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Biotechnology: A New Frontier for Molecular Thermodynamics

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BIOTECHNOLOGY: A NEW FRONTIER FOR MOLECULAR THERMODYNAMICS

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Abstract

Thanks to growing scientific knowledge at the molecular level and to the awesome growing power of computers, it may now be possible to apply molecular thermodynamics toward the development and production of biochemicals. To illustrate that possibility, a few examples are presented; these include extraction for isolating solutes from dilute aqueous solution; extraction using organic solvents containing reverse micelles; phase separation in liquid solutions of large molecules using shear; entropy-driven adsorption of enzymes and finally, molecular-simulation calculations to determine the catalytic properties of a mutant enzyme.

While novel future applications will require much dedicated research, it is necessary to start now to build the necessary foundations. Emphasis must be directed toward establishing a representative data base and toward increasing familiarity with new experimental methods and new theoretical concepts. To apply molecular thermodynamics to biotechnology, it is particularly important for chemical-engineering thermodynamicists to give attention to the properties of aqueous systems containing salts and large, charged molecules.

The prevailing goal of molecular thermodynamics is to interpret, correlate and predict thermodynamic properties for development of chemical products and for design of chemical operations, especially separation operations. During the past two generations, molecular thermodynamics has contributed primarily to chemical engineering in the petroleum and petrochemical industries. In the next generation, it is likely that molecular thermodynamics can make similar contributions to the biotechnical industries.

Molecular thermodynamics uses microscopic knowledge toward better understanding of macroscopic thermodynamic properties. In biotechnology, the molecules are more complicated than those in conventional chemical technology but, thanks to growing scientific knowledge at the molecular level and, thanks to powerful computers, it should be possible to direct that knowledge toward more efficient production of biochemicals.

Before presenting some illustrations, we must ask: what can we expect? If molecular thermodynamics is extended to biotechnology, what benefits can we

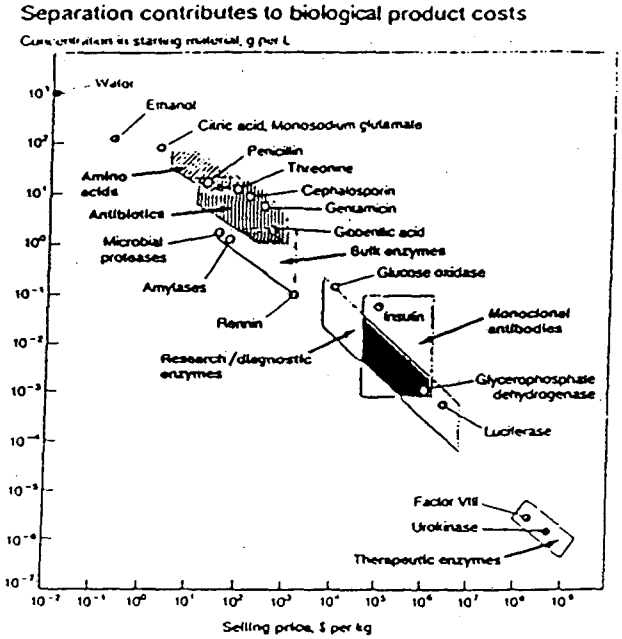
anticipate? We must not expect too much, at least not soon. Much of biotechnology is recent; therefore, at present, primary attention of bio-scientists is directed toward creating new products, not toward large-scale economic production. However, history shows that molecular thermodynamics has been most useful for mature industries; it is only when competition becomes important and when production is scaled up, that molecular thermodynamics can provide important economic benefits. It is sobering to remember that successful oil refineries were built many years before chemical engineers used chemical potentials or fugacities and that sulfuric-acid and nitric-acid plants existed long before the Debye-Hückel equation. Nevertheless, for those biotechnical industries which have reached maturity and for those that are expected to do so in the next generation, it is likely that molecular thermodynamics can provide a useful service, provided that we make an effort now to build the necessary foundations.

The main application of molecular thermodynamics is likely to be for design of separation operations, in particular for separating products from dilute aqueous solutions. There is much economic incentive to perform such operations efficiently, as shown in Figure 1. Many valuable biochemicals are produced in reactors where the product concentration is very small. The cost of production rises rapidly as the concentration falls.

Molecular thermodynamics is synthetic; it integrates molecular knowledge toward a useful goal. A few examples serve to illustrate how molecular thermodynamics can yield useful results for engineering operations in biotechnology.

It is convenient to present these examples under three headings corresponding to three time frames: short-range, intermediate-range and long-range research. We begin with short-range research, i.e. research where much of the basic knowledge is already available.

Figure 1



The selling price of products is a strong function of product concentration and consequently the cost of separation and/or purification. The reactor cost is typically less than 25% of total production cost. Three distinct categories are evident.

