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Energetics of Cell–Cell and Cell–Biopolymer Interactions

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ABSTRACT

The energy vs distance balance of cell suspensions (in the presence and in the absence of extracellular biopolymer solutions) is studied, not only in the light of the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (which considered just the electrostatic (EL) and Lifshitz-van der Waals (LW) interactions), but also by taking electron-acceptor/electron-donor, or Lewis acid-base (AB) and osmotic (OS) interactions into account. Since cell surfaces, as well as many biopolymers tend to have strong monopolar electron-donor properties, they are able to engage in a strong mutual AB repulsion when immersed in a polar liquid such as water. The effects of that repulsion have been observed earlier in the guise of hydration pressure. The AB repulsion is, at close range, typically one or two orders of magnitude stronger than the EL repulsion, but its rate of decay is much steeper. In most cases, AB interactions are quantitatively the dominant factor in cell stability (when repulsive) and in "hydrophobic interactions" (when attractive). OS interactions exerted by extracellularly dissolved biopolymers are weak, but their rate of decay is very gradual, so OS repulsions engendered by biopolymer solutions may be of importance in certain long-range interactions. OS interactions exerted by biopolymers attached to cells or particles (e.g., by glycocalix glycoproteins), are very short-ranged and usually are negligibly small in comparison with the other interaction forces, in aqueous media.

Index Entries: Cell biopolymer interactions; cell–cell interactions; DLVO theory; electrostatic interactions; glycocalix; Lewis acid base interactions; Lifshiftz van der Waals interactions; monopolar (electron donor) interactions; Van der Waals forces.

INTRODUCTION

The quantitative determination of, and the differentiation between apolar and polar surface tension components and parameters of liquids and solids has recently become possible. It can be shown, via the Lifshitz approach, that the surface tension components associated with the three electrodynamic (van der Waals) interactions, i.e., the dispersion (London), induction (Debye), and orientation (Keesom) forces, obey the same equations and should be treated in the same manner as essentially "apolar" interactions (*1*), and together may be designated as Lifshitz-van der Waals (LW) interactions. Once this point is clarified, the "polar," electron-donor/electron-acceptor, or Lewis acid-base (AB) interactions can be studied as separate phenomena that obey a different set of equations (*2–5*).

THEORY AND DETERMINATION OF SURFACE TENSION COMPONENTS

The apolar and the polar surface tension components are additive:

$$\gamma = \gamma^{LW} + \gamma^{AB} \quad [1]$$

However, the polar interactions are fundamentally asymmetrical (*6*), so that it is necessary to express the polar (AB) interfacial free energy between substances 1 and 2 in terms of the products of their electron-acceptor (γ^+) and electron-donor (γ^-) parameters (*6,7*) as

$$\Delta G_{12}^{AB} = -2\left(\sqrt{\gamma_1^+ \gamma_2^-} + \sqrt{\gamma_1^- \gamma_2^+}\right) \quad [2]$$

The polar component of the surface tension of a substance i then can be defined as

$$\gamma_i^{AB} = 2\sqrt{\gamma_1^+ \gamma_2^-} \quad [3]$$

It then becomes possible to express Young's Equation (1805) (*8*) as

$$(1 + \cos\theta)\,\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^+ \gamma_L^-}\right) \quad [4]$$

where θ is the advancing contact angle of drops of liquid L on a flat surface of a solid S. By contact angle measurement with three different liquids L, each with known values of γ_L^{LW}, γ_L^+, and γ_L^-, the three Eq. (4) thus obtained can be solved for the three unknown entities γ_S^{LW}, γ_S^+, and γ_S^+ (*2,5*).

This approach for the determination of the apolar (LW) as well as the polar components (AB) and parameters (γ^+ and γ^-) of various polymers and biopolymers, has become a powerful tool for elucidating various colloidal and biological phenomena, such as phase separation in aqueous polymer solutions (*9*), the mechanism of Southern (DNA) and Western

(protein) blotting (*10*), protein solubility (*11*), particle stability in aqueous suspensions (*4*), and phospholipid membrane fusion (*12*).

Lifshitz-van der Waals (LW) Interactions

Using an apolar liquid for contact angle measurement, in conjunction with Eq. [4], the two right hand (polar) terms remain zero, so that one apolar (LW) liquid suffices for determining γ_S^{LW}. In order to obviate spreading (i.e., generating a zero degree contact angle), γ_L^{LW} must be larger than γ_S^{LW}. Most biological materials have γ_S^{LW} values of the order of 38–42 mJ/m^2. Thus (virtually) apolar liquids such as α-bromonaphthalene (γ_L=44.4 mJ/m^2) and diiodomethane (γ_L=50.8 mJ/m^2) are among the most useful ones for measuring γ_S^{LW}.

The apolar component of the interfacial tension between substances 1 and 2 is expressed as

$$\gamma_{12}^{LW} = (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2 \quad [5]$$

From this the interaction energy of substance 1 with substance 2 is expressed by using the Dupré equation:

$$\Delta G_{12} = \gamma_{12} - \gamma_1 - \gamma_2 \quad [6]$$

The interaction energy of molecules or particles of substance 1 with each other, while immersed in liquid 2 is expressed as

$$\Delta G_{121} = -2\gamma_{12} \quad [7]$$

and the interaction energy of molecules or particles 1 and 2, both immersed in liquid 3 is

$$\Delta G_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \quad [8]$$

(Where the superscripts LW or AB are not used, the equations are valid for either kind of interaction).

γ_L^{LW} of liquids with a known γ_L can be obtained by contact angle determination on low-energy apolar surfaces, such as teflon ($\gamma_S = \gamma_S^{LW}$ 18.5 mJ/m^2), using Eq. [4] and neglecting its polar terms. From γ_L and γ_L^{LW}, γ_L^{AB} can be obtained from the difference between the first two, *see* Eq. [1].

