the visual display unit.

(6) Proceed to the next stage of refinement by referring back to the original photograph. To do this, add the base-line for $t$, to the scaled simulation, plot the result, and compare by superposition on a projection micro-comparator, as in Fig. 1.

Conclusion

Besides its use for assessing interaction in protein systems, interactive computer simulation would seem to be a useful accessory for interpreting any centrifuge experiment. It can provide the norm, departures from which imply interaction or some unusual behaviour. It may be noted that the greatest limitation to interpreting past experiments is the absence of appropriate base-lines, and it is to be hoped that in future base-lines will be obtained by re-runs at exactly equivalent times with solvent only and without disassembling the cell.

This work was supported throughout by the Science Research Council. We thank Dr. K. A. Cammack and Dr. D. S. Miller for providing extensive experimental data, and Professor D. J. Cox for a copy of his program. Mr. R. J. Chance, Miss C. E. Evans and Mr. W. R. Kissack for valuable help, and Dr. S. P. Spragg for continual advice.

Adams, E. T., Jr. (1967) *Fractions No. 3*, pp. 1–12, Spinco Division, Beckman Instruments, Palo Alto

Novel applications of the analytical ultracentrifuge: molecular-weight and morphological studies on phospholipid vesicles

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Solution studies of membranes and their different components have been hampered by problems of homogeneity, of obtaining accurate partial-specific-volume determinations and by the dynamic molecular behaviour of lipid molecules. Recently, purified membrane proteins have been studied in detergents to give specific structural information about the protein in a solvating micellar shell. Many phospholipids, on the other hand, spontaneously form bilayers that have a wide variety of different dimensions and physical properties, making them, as such, quite unsuitable for direct investigation.

In an effort to use ultracentrifugation and other solution techniques to gain an insight into the molecular properties of phospholipid bilayers, a basic feature of natural membranes, the phospholipid dimyristoyl phosphatidylcholine was chosen for study (Watts et al., 1978). Bilayers of this lipid in aqueous solution underwent an ordered-to-disordered phase transition at the readily accessible temperature of 23°C. On ultrasonic radiation above this temperature, small single bilayered vesicles are formed, and these by chromatography are shown to possess a high degree of homogeneity. Molecular-weight determinations from sedimentation and diffusion experiments at 5°C intervals from 10 to 30°C show that the vesicles have a molecular weight of 1–93 (±0.10) x 10^6, corresponding to 2850 ± 140 molecules per vesicle. Partial specific volumes were measured by determining the vesicle iso-density point through interpolation, when sedimented in H_2O/H_2O-buffered solutions. The bilayer phase transition is then shown by a change in bilayer density.

Electron-microscopic evidence showed that the vesicles are spherical, which was also confirmed by solution studies from calculation of the $\beta$-function and by using additional measured viscosity data. Assuming vesicle sphericity then allows information about the bilayer hydration to be obtained by using standard analyses. The degree of hydration was found to increase markedly on lipid-chain 'melting'. The vesicle dimensions were then obtained by assuming a three-compartment model for the vesicle, comprising the lipid bilayer itself, the occluded vesicle volume and the hydration layer on the vesicle surface. It was found that, concomitant with acyl-chain 'melting' at 23°C, the vesicle trapped volume was measured to increase 6-fold, the vesicle outer radius was calculated to increase from

1980