Figure 2

Correlation of Solubilities of Amino Acids

Amino-Acid/Water Chemistry

(1) $A_{(s)} \leftrightarrow A^{\pm}_{(l)}$ (2) $A^{\pm}_{(l)} \leftrightarrow A^{-}_{(l)} + H^{+}_{(l)}$

(3) $A^{\pm}_{(l)} + H^{+}_{(l)} \leftrightarrow A^{+}_{(l)}$ (4) $H^{+}_{(l)} + OH^{-}_{(l)} \leftrightarrow H_2O$

$K_i = a_{A^\pm} = x_{A^\pm}\gamma_{A^\pm}$

- Measure x_A (total) at known pH
- Obtain relative amounts of A^+, A^-, and $A^\pm$ from mass balance and K for reactions 1 thru 4
- Calculate γ's from molar excess Gibbs Energy, g^E

$$g^E = g^{EDH} + g^B + g^{Conv}$$

EDH = Extended Debye-Hückel
B = Born (for mixed solvents)
Conv = Conventional (e.g. NRTL or Wilson)

(Note that ions also have non-Coulombic interactions)

- Given limited data, we can calculate the effects of pH, ionic strength, or solvent.

Short-Range Research

Three examples illustrate short-range research. One is concerned with correlating solubilities and the others are concerned with extraction.

In biotechnology, many molecules of interest have electric charges. Chemical engineering thermodynamicists have traditionally preferred to restrict attention to nonelectrolytes but that restriction must be removed if we are to understand biotechnical systems.

A relatively simple system is provided by aqueous solutions of amino acids. The solubilities of these acids is strongly dependent on pH because, in solution, amino acids can exist as positive ions, negative ions or zwitterions, as indicated in Figure 2. For many amino acids, the equilibrium constants are known for the indicated equilibria 1-4. If some experimental solubilities x_A are known at fixed pH, it is possible to calculate the solubilities at other pH as shown by Nass (1988) and by Chen (1988). For this calculation it is necessary to calculate activity coefficients from a suitable model for the excess Gibbs energy; the essentials of such a model are outlined in Figure 2.

As shown by Chen (1988), it is also possible to calculate solubilities in mixed solvents by using the Born equation first, to discharge the ions in the aqueous solvent and then to recharge them in the mixed solvent; for that calculation, we require data (or estimates) of the dielectric constant of the mixed solvent.

Some results are shown in Figure 3. These results show that, in aqueous solution, the effect of pH is large and not monotonic and that the addition of ethanol has a dramatic effect on solubility (note logarithmic scale).

The second example applies to the production of succinic acid by fermentation. An economically important step is to separate succinic acid from a dilute aqueous solution. Extraction provides a promising method for separation when the extracting solvent contains a water-insoluble basic solute which complexes readily with the acid. As shown by Tamada, Kertes and King (1986), a suitable basic solute is a commercial aliphatic tertiary amine (Alamine 336) with average molecular weight 392.

Experimental studies by Tamada (1989), shown in Figure 4, indicated that highly favorable distribution coefficients are obtained with (about) 1 molal solutions of Alamine 336 in an organic solvent here called the diluent. The interesting and challenging feature of the experimental results is that the distribution coefficient for succinic acid in chloroform diluent is much larger than that in methyl-isobutyl ketone. The acid-amine complex appears to be appreciably more stable in chloroform than in ketone but it is not clear why. Some fundamental physico-chemical studies will be required to answer that question. Nevertheless, for industrial-process development, conventional experimental studies in complex-forming extraction may be useful for recovery operations in processes where chemical products are made by biological methods.

A third example is concerned with extraction of biomolecules in two-phase aqueous systems containing polymers and salts. In recent years, two-phase aqueous systems have received much attention from chemical engineers but most of that attention has been restricted to the case where the aqueous system contains only nonelectrolyte polymers (no salts and no charged biopolymers). When no permanent electric charges are present, it is not difficult to describe phase behavior. However, when salts are present, they may have a large effect on the distribution coefficient of a charged biomolecule.

Figure 3

Solubilities of Amino Acids at 25° C

(K. Nass, C. Chen)

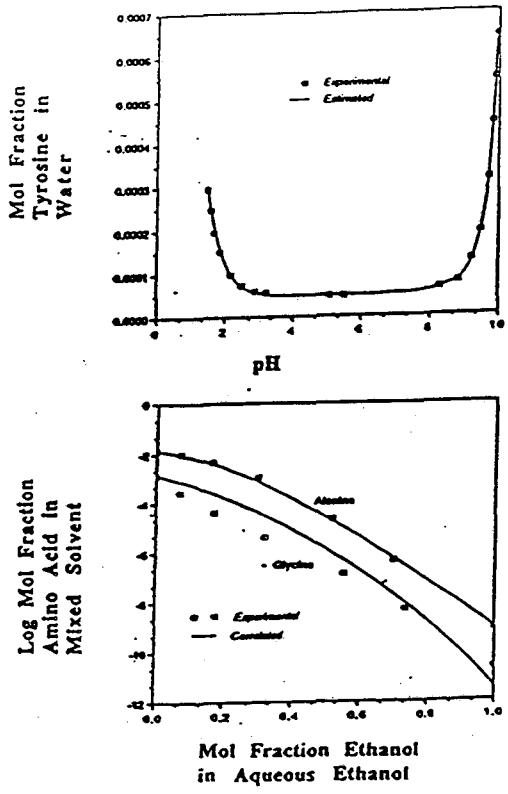
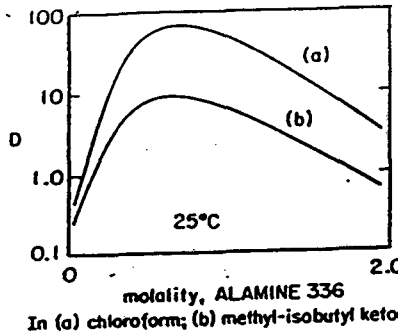