Polar or Electron-Donor/Electron-Acceptor (Lewis Acid-Base: AB) Interactions

Once γ_S^{LW} of a substance is known by contact angle measurement with an apolar liquid (*see above*), contact angle measurements with two well characterized polar liquids (such as water and glycerol), will then, using Eq. [4] twice, yield γ_S^+ and γ_S^-. For water, γ_W^{LW}=21.8 mJ/m^2, and γ_W^{AB}=51 mJ/m^2 (*1*); its γ_W=72.8 mJ/m^2. In the absence of other data, it is assumed

that $\gamma_W^+ = \gamma_W^- = 25.5$ mJ/m²*. For glycerol (GL) these values are then: $\gamma_{GL}^{LW} = 34$ mJ/m² (2,4) and $\gamma_{GL}^+ = 3.9$ and $\gamma_{GL}^- = 57.6$ mJ/m² (2,4); its $\gamma_{GL} = 64$ mJ/m².

The polar component of the interfacial tension between substances 1 and 2 is expressed as (2–6)

$$\gamma_{12}^{AB} = 2\,(\sqrt{\gamma_1^+ \gamma_1^-} + \sqrt{\gamma_2^+ \gamma_2^-} - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_\gamma^- \gamma_2^+}) \qquad [9]$$

It is easily shown that, in contrast with γ_{12}^{LW} (Eq. [5]), which is always positive or zero, γ_{12}^{AB} (Eq. [9]) readily can be negative (2–6). The implications of this are important, because when $\gamma_{12}^{AB} < 0$, $\Delta G_{121}^{AB} > 0$ (Eq. [7]), which means that polar molecules, cells or particles 1, immersed in a polar liquid 2 (e.g., water), then can *repel* each other, even in the absence of any electrical surface charge.

Monopolar Surfaces and Negative Interfacial Tensions

The most favorable condition under which negative interfacial tensions between substance *S* and water (*W*) occur, prevails when *S* is monopolar, i.e., when either $\gamma_S^+ = 0$, or $\gamma_S^- = 0$. In actuality, the first contingency occurs the most frequently, i.e., many biological compounds have a sizable γ_S^-, with a γ_S^+ that is negligibly small or zero (2,4). For substances with $\gamma_S^{LW} \approx 40$ mJ/m², which is, on average, the most frequently occurring value for typical monopolar substances, γ_S^- must exceed 28 mJ/m² for γ_{SW} to be negative (4). Many materials of biological or organic origin are γ^- monopolar and have γ^- values well above 28 mJ/m², e.g.: agarose, gelatin, polyethylene glycol, dextran, ribosomal RNA, cellulose acetate, and polyvinyl alcohol (4). To obtain the total interfacial tension, Eqs. [5] and [9] must be combined to

$$\gamma_{12} = (\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}})^2 + 2\,(\sqrt{\gamma_1^+ \gamma_1^-} + \gamma_2^+ \gamma_2^- - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_1^+ \gamma_2^-}\,) \qquad [10]$$

Total Interfacial Interactions

The total interfacial attraction energy between substances 1 and 2 *in vacuo* (Eq. [6]) becomes

$$\Delta G_{12}^{TOT} = \Delta G_{12}^{LW} + \Delta G_{12}^{AB} = \Delta G_{12}^{TOT} = \gamma_{12}^{LW} - \gamma_1^{LW} - \gamma_2^{LW} - 2\sqrt{\gamma_1^+ \gamma_2^-} - 2\sqrt{\gamma_1^- \gamma_2^+} \qquad [11]$$

*It can be shown (2,4,5) that this assumption only impinges on the expression of the absolute values of γ_S^+ and γ_S^-. When used to express γ_{12}^{AB}, ΔG_{12}^{AB}, ΔG_{121}^{AB}, or ΔG_{132}^{AB}, only the (known) *ratios* of γ_S^+ and γ_S^- with, respectively, γ_W^+ and γ_W^- are needed; these ratios can be exactly determined and are not based on any assumptions for γ_W^+ and γ_W^-. We only make the assumption for water that $\gamma_W^+ = \gamma_W^-$, in order to give the order of magnitude of γ_S^+ and γ_S^-, for the sake of comparison (e.g., with the values for water). When it is found that for a substance *S* either γ_S^+ or γ_S^- is zero, that is not based on the $\gamma_W^+ = \gamma_W^-$ assumption.

This value is always negative. (It should be noted that electrostatic interactions are not taken into account here.)

The total interfacial interaction energy between substances 1 and 2, immersed in polar liquid 3 (Eq. [8]) is

$$\Delta G_{132}^{TOT} = \Delta G_{132}^{LW} + \Delta G_{132}^{AB} = \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} + 2\left[\sqrt{\gamma_3^+}\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}\right) + \sqrt{\gamma_3^-}\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}\right) - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}\right] \quad [12]$$

and, according to Eq. [7]

$$\Delta G_{121}^{TOT} = \Delta G_{121}^{LW} + \Delta G_{121}^{AB} = -2\gamma_{12} = -2\gamma_{12}^{LW} - 2\gamma_{12}^{AB} \quad [13]$$

(*See* Eq. [10] for the expression for γ_{12}). Equations [12] and [13] are the crucial general equations for the total interfacial interaction energies between two different, resp. two identical substances, immersed in a liquid. The values for ΔG_{132}^{TOT} and ΔG_{121}^{TOT} can be negative, zero, or positive. When negative, that (attractive) energy corresponds to the "hydrophobic interaction" energy (*2–6*). When positive, that energy connotates the repulsion between two particles or molecules immersed in a polar liquid (*2–6*).