Figure 4

EXTRACTION OF SUCCINIC ACID FROM DILUTE AQUEOUS SOLUTION

(Tamada, Kertes and King)



In (a) chloroform; (b) methyl-isobutyl ketone

D = Distribution coefficient of acid (moles-liter^{-1}/moles-liter^{-1})

ALAMINE 336 (Henkel) is a tertiary amine, C_8–C_{10} range, $\overline{M}_w$ = 392 g/mole.

Succinic acid: $HO-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-OH$

Why is chloroform a much better diluent than MIB ketone?

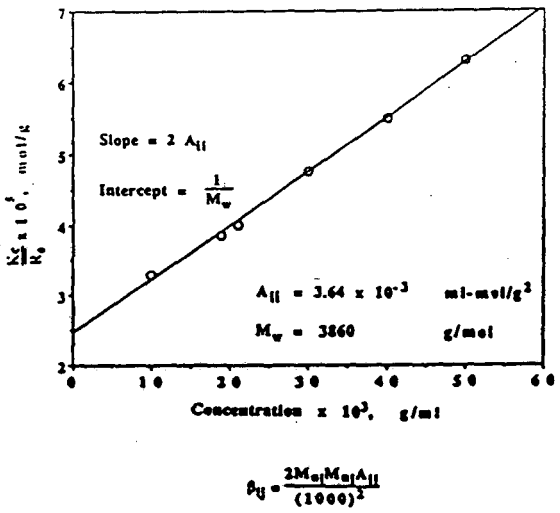
We now have a reasonable first-order framework for calculating phase behavior of semi-dilute, two-phase aqueous systems containing polymers, salts and biomolecules. That framework is based on the osmotic virial expansion as discussed by Haynes et al. in one of the posters at this meeting (1989). The required parameters are obtained from low-angle light-scattering data as indicated in Figure 5 where K is the optical constant, c is the (mass) concentration of the solute and $\underline{R}$ is the reduced Rayleigh ratio. The intercept of this plot gives the weight-average molecular weight and the slope gives the osmotic second virial coefficient A. That virial coefficient, in turn, gives one of the parameters required in the thermodynamic framework.

Figure 5 shows results for polyethylene glycol (PEG 3350) in water. Similar light-scattering data give results for other polymers and for the second virial cross-coefficients of aqueous polymer mixtures. These are the data required to obtain the phase diagram for a two-phase aqueous system, as shown in Figure 6, where the top aqueous phase is rich in polyethylene glycol (PEG) while the bottom aqueous phase is rich in Dextran. For nonelectrolyte-polymer solutes, this phase diagram is not appreciably changed when salts are present at low or moderate concentrations. However, the presence of salts can have a major influence on the distribution coefficient of a charged biomolecule because salts may partition between the two phases and set up a small difference (a few millivolts) in electrical potential. That difference does not affect the behavior of the two-phase aqueous polymer system but it has a large effect on the partition properties of a charged biomolecule.

In aqueous solution, interactions between biomolecules and polymers can also be measured by low-angle light scattering. Further, ion-ion interactions in aqueous salt solutions can be characterized by vapor-pressure or electric-cell measurements; fortunately, data for most common salts are available in the literature. Finally, interactions between ions and charged biomolecules can be characterized by osmometry; it is relatively easy to find a semi-permeable membrane that rejects biomolecules but is permeable to water and small ions.

Figure 5

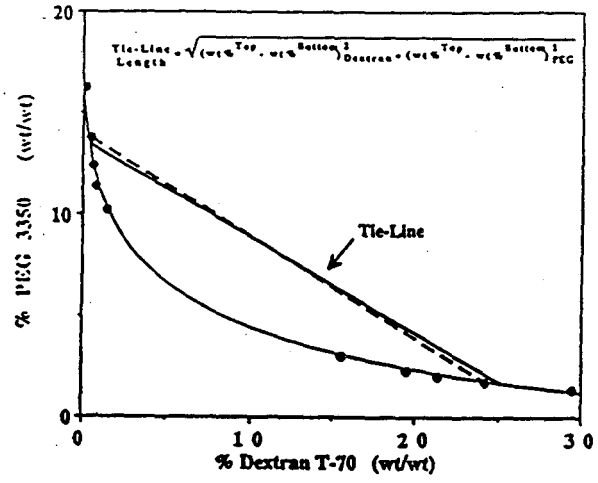
Low-Angle Laser-Light Scattering Data for PEG 3350 in Pure Water



$$\beta_{ij} = \frac{2M_{oi}M_{oj}A_{ij}}{(1000)^2}$$

Figure 6

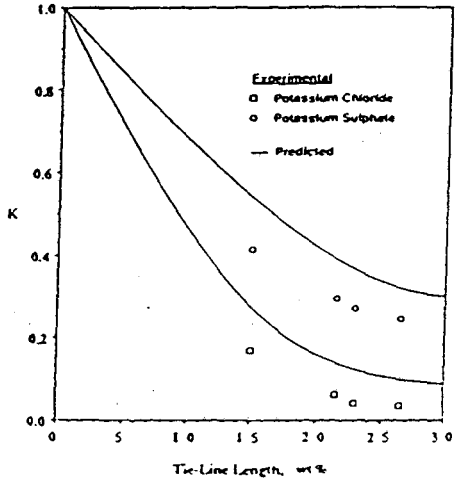
Calculated (—) and Experimental (—) Phase Equilibria for the Polyethylene Glycol (PEG) 3350/ Dextran T-70/ Water System at 25 °C



We do not here go into details that are described by Haynes et al. (1989). The essential point is that, in a dilute or semidilute aqueous solution, the various interactions between solute species can be measured independently; by summing these interactions in a suitable virial expansion, it is possible to calculate chemical potentials and hence, phase equilibria and distribution coefficients. To illustrate, Figure 7 compares predicted and measured distribution coefficients for bovine serum albumin; the calculations follow from the osmotic virial expansion with all parameters found from independent measurements: light-scattering, osmometry and vapor-pressure (or equivalent) data for salt solutions. [The electric charge on albumin depends on pH; it is determined from an independent electrophoresis experiment]. Agreement is good but not perfect because improvements are necessary in the theoretical framework, in the experimental procedures and in methods for data reduction. But the fundamental molecular-thermodynamic framework is now well-established; most needed now is a larger data base.

Figure 7

Experimental and Predicted Partition Coefficients for Bovine Serum Albumin in the Aqueous Two-Phase System Containing PEG 3350, Dextran T-70 and a 50-mM Potassium Salt at 25 °C

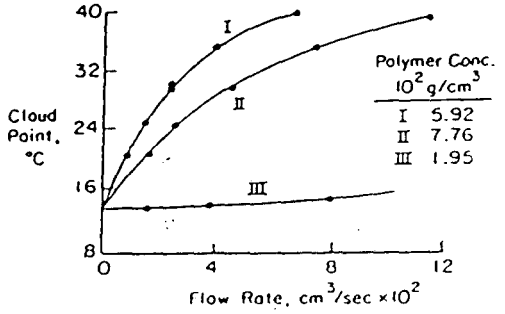


$$K = \frac{\text{Molality of the Protein in the Top Phase}}{\text{Molality of the Protein in the Bottom Phase}}$$

Figure 8

CLOUD-POINT TEMPERATURES FOR FLOWING SOLUTIONS OF POLYSTYRENE IN DIOCTYL PHTHALATE

(Rangel-Nafaile *et al.*, 1984)



$\bar{M}_w$ of polymer = 1.8×10^6

Capillary-Tube Int. Diameter = 0.146 cm

Intermediate-Range Research

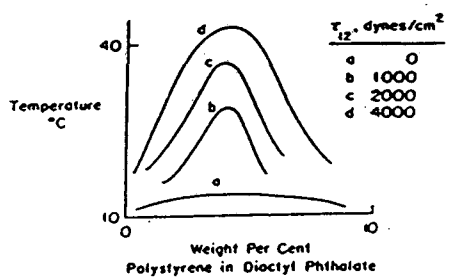
Next, we consider two examples for intermediate-range research. The first, concerned with the effect of shear on phase equilibria, was discussed by Matthew Tirrell at the previous conference in Denmark in 1986; in his article "Future Problems", Tirrell (1986) indicated that the phase behavior of polymer solutions can be significantly influenced by shear forces. We recall this article now because the phenomena he described are important also for solutions of large biomolecules.

Figures 8 and 9 show the effect of shear on liquid-liquid equilibria in a solution of polystyrene in dioctylphathalate. When there is no shear, the two components are completely miscible in all proportions at temperatures above (about) 16° C. However, as the solution is stirred vigorously, two phases appear; the upper consolute temperature increases as the shear rate rises. In these experiments, (Rangel-Nafaile, 1984), the molecular weight of polystyrene is about 1.8 x 10^6. Shear changes the configuration of the polymer molecules and those changes affect the Gibbs energy of the solution. The change in Gibbs energy upon mixing now contains not only the customary Flory-Huggins expression but, in addition, Gibbs energy of stretching which, under well-defined assumptions, can be related to the equilibrium compliance illustrated in Figure 10. That equilibrium compliance depends on temperature and composition. Therefore, an appreciable amount of experimental rheological work is needed to predict the results shown in Figure 9. Nevertheless, at least for some situations, it is now possible to predict the effect of shear on phase equilibria from independent experiments.