Electrostatic (EL) Interactions

Once immersed in polar liquids (and especially in water), few macromolecules, particles, or cells are totally lacking in electrical surface charge. Thus, such charged entities, when immersed in water, will tend to repel each other. In most cases the repulsive energy engendered by the electrostatic surface potential of charged entities must therefore be taken into account in determining the total interaction energy (above and beyond the total interfacial, or LW+AB interaction energy, treated above). The (electrokinetic) methods for determining surface potentials have been amply described elsewhere (*13–14*), as have the approaches for deriving repulsion (or attraction) energies from them (*15–17*). It should be recalled that electrostatic interaction energies tend to be proportional to the *square* of the electrokinetic (or ζ) potential, and ζ-potentials themselves are, as a first approximation, simply proportional to electrokinetic mobilities. Thus, generally, below a certain value of the ζ-potential (e.g., 10–14 mV), electrostatic repulsions quickly become negligible.

Osmotic (OS) Interactions

The second non-LW and non-AB interaction that can play a role in cell-cell and cell-protein energetics is the osmotic pressure (*18*) engendered by macromolecules, dissolved in the aqueous medium, and/or macromolecules attached to or adsorbed to particles or cells, immersed in the liquid. The total OS interaction energy level at close range to the particles or cells generally is much lower than the *LW*, AB, or EL interaction ener-

gies. However, especially with high molecular weight polymers and/or very asymmetrical macromolecules, the rate of decay of OS interactions with distance tends to be much less steep, especially as compared to AB interactions (*see below*).

Energy Balance

Rate of Decay with Distance

Considering, for all categories, the interaction energy between two (semi-infinite) plane parallel surfaces, the rate of decay with distance (d) of the various interaction categories may be expressed as

1. *LW* Interactions

$$\Delta G_d^{LW} = \Delta G_{do}^{LW} (d_o / d^2) \qquad [14]$$

where ΔG_{do}^{LW} is the free energy of interaction at the minimum equilibrium distance (d_o), i.e., at contact (*6*). Equation [14] holds only for unretarded Lifshitz-van der Waals interactions, i.e., at distances d from d_o (≈ 1.58 Å) to ≈ 100 Å. For $d > 100$ Å, the retarded regime sets in, so that

$$\Delta G_d^{LW} = \Delta G_{do}^{LW} (d_o / d^3) \qquad [15]$$

2. *AB* Interactions (for $d >> d_o$)

$$\Delta G_d^{AB} = \Delta G_{do}^{AB} \exp [(d_o - d)/\lambda) \qquad [16]$$

where λ is the correlation distance (*19*) for the suspending liquid. There are two possibilities to be considered.

When $\Delta G^{AB} > 0$, λ is close to 2 Å, which is of the order of magnitude of the radius of gyration of water molecules. The repulsion here is principally propagated by hydration pressure (*20,21*), engendered by the orientation of the water molecules of hydration (*21,22*). However, at high ionic strengths, instead of a correlation length that corresponds roughly to the radius of the liquid molecules, λ becomes a measure of the dimension of the hydrated ions, so that λ can approach ≈ 12 Å (*23*). Thus in water, ΔG_d^{AB} decays to $10^{-4} \times \Delta G_{do}^{AB}$ in 18 Å, whereas at high salt concentrations, $10^{-4} \times \Delta G_{do}^{AB}$ is only reached at ≈ 108 Å. When $\Delta G^{AB} < 0$, a "hydrophobic" attraction prevails, which is, energetically, linked to the cohesion force of water (*6*). Here the value for λ appears to be of the order of 10 to 14 Å, according to empirical estimations (*24,25*). More recently, values for λ as high as 130 Å have been reported (*46*).

3. *EL* Interactions

As a first approximation

$$\Delta G_d^{EL} = \Delta G_{do}^{EL} \exp (-\kappa d) \qquad [17]$$

(*see 15,16*), where $1/\kappa$ (the Debye length) is expressed as

$$1/\kappa = \sqrt{\epsilon k T / 4\pi e^2 \Sigma v_i^2 n_i} \qquad [18]$$

where ϵ is the dielectric constant of the liquid medium ($\epsilon = 80$ for water), k is Boltzmann's constant ($k = 1.38 \times 10^{23}$ J/°K), T the absolute temperature in degrees K, e is the charge of the electron ($e = 4.8 \times 10^{-10}$ e.s.u.), and $\Sigma v_i^2 n_i$ the ionic strength, with v_i the valance of each ionic species and n_i the number of ions of each species per cm^3 of bulk liquid.

Thus, ΔG^{EL} is strongly dependent on the ionic strength and, contrary to ΔG^{AB}, decreases more steeply at high than at low salt concentrations.

4. *OS* Interactions

The osmotic energy of polymer solution (or of a layer of polymer molecules) may be approximated as

$$\Delta G_d^{OS} = \Delta G_{d_0}^{OS} \exp(-d/Rg) \qquad [19]$$

where Rg denotes the radius of gyration of the polymer molecules. For dextran molecules with a molecular weight of 500,000, and an average molecular length $L \approx 2{,}140$ Å (*26*), as a first approximation the radius of gyration of oblong asymmetrical molecules may be expressed as (*27*)

$$Rg = L/\sqrt{12} \qquad [20]$$

so that for these dextran molecules, $Rg \approx 620$ Å. $\Delta G_{d_0}^{OS}$ is obtained by integrating the osmotic pressure function; as a first approximation, by multiplying the osmotic pressure by Rg. For a very asymmetrical macromolecule, such as dextran (Mw $\approx$ 500,000), even at $d = 1{,}000$ Å, ΔG_d^{OS} still is 18% of $\Delta G_{d_0}^{OS}$, although it should be noted that $\Delta G_{d_0}^{AB} = 1.6 \times 10^2 \times \Delta G_{d_0}^{OS}$ at 10% dextran. Thus, from $d \geq 65$ Å, ΔG_d^{OS} surpasses ΔG_d^{AB}. Similarly, for $\Delta G_{131}^{LW} = -5$ mJ/m^2 (typical for many biopolymers in water), the *LW* attraction is superseded by an *OS* repulsion (caused by 10% dextran 500,000) at $d \geq 20$ Å.