Figure 9

EFFECT OF SHEAR ON PHASE BEHAVIOR OF SOLUTIONS CONTAINING MACROMOLECULES

(Rangel-Nafaile *et al.*, 1984)



ΔG_{mixing} = Flory–Huggins Expression + G^S

G^S = Gibbs energy of stretching

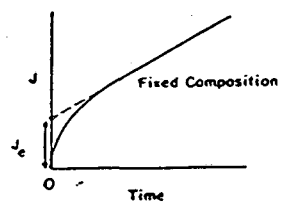
For Gaussian chains, $G^S = \frac{V}{2} J_e \tau_{12}^2 > 0$

V = Total Volume τ_{12} = Shear Stress

J_e = Equilibrium Compliance

Figure 10

EQUILIBRIUM COMPLIANCE J_e CAN BE MEASURED BY RHEOLOGICAL EXPERIMENTS



$J = \frac{\text{strain}}{\text{stress}}$ at constant stress

J_e is similar to (Young's modulus)$^{-1}$

J_e depends on composition

We know that shear can have a profound effect on the properties of large biomolecules; an early study of this effect was reported by Joly (1962). It is likely that better understanding of this effect can suggest now separation methods in biotechnology.

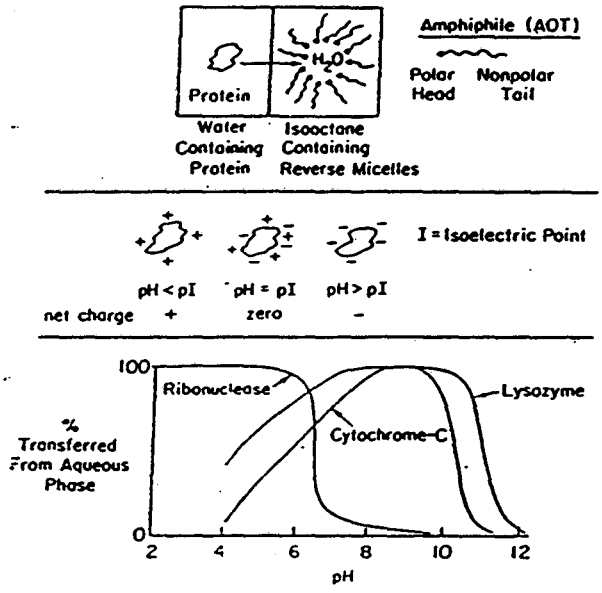
A second example is provided by the fascinating properties of reverse micelles. We usually think of micelles as small organic clusters, stabilized by a surface-active agent in a continuous aqueous phase. A reverse micelle is a small cluster of water, stabilized by a surface agent, in a continuous nonpolar organic liquid, e.g. iso-octane. Reverse micelles have useful properties for selectively extracting biomolecules from aqueous solution as shown in Figure 11. The bottom of that figure shows some results reported

by Göklen and Hatton (1987). By adjusting the pH of the aqueous phase, it is possible to separate from aqueous solution a mixture of ribonuclease, lysozyme and cytochrome C.

Recent experiments by Smith (1989) have shown that selective extraction of proteins from aqueous solution can also be achieved by reverse micelles in supercritical gases at high pressures. Supercritical gases may be more versatile micelle-containing solvents than conventional organic liquids because, at constant temperature, the density of a supercritical gas can be varied continuously by changing the pressure. When the solvent is highly volatile, solvent regeneration is much simplified.

Figure 11

LIQUID-LIQUID EXTRACTION OF PROTEINS USING REVERSE MICELLES



(From Göklen and Hatton)

Figure 12

Catalytic Properties of Enzymes in Reverse Micelles

enzyme	turnover number (compared to aqueous solution)
peroxidase	100x
acid phosphatase	300x
lactase	60x

ref: Martinek, K., et al., Coll. Czech. Chem. Commun. (1987) 52, 2589-2602.

Reverse micelles are also potentially useful for enzyme-catalyzed reactions: in some cases, reaction rates in reverse micelles are much larger than those in aqueous solution, as shown in Figure 12. (The turnover number is the number of reactant molecules converted per unit time, per catalyst site when the enzyme is fully saturated with reactant.) This increased catalytic activity follows from favorable conformations of the enzyme or water or reactant in the reverse micelle. Conventional thermodynamic measurements cannot provide significant information on these conformations; other experimental methods must be used. A particularly useful method for this purpose is EPR (electron paramagnetic resonance) which provides insight on the ability of a molecule to rotate in a specified environment. To illustrate, Figures 13 and 14 show recent experimental results obtained by Louise Creagh (1988). Figure 13 gives EPR spectra for the enzyme trytophanase where the lysine segments of that enzyme have been linked to a nitroxide spin-label. When the enzyme is entrapped in a reverse micelle, the spectrum is broadened and shows fine structure, indicating that the enzyme structure is more rigid in the reverse micelle.