Relative Importance of the Contributing Forces

Pseudoattachment at the Secondary Minimum of Attraction

Real "attachment" between cells and/or biopolymers at the secondary minimum does not occur. However, pseudoattachment, in the guise of a labile temporary immobilization brought about by long, asymmetrical, crossbinding polymers, is possible at the secondary minimum (*28*). For example, in the case of rouleau-formation by erythrocytes, this can readily take place in the presence of asymmetrical polymers (*see below*) (*28*). Such pseudoattachment, however, is easily disturbed by shaking, dilution, and so on.

True Attachment at the Primary Minimum of Attraction

The only possible condition for real attachment is adhesion at the primary minimum of attraction, to which effect the total energy at the secondary maximum of repulsion must be less than 1½ kT, *and* the total energy at the primary minimum of attraction must be more than 1½ kT (*see* Fig. 1.).

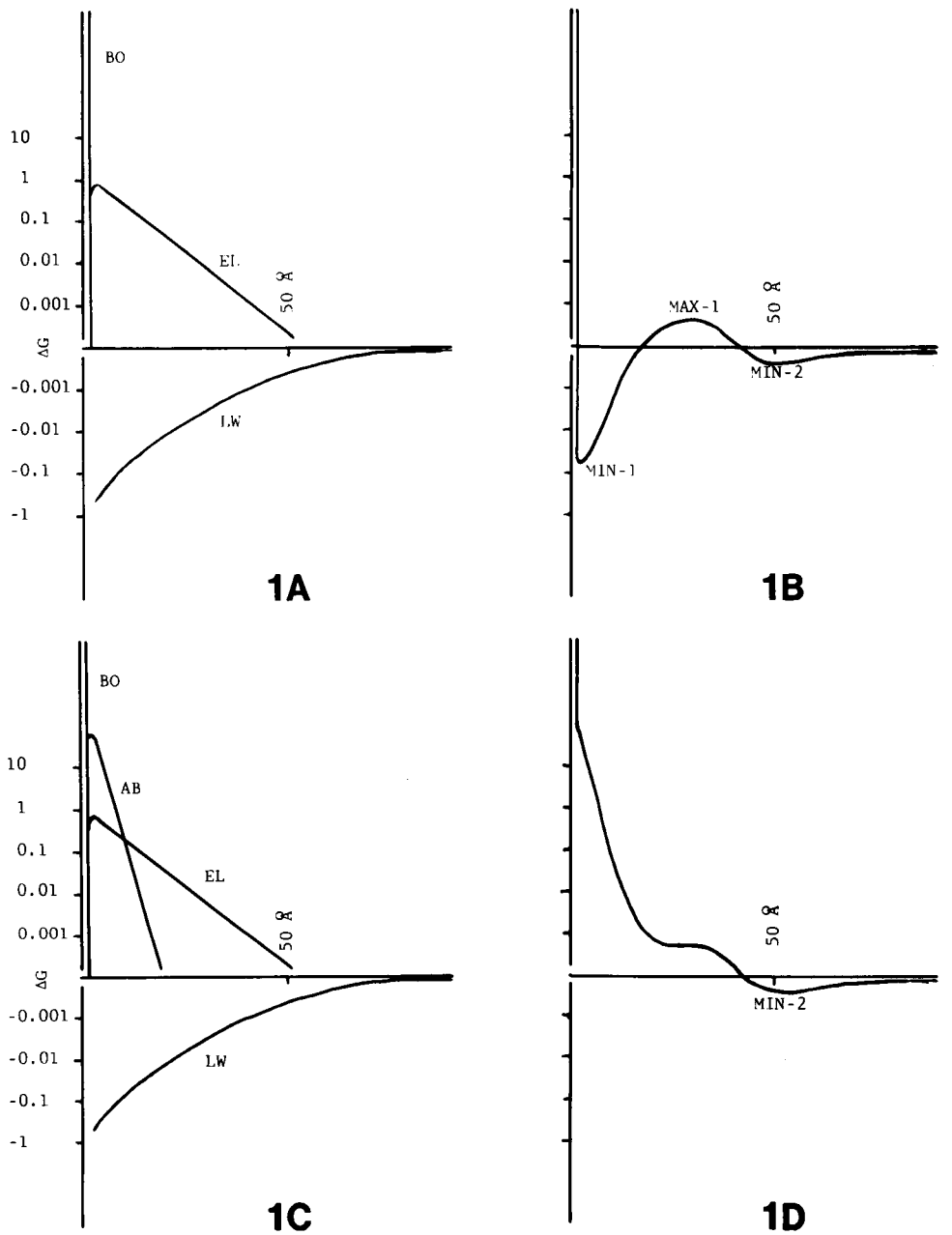

Fig. 1. Energy balance diagrams (ΔG vs d, with ΔG on a logarithmic scale, in mJ/m^2) of erythrocyte suspensions. 1A: Electrostatic (EL) and Lifshitz-van der Waals (LW) interactions, combined in 1B: The classical DLVO plot with the primary minimum of attraction (MIN-1), the primary maximum of repulsion (MAX-1) and the secondary minimum of attraction (MIN-2). BO indicates the Born repulsion, which prevents all atoms and molecules to approach each other to a distance smaller than ≈ 1.4 Å. 1C: The AB interaction (in this case a repulsion) is depicted here, in addition to the EL repulsion, yielding in 1D: The combined curve that depicts the total interaction more realistically than the DLVO

Thus, for true attachment to occur, ΔG^{EL} must be low (or the particles must be small, or have protruding processes of a small radius of curvature). For biological entities in aqueous media, ΔG_{131}^{LW} usually is of the order of -0.5 to -5 mJ/m^2. For attachment to occur at surface areas smaller than 120 Å^2, an additional significant negative ΔG_{131}^{AB} ("hydrophobic attraction") is required, and or an *attractive* (negative) ΔG^{EL} between charged moieties of opposite signs of charge.

Stabilization through Net Repulsion

In aqueous media, electron-donor monopolar surfaces of biological origin, with $\gamma^- > 28$ mJ/m^2, will give rise to a positive ΔG_{131}^{AB} value, upon which attachment is no longer favored. High ζ-potentials (typically $\zeta > 15$ mV), of course, also will cause an effective repulsion, leading to stabilization, at low to moderate ionic strengths. In biological systems, ΔG^{OS} usually plays only a minor role in cell stability, but in apolar liquids, ΔG^{OS} repulsion may be the principal stabilizing force for suspensions of particles stabilized by soluble polymers.

METHODOLOGY

Contact angle determination on cells is best done on flat layers of cells obtained by filtering the cell suspension, by suction, onto microporous membranes, e.g., cellulose acetate Millipore (Bedford, MA) or porous silver Flotronics-Selas (Dresher, PA) membranes, with pore diameters of 1 to 3 μm. Contact angle determination on (hydrated) proteins is best done on flat layers of hydrated protein obtained by ultrafiltering the protein solution, on anisotropic membranes (22), either especially prepared for that purpose (29), or commercially obtained (e.g., Millipore, Bedford, MA; Amicon, Danvers, MA). The advancing contact angle should be utilized (*see 30*).

For the determination of electrokinetic potentials, *see* (*13,14*). A method for analytical cell electrophoresis, which overcomes the problem of electroosmotic backflow in a simple manner has been described earlier (*31*); this method requires no special apparatus apart from a microscope and a DC powersupply.

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plot. The most important aspect of the complete curve shown in Fig. 1D, is the absence of a primary minimum of attraction (still visible in Fig. 1B), owing to the AB-repulsion, which gives rise to superstability in vivo.

At higher exterior albumin concentrations ($\approx 12\%$ BSA), applied in vitro, the colloid-osmotic repulsion engendered by the $\approx 6\%$ excess extracellular albumin concentration must be taken into account (*see text*). The main long-range osmotic effect is to abolish the residual long-range LW attraction and with it the secondary minimum, which is still visible in Fig. 1D. The short-range osmotic effect still is negligible compared to the AB repulsion, which pushes the cells tegether into miniagglutinates engendered by the phase separation.

RESULTS AND APPLICATIONS

Cell–Cell Interactions

Stability of Blood Cells

If the stability (i.e., the nonclumping) of human peripheral blood cells in vivo obeyed the classical DLVO theory, that is, if that stability were dependent on just the electrostatic repulsion vs van der Waals attraction (*15,16*), we would all be in trouble, because only erythrocytes (ER) would be (barely) stable, whereas the lymphocytes (LY) and polymorphonuclear leukocytes (PMN) would aggregate, not only with each other, but also with ER (*see* Table 1).

This does not signify that there is not a small primary maximum of repulsion (of about $+0.2$ mJ/m^2 for ER, at ≈ 10 Å distance) that has to be overcome first and would under normal circumstances assure stability for totally smooth cells (*see* Fig. 1). However, few cells are totally smooth. Erythrocytes with echinocytic protruberances with a diameter slightly under 10 nm, would be able to make contact, and then remain attached to each other. Leukocytes (both LY and PMN) with villi somewhat thicker than that (e.g., up to 100 nm), would be able to overcome the primary maximum of repulsion, and irreversibly attach to each other, or to ER, and form largely irreversible clumps. Of course, in the normal course of events, none of this actually happens and the reason why it does not happen lies in the very steep repulsion at close range, engendered by the AB repulsion (*see* Fig. 1), which makes it virtually impossible for any of the blood cells to adhere to each other. By ultracentrifugation at $260{,}000 \times g$, we have shown earlier that it is indeed impossible to make erythrocytes to adhere to each other by the application of purely physical forces (*33*).

Phagocytosis

However, in the case of interaction of bacteria with PMNs (and also with monocytes and macrophages), in many cases attachment (and subsequent engulfment) takes place, owing to the much feebler AB parameters

Table 1
The Components of Cell Stability

Cell Type	ζ-potential, in mV	ΔG^{LW}	ΔG^{EL}	ΔG^{AB}	ΔG^{TOT}	ΔG^{DLVO}
		in mJ/m^2				
ER	−18	−0.56	+0.47	+25[a]	+25	−0.1
LY	−14	−0.70	+0.29	+15[b]	+14.6	−0.1
PMN	−12	−0.70	+0.21	+10[b]	+ 9.5	−0.5

[a] van Oss and Cunningham, unpublished results.
[b] Estimated, by comparison with (ER), from earlier contact angle data (32).

of most bacteria (*32,34,35*). Only strongly electron-donor monopolar, encapsulated, bacteria escape phagocytic engulfment by PMNs, and thus are pathogenic (*32,34,35*). In the bloodstream this picture becomes more complicated through the intermediate of aspecific adsorption of immunoglobulins onto bacteria of low polarity, which adsorbed immunoglobulins, via their Fc-moiety, then attach to the Fc-receptors on the PMNs (*36,37*). Again, monopolar encapsulated bacteria, even though they also aspecifically adsorb immunoglobulins, escape phagocytosis, as the adsorbed immunoglobulins remain inside the monopolar capsule and their Fc-moieties thus cannot achieve contact with Fc-receptors on phagocytic cells. Only after the formation of specific anticapsular antibodies, which attach to the outer rim of the monopolar capsule, does Fc-receptor mediated phagocytosis takes place (*38*).