Figure 14 gives EPR spectra for Mn $(H_2O)_6 Cl_2$ dissolved in bulk water and in reverse micelles with different w_o which is the molar ratio of water to surface-active agent. As w_o falls, the spectra broaden, indicating that rotation is inhibited by an increasingly rigid water structure in the reverse micelle.

Figure 13

Nitroxide EPR Spectra of Lysine-Labelled Tryptophanase

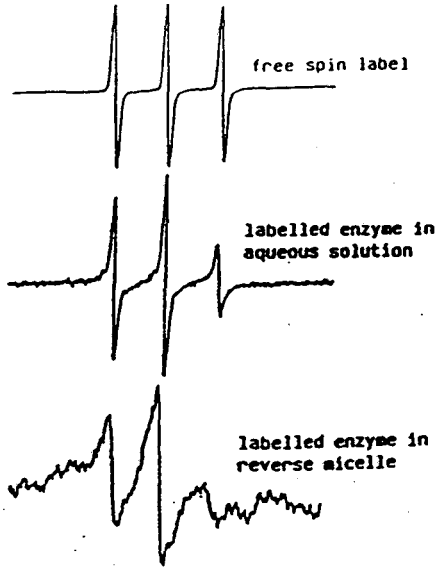
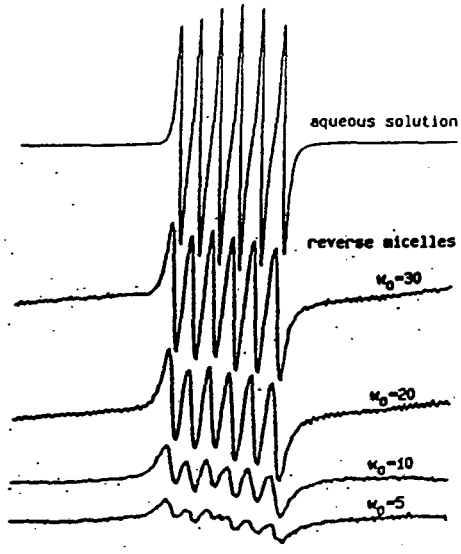


Figure 14

Manganese EPR Spectra



Studies by Blankschtein (1986) provide a start toward a molecular-thermodynamic framework for describing the equilibrium properties of micelle systems. Blankschtein gives the Gibbs energy of a binary system containing water and surface-active agent (amphiphile) as the sum of three contributions, as briefly indicated in Figure 15. He then shows how this Gibbs energy is used to establish the coexistence curve for aqueous mixtures of a micelle-forming solute, C8-lecithin, shown in Figure 16. Based on ideas similar to those of Blankschtein, Hu (1988) considered ternary systems containing water, a hydrocarbon and an amphiphile, and Maestro and coworkers (Caselli 1988) considered systems containing water, a hydrocarbon, an amphiphile and an enzyme.

Much remains to be done but it is clear that the micellar state is likely to become increasingly important in biotechnology and it is encouraging to see that molecular-thermodynamic models are now in an early stage for increasing our quantitative understanding of micelle properties.

Figure 15

PHASE EQUILIBRIA IN AQUEOUS MICELLE SOLUTIONS

(Blankschtein *et al.*, *J. Chem. Phys.*, Dec. 15, 1986)

G = Gibbs Energy of Mixture

$G = G_f + G_m + G_{int}$

G_f: Formation of micelles; each micelle contains n amphiphiles.

G_m: Mixing of micelles with water and monomeric amphiphiles.

G_{int}: Interactions between micelles, water and monomeric amphiphiles.

Chemical Equilibria

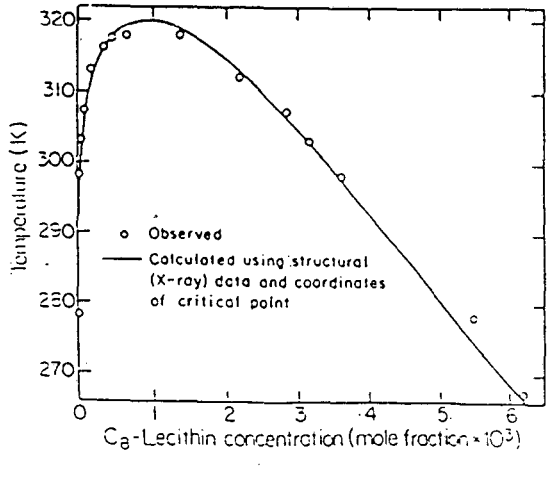
$\mu_n = n\mu_1$

μ_1 = Chemical potential of monomeric amphiphile.

Figure 16

COEXISTENCE CURVE FOR MICELLAR SOLUTIONS OF C_8-LECITHIN/WATER

(Blankschtein *et al.*)



Long-Range Research

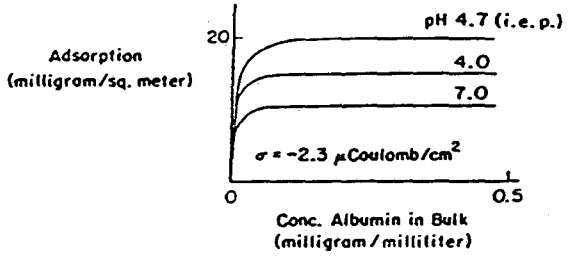
For long-range research, we have in mind primarily studies to improve our understanding of conformation of large biomolecules (e.g. folding of proteins). Such understanding is needed for design of those technical processes that manufacture sensitive biological products whose function is critically dependent on conformation. Such products are likely to become increasingly important for medical applications.

To illustrate, Figure 17 shows some experimental results reported by Norde and Lyklema (1987) who studied the adsorption of human plasma albumin on electrically-charged polystyrene latex. Albumin is an elliptically-shaped protein with a molecular weight of about 70,000. (The charge density is controlled by the density of sulfate groups on the latex.) Adsorption is very strong, especially at the isoelectric point where the protein is most stable. Norde and Lyklema also measured the enthalpy of adsorption (at complete monolayer coverage) and, surprisingly, found that, for some conditions, it is endothermic, as shown in Figure 18. For such conditions, therefore, adsorption is entropy-driven, that is, the entropy of adsorbed albumin is larger than that of "free" albumin in aqueous solution. This result is contrary to our usual concepts of adsorption.

Figure 17

STRONG ADSORPTION OF HUMAN PLASMA ALBUMIN ON CHARGED POLYSTYRENE LATEX AT 37°C

(Norde and Lyklema)



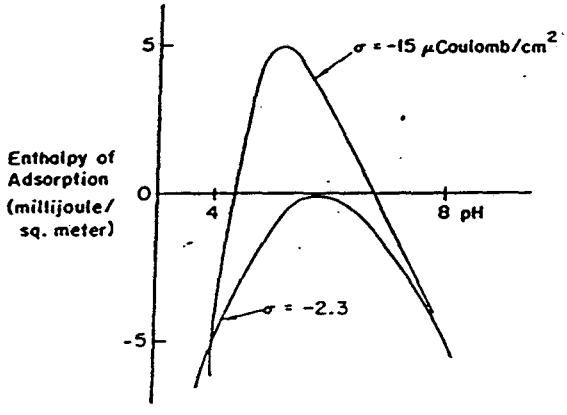
Similar results are obtained when $\sigma = -15.5$.

Since adsorption proceeds readily, $\Delta G < 0$.

Detailed research shows that adsorption is entropy-driven for some conditions (T, pH, σ).

Figure 18

ENTHALPY OF ADSORPTION AT 37°C OF *HUMAN PLASMA ALBUMIN* ON POLYSTYRENE LATEX NEGATIVELY CHARGED WITH SULFATE GROUPS



Microcalorimetry for adsorption: For some pH and σ, the enthalpy of adsorption is endothermic.

Norde and Lyklema present a careful analysis of the numerous possible contributions to both the enthalpy and the entropy of adsorption. Their conclusion is that the dominant contribution to the observed positive entropy change must follow from a change in albumin conformation such that adsorbed albumin is more disordered (unfolded) in the adsorbed state than it is in solution. This conclusion can have important technical implications when designing a process where a valuable protein is in contact with a solid surface. The work of Norde and Lyklema indicates that our old models for adsorption (e.g. the Langmuir isotherm) are seriously inadequate; we need a totally new molecular-thermodynamic framework for describing adsorption equilibria in aqueous systems containing large proteins and salts.

Finally, we briefly present an example which shows how molecular thermodynamics, aided by computer simulation, can be applied to establish enzyme conformation and thereby produce useful results for chemical technology.

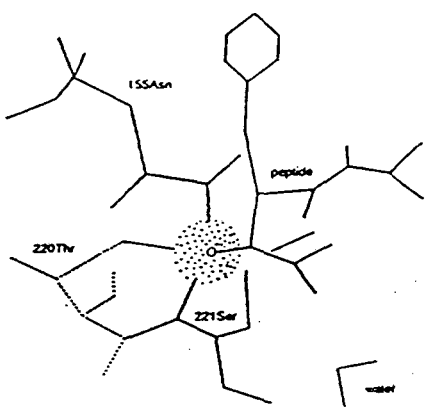
In recent years, we have seen impressive advances in computer simulations for calculating the equilibrium properties of fluids; since there is good reason to believe that these advances will continue at an accelerating rate, we should now start to apply computer simulations to problems in biotechnology. Researchers in molecular biology and in pharmacology are already far along in constructing techniques for applying computer simulations to large molecules. The necessary computer software is formidable but much of that can be obtained from software libraries, often at nominal cost. With some- but not excessive- effort, chemical engineering thermodynamicists can apply these computing techniques to problems of industrial interest.

To illustrate, consider an engineering-oriented calculation reported by Kollman and coworkers (Rao 1987). While numerous simplifying assumptions are required for the present state of the art, computer simulation can be used to indicate how a directed mutation in an enzyme is likely to influence the kinetics of a particular enzyme-catalyzed reaction.