Cell-Sorting

Cell-sorting in the sense of preferential agglomeration of cells of the same type, from mixed suspensions of different cell types (in the absence of specific auto-recognition receptors), is closely linked to the energy-balance characteristics of each different cell type (*28*). It can easily be shown that, in mixed cell populations, where all cells can attract one another, the final outcome will tend to be the formation of multiple agglomerates, each consisting of only one cell type, on account of the fact that each type of cell has its own characteristic energy level at the primary minimum of attraction and rate of decay with distance, of the total interaction energy (*see* Fig. 1). An interesting example is the sorting of human and rabbit erythrocytes into separate, exclusively human, and exclusively rabbit rouleaux (*28*), although, exceptionally, the (labile) attachment in this case occurs at the secondary minimum of attraction.

Membrane Fusion

To fuse two cells, or two vesicles surrounded by phospholipid membranes, a strong monopolar (and therefore hydration pressure) repulsion must first be overcome. The admixture of Ca^{++} can bring about the fusion of charged as well as of uncharged phospholipid membranes, whereas the addition of polyethylene glycol facilitates, but cannot by itself alone cause membrane fusion. The role Ca^{++} plays in membrane fusion (apart from decreasing the negative ζ-potential, if any) lies mainly in its capacity for neutralizing the electron-donor monopolar energy of, e.g., phospholipid layers, which, in the process, it appears to render more "hydrophobic." Thus, the admixture of Ca^{++} causes the monopolar repulsion to change via a (hydrophobic) AB attraction, ultimately into an apolar (LW) attraction, which favors fusion (*12*). The role of polyethylene glycol in facilitating membrane lies in its capacity to force two or more cells or vesicles together by phase separation caused by electron-donor monopolar repulsion, further aided by its strong dehydrating power (*12*).

Cell–Biopolymer Interactions

Even without considering specific ligand-receptor interactions (e.g., antigen–antibody, or lectin–carbohydrate interactions), the aspecific interactions between cells and biopolymers (especially proteins) in vivo as well as in vitro are manifold. There are several aspects to these interactions, i.e., polymer binding (or crosslinking of cells), charge effects caused directly or indirectly by bound polymers and repulsions exercised by polymers on cells, owing to monopolar polymer–cell interactions, as well as to osmotic pressures caused by extracellular dissolved polymers (e.g., the influence of plasma proteins on circulating blood cells).

Action of Neutral Polymers:
Dextran and Polyethylene Glycol

There are three entirely different effects that dextran 500 (Mw ≈ 500,000) (DEX) can have on suspensions of human erythrocytes (ER) that manifest themselves at different concentrations of DEX.

At 1–2% (w/v) DEX, the markedly asymmetrical polysaccharide molecules with dimensions of approximately 2,140 Å long and 21 Å diameter (*26*) loosely crossbind the ER at an intercellular distance corresponding to the distance of their secondary minimum of attraction (≈ 60 Å), which organizes the ER in parallel arrays of biconcave discs, or "rouleaux," since at the secondary minimum the flat-flat position of the discoids is energetically strongly favored over any other intercellular configuration (*28*).

At 5–8% DEX rouleaux are no longer formed and one only observes ER doublets or triplets in which at first the cells only appear to adhere to each other via a hingeing one-point attachment, but after some time suddenly coalesce into tight spherical miniagglutinates consisting of two or three ER each (*39*). This formation of agglutinates that form an interfacial boundary with the suspending medium of a minimum surface area, is strongly indicative of a phase separation. And indeed, the polar (AB) repulsion between ER (through their polysaccharide glycocalix as well as through their surface-adsorbed DEX) and 5 or more percent DEX dissolved in the extracellular liquid medium, energetically suffices to induce a phase separation (*40*). A certain degree of osmotic repulsion between ER and 5–8% DEX also occurs, but in this case the energy of OS repulsion is only about 0.7% of the energy of the AB repulsion. The rate of decay of the OS repulsion, however, is much more gradual than the very steep decay of the AB repulsion.

Nevertheless as, owing to their virtually complete absence of electric charge (*28*), DEX molecules can approach ER extremely closely, the strong close range AB repulsion rather than the very weak, long range, OS repulsion must be held to be the main driving force in the phase separation between the spherical ER agglutinates and the bulk DEX solution.

At > 10% DEX small spherical agglutinates no longer form and all ER are again in a stable monodisperse suspension. This is owing to the Brooks

effect (*41*), in which high DEX concentrations cause a marked increase in the ζ-potential of ER. That ζ-potential becomes elevated enough at 10% DEX to allow the individual ER cells to repel each other electrostatically sufficiently strongly to overcome the outside AB repulsion that still could push the ER together at 5–8% DEX.

Polyethylene glycol (Mw $\approx$ 8,000) (PEG) manifests only the second effect of DEX on ER, but not the last (resuspending) effect, at high concentrations, nor the first, crossbinding effect, at low concentrations (*39*). For the formation of spherical agglutinates by phase separation, owing to PEG, a concentration of 18% PEG is needed. Here also the electron-donor monopolarity of PEG (i.e., its AB repulsion) is the main driving force for the phase separation (*40*). The OS repulsion by 18% PEG, although stronger than in the case of DEX, still is not more than 7% of the outside AB repulsion energy engendered by the PEG. The molecular dimensions of PEG are too small to allow it to crossbind erythrocytes, and they also appear to be too small to have an influence on the ζ-potential of ER at high PEG concentrations.