Figure 19 shows the structure of subtilisin, a readily available enzyme with a molecular weight of about 28,000. Subtilisin is used in common detergents for domestic washing machines; subtilisin catalyzes hydrolysis of peptides typically found in common foods that soil our dishes and stain our clothing. Unfortunately, subtilisin's stability decreases with rising temperature; it is therefore of practical interest to consider a mutant of subtilisin which is likely to be stable at temperatures used in a washing machine. Kollman and coworkers investigated the effect of substituting alanine for asparagine at the 155 position in subtilisin; such substitution increases stability at higher temperatures. But what does this substitution do to the kinetics of peptide hydrolysis?

Figure 19

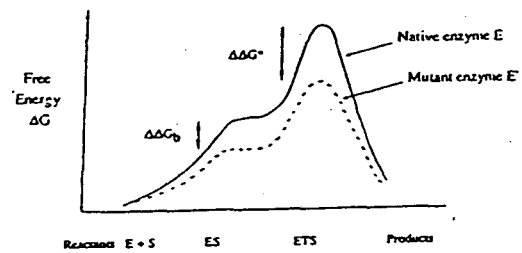
Active Site of the Transition State for the Hydrolysis of a Peptide Bond by Subtilisin



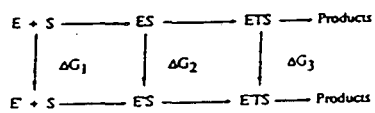
The dots represent the van der Waals radius of the oxygen of the scissile peptide bond (indicated by an arrow). Shown only are those residues containing a hydrogen atom which interacts with that oxygen.

Figure 20

FREE-ENERGY CALCULATION FOR A PERTURBED (MUTANT) ENZYME-CATALYZED REACTION



* = Activation b = Binding



S = Substrate ETS = Transition state

Find ΔG_1, ΔG_2 and ΔG_3 by computer simulation.

$\Delta\Delta G_b = \Delta G_2 - \Delta G_1$; $\Delta\Delta G^* = \Delta G_3 - \Delta G_2$

Kollman's computer-simulation calculations are schematically summarized in Figures 20 and 21. Native enzyme E reacts with substrate S in several steps: first, a non-bonded complex ES is formed; this is the binding step. Next, a transition state ETS is formed; this is the activation step. Finally, the activated state decays to products.

The top and middle of Figure 20 indicate a perturbation calculation; in this case, the perturbation is provided by substituting mutant enzyme E' for native enzyme E. (For illustrative purposes, the diagram optimistically assumes that the line for the mutant

enzyme lies below that for the native enzyme.) Computer simulations are used to calculate the Gibbs energy changes ΔG_1, ΔG_2 and ΔG_3. Since Gibbs energy is a state function, these calculated quantities then give the change in the Gibbs energy of binding $\Delta(\Delta G_b)$ and the change in the Gibbs energy of activation $\Delta(\Delta G^*)$. The first of these changes is negligible but the second is not. Kollman's calculations show that the dotted line in Figure 20 should lie above the continuous line; while the substitution of alanine for aspargine at position 155 increases thermal stability, the rate of hydrolysis decreases by a factor of about 600. This prediction was later confirmed by kinetic experiments.

FIGURE 21

HYDROLYSIS OF A TRIPEPTIDE

CATALYZED BY SUBTILISIN

E = Native Subtilisin S = Ala-Ala-Phe

E* = Subtilisin with one mutation in the 155 position:

Asparagine (Asn155) replaced by Alanine (Ala155)

	$\Delta\Delta G_b$	$\Delta\Delta G^*$
	(kcal/mole)	
Simulation	0.11 ± 0.80	3.40 ± 1.13
Kinetic data	0.41	3.67

In this case, the mutant enzyme is less effective (ratio of rate constants is about 600). Kinetic data, obtained after simulation, agree with the prediction.

Computer simulations cannot replace essential laboratory experiments but they can significantly reduce experimental effort by screening, i.e. by indicating which experiments are most likely to give the most useful information. Computer calculations for physical properties of biological systems are now in an early but highly promising stage. It is likely that the solution to numerous problems in biotechnology will be obtained, at least in part, by computer-generated thermodynamic properties of biomolecules.

Conclusion

The history of chemical engineering in the United States indicates that chemical engineers possess a set of cardinal virtues: flexibility, versatility and optimism, as expressed by an attitude which is goal-oriented, which does not hesitate to use whatever is available to get the job done.

As the biotechnical industries mature, we may expect that molecular thermodynamics can make a significant contribution toward improving the

development of new products and toward their economic production. But that contribution can only become real if we are now willing to make the necessary investment in patient and dedicated research. Our main tasks are first, to expand the required data base and second, to become familiar with experimental, computational and theoretical methods that are now only at the periphery of conventional chemical engineering thermodynamics. Special attention must be given to aqueous systems containing electrolytes and large molecules. These tasks are difficult but, given our traditions, they are within our grasp.

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