Action of Basic Polymers

Polylysine (Mw $\approx$ 14,000) (PL), like PEG, only induces formation of spherical cell doublets, or slightly larger agglomerates, but PL causes this type of agglutination at very low concentrations (8–10 μg/mL) (*42*). A phase separation inducing force is also active in the case of PL, but here the origin of that force is electrostatic (*40*). At these very low polymer concentrations the AB and OS energies are negligible; it is only the electrostatic repulsion engendered by the strongly positively charged PL (which binds avidly to the negatively charged ER) that causes the interaction between PL-coated ER and the bulk PL molecules, thus generating a mutual repulsion of the order of $\Delta G^{EL} + 1.3$ mJ/m^2, which suffices to cause a phase separation (*40*).

Polybrene (Mw $\approx$ 6,000) (PB) is another positively charged polymer, which is frequently used in blood banking practice as an aid to hemagglutination testing for the Rh_o (D) blood group antigen (*43*). PB agglutinates by crosslinking the ER, in an nonrouleau-like fashion. The agglutinates can be made to dissociate again, on increasing the ionic strength. The convex shape of the PB-induced agglutinates (*43*) is also indicative of phase separation.

Action of Serum Albumin in Vitro

For several decades already it has been a general laboratory practice to add high concentrations (12–18%) of bovine serum albumin (BSA) to ER, in order to bring the red cells close enough together for IgG-class anti-Rh_o (D) antibodies to achieve specific hemagglutination. The mechanism of action of BSA addition has for long been a matter of some discussion, but until recently the high protein extracellular osmotic pressure was thought, on balance, to be the major driving force (*43*). However, since the recent discovery of the strong electron-donor monopolarity of serum albumin

(22), that theory should be somewhat updated. Clearly, in addition to OS forces, there are strong AB forces between the bulk BSA (e.g., 15%, w/v) and the BSA adsorbed onto the ER, which, at 15% bulk concentration, corresponds to ≈3% surface concentration (44). Thus, an *excess* bulk concentration of ≈12% may well exert a significantly stronger AB than OS pressure on the cells. In addition, the electrostatic (EL) repulsion exerted on the ER by the excess bulk BSA must be taken into account.

However, it is only at 7% (BSA) that one begins to encounter phase separation (45). We may therefore estimate that at roughly 6% albumin an equilibrium exists at which the extracellular colloid osmotic pressure equals the osmotic pressure exerted by the glycoproteins of the glycocalix together with the albumin adsorbed by the cells. For 6% albumin it is known that the ER surface concentration of adsorbed albumin is approximately 1.5% (44). We may thus conclude that the colloid-osmotic pressure exerted by the glycocalix glycoproteins is about the same as the pressure that would be exerted by 6–1.5=4.5% albumin (supposing that the *AB* interaction of these glycoproteins is about the same, per unit surface area, as the *AB* interaction of albumin). Relatively little is as yet known about the physicochemical properties of ER glycocalix glycoproteins, but from the above datum it would become possible to determine the ER surface concentration of these glycoproteins, provided their molecular weight is known (or vice versa).

At BSA concentration of 7% and higher, the long-range repulsive effect of ΔG^{OS} soon becomes quantitatively greater than the long-range LW attraction. At 12% BSA, long-range OS forces suppress the entire long-range attraction and even abolish all vestiges of a secondary minimum of attraction. Thus, with these strongly negatively charged proteins, the distance of approach would become so great (especially under the influence of the electrostatic repulsion), that *AB* forces at first sight would appear to be negligible. However, owing to their relatively small average contactable surface area (of about 1,000 Å^2), the total energy of repulsion per pair of BSA molecules would not amount to more than 10^{-3} kT. Even at 10 Å, where the ΔG^{EL} *and* ΔG^{AB} both are of the same order of magnitude, i.e., ≈0.2 mJ/m^2, these repulsive forces together only represent ≈1 kT. Thus, BSA molecules are capable of approaching each other even more closely than within 10 Å, at which point the AB repulsion is by far the dominating force. This gives rise to superstability, and enhances the phase separation between BSA and ER (with adsorbed BSA). At very close distances (<10 Å) ΔG^{OS} is only of the order of +0.1 mJ/m^2, and as such, still negligible as a short-range force. Thus, in inducing ER agglutination through phase separation by extracellularly dissolved BSA, at concentrations of 12% or more, the order of influence of the various forces is: AB > ER > OS > LW.

Action of Serum Albumin in Vivo

As shown above, the influence of high concentrations of BSA on ER can be considerable, and easily leads to phase separation, which can in-

duce cell-clumping. However, in vivo, the concentration of human serum albumin (HSA), whose physical properties are very similar to those of BSA, is much lower than those discussed above. The normal in vivo HSA concentration is ≈4.5% (w/v) and it is only at 7% (BSA) that one begins to encounter phase separation (*45*). At 4.5% HSA the concentration the total colloid (adsorbed protein + glycocalix glycoprotein) concentration at the ER surface still is higher than the bulk protein concentration in the surrounding plasma, which ensures complete cell stability. But it seems probable that approximately 5.5% HSA in peripheral blood is close to the highest safe concentration above which a variety of life-endangering cell-clumping accidents could begin to occur. The colloid-osmotic pressure of the other plasma proteins does not add much to that of HSA, on account of their lower concentration and their (generally) much higher molecular weight.

It is also clear that even in the stability of high ζ-potential proteins such as serum albumin, *AB* forces play a preponderant role.

REFERENCES

1. Chaudhury, M. K. (1984), Ph.D. Thesis, State University of New York at Buffalo, Buffalo, NY.
2. Chaudhury, M. K., van Oss, C. J., and Good, R. J. (1988), *J. Colloid Interface Sci.* (submitted).
3. van Oss, C. J., Good, R. J., and Chaudhury, M. K. (1987), *Separ. Sci. Technol.* **22,** 1–24.
4. van Oss, C. J., Chaudhury, M. K., and Good R. J. (1987), *Adv. Colloid Interface Sci.* **28,** 35.
5. van Oss, C. J., Ju, L., Chaudhury, M. K., and Good, R. J. (1988), *J. Colloid Interface Sci.* (in press).
6. van Oss, C. J., Chaudhury, M. K., and Good, R. J. (1988), *Chem. Revs.* (in press).
7. Small, P. A. (1953), *J. Appl. Chem.* **3,** 71.
8. Young, T. (1805), *Phil. Trans. Roy. Soc.* **95,** 65.
9. van Oss, C. J., Chaudhury, M. K., and Good, R. J. (1987), *Separ. Sci. Technol.* **22,** 1515.
10. van Oss, C. J., Good, R. J., and Chaudhury, M. K. (1987), *J. Chromatog.* **391,** 53.
11. van Oss, C. J., Good, R. J., and Chaudhury, M. K. (1986), *J. Prot. Chem.* **5,** 385.
12. van Oss, C. J., Chaudhury, M. K., and Good, R. J. (1987), *Membrane Fusion,* S. Ohki, ed., Plenum, New York (in press).
13. Righetti, P. G., van Oss, C. J., and Vanderhoff, J. W., eds. (1979), *Electrokinetic Separation Methods,* Elsevier, Amsterdam.
14. Overbeek, J. Th. G. and Wiersema, P. H. (1967), *Electrophoresis,* M. Bier, ed., vol. 2, Academic, NY, p. 1.
15. Verwey, E. J. W. and Overbeek, J. Th. G. (1948), *Theory of the Stability of Lyophobic Colloids,* Elsevier, Amsterdam.

16. Overbeek, J. Th. G. (1952), *Colloid Science,* M. R. Kruyt, ed., vol. 1, Elsevier, Amsterdam, p. 245.
17. Kitahara, A. and Watanabe, A. (1984), *Electrical Phenomena at Interfaces,* Marcel Dekker, New York.
18. Hirtzel, C. S. and Rajagopalan, R. (1985), *Advanced Topics in Colloidal Phenomena,* Notes Pub., Park Ridge, NJ, p. 54.
19. Chan, D. Y. C., Mitchell, D. J., Ninham, B. W., and Pailthorpe, B. A. (1979), *Water,* F. Franks, ed., vol. 6, Plenum, NY, p. 239.
20. LeNeveu, D. M., Rand, R. P., and Parsegian, V. A. (1977), *Biophys. J.* **18,** 209.
21. Parsegian, V. A., Rand, R. P., and Raw, D. C. (1985), *Chemica Scripta* **25,** 28.
22. van Oss, C. J. and Good, R. J. (1988), *J. Protein Chem.* **7,** 179.
23. Ruckenstein, E. and Schiby, D. (1983), *Chem. Phys. Lett.* **95,** 439.
24. Claesson, P. M., Blom, C. E., Herden, P. C., and Ninham, B. W. (1986), *J. Colloid Interface Sci.* **114,** 234.
25. Pashley, R. M., McGuiggan, P. M., Ninham, B. W., and Evans, D. F. (1986), *Science* **229,** 1088.
26. Edberg, S. C., Bronson, P. M., and van Oss, C. J. (1972), *Immunochemistry* **9,** 273.
27. Hiementz, P. C. (1986), *Principles of Colloid and Surface Chemistry,* Marcel Dekker, N Y, p. 266.
28. van Oss, C. J. and Absolom, D. R. (1985), *J. Dispersion Sci. Technol.* **6,** 131.
29. van Oss, C. J. and Bronson, P. M. (1970), *Separ. Sci.* **5,** 63.
30. Good, R. J. (1979), *Surface and Colloid Science,* R. J. Good and R. R. Stromberg, eds., vol. 11, Plenum, NY, p. 1.
31. van Oss, C. J., Fike, R. M., Good, R. J., and Reinig, J. M. (1974), *Analyt. Biochem.* **60,** 242.
32. van Oss, C. J., Gillman, C. F., and Neumann, A. W. (1975), *Phagocytic Engulfment and Cell Adhesiveness,* Marcel Dekker, NY.
33. van Oss, C. J. (1985), *J. Dispersion Sci. Technol.* **6,** 139.
34. van Oss, C. J. (1978), *Ann. Rev. Microbiol.* **32,** 19.
35. van Oss, C. J. (1986), *Meth. Enzymol.* **132,** 3.
36. Absolom, D. R., van Oss, C. J., Zingg, W., and Neumann, A. W. (1982), *J. Reticuloendothelial Soc.* **31,** 59.
37. van Oss, C. J., Absolom, D. R., and Neumann, A. W. (1984), *The Reticuloendothelial System,* S. M. Reichard and J. P. Filkins, eds., vol. 7A, Plenum, NY, p. 3.
38. Stinson, M. W. and van Oss, C. J. (1971), *J. Reticuloendothelial Soc.* **9,** 503.
39. Tilley, D., Coakley, W. T., Gould, R. K., Payne, S. E., and Hewison, L. A. (1987), *Eur. Biophys. J.* (in press).
40. van Oss, C. J. and Coakley, W. T. (1988), *Cell Biophys.* (in press).
41. Brooks, D. E. and Seaman, G. V. F. (1973), *J. Colloid Interface Sci.* **43,** 670.
42. Coakley, W. T., Hewison, L. A., and Tilley, D. (1985), *Eur. Biophys. J.* **13,** 123.
43. van Oss, C. J., Mohn, J. F., and Cunningham, R. K. (1978), *Vox. Sang.* **34,** 351.
44. Janzen, J. and Brooks, D. E. (1988), *Interfacial Phenomena in Biological Systems,* M. Bender, ed., Marcel Dekker, NY (in press).
45. Mohn, J. F. and Lambert, R. M., private communication.
46. Christenson, H. K. (1988), *J. Dispersion Sci. Technol.* **9,** 171.