Van der waals parameters, refractive indices and dispersion equations of spectrin, actin and other mammalian proteins

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Van der Waals' parameters and dispersion equations of some mammalian proteins including actin, spectrin, haemoglobin, myoglobin, fibrinogen, ribonuclease A, and a calf serum protein are determined from refractive indices of their solutions and the solvents. The results indicate that van der Waals' parameters of these proteins, and also those of bovine serum albumin, fall within a relatively narrow range of values; which slightly overlap those of sugars and are above those of phospholipids.

INTRODUCTION

The main purpose of this study is to obtain an estimate for the effect of proteins present at cellular surfaces and in intercellular media on the magnitude of long-range van der Waals' interactions between cellular surfaces. Recently, a detailed study has been published on the determination of van der Waals' parameters of bovine serum albumin[1]. Detailed calculations of van der Waals' interactions between cellular surfaces have been given previously[2,3]. These calculations make use of the parameters of water, phospholipids, sugars, cholesterol and a protein (bovine serum albumin) which have been chosen to represent major components at the cell surface. In this work we determine the van der Waals' parameters of some other proteins, including spectrin, which was extracted from the membranes of erythrocytes.

EXPERIMENTAL

The following proteins were studied:

spectrin (components 1 and 2 of the spectrin complex), the most abundant protein on the cytoplasmic side of the erythrocyte membrane[4] which is thought to have a major role in determining the shape of the erythrocyte[5] (in this work, it was extracted from human erythrocytes using a modification[6] of the procedure described in Marchesi *et al.*[7]);

actin, extracted from rabbit muscle[8], donated by Dr K. Laki (NIH);

ribonuclease A from bovine pancreas obtained from P. L. Biochemicals (Milwaukee, Wisconsin);

crystalline human haemoglobin and human fibrinogen, obtained from R-plus Laboratories (Denville, N. J.);

sperm whale myoglobin obtained from Schwarz–Mann Laboratory (Orangeburg, New York);

a calf serum protein of molecular weight of about 500 000 which is a factor enhancing spreading *in vitro* of substratum attached baby hamster kidney cells, Chinese hamster ovary cells, HeLa cells and L cells[9], donated by Dr F. Grinnell (University of Texas Southwestern Medical School, Dallas)[10].

Refractive index measurements were made with a precision Abbé refractometer at three visible light wavelengths, 5893, 5460 and 4358 Å using sodium vapour and mercury vapour light sources. Filters were used to obtain the 4358 and 5460 Å light from the mercury vapour lamp.

THEORETICAL

In this work refractive indices of solutions of proteins were measured and used to calculate refractive indices of the pure proteins from the Lorentz–Lorenz relation:

$$\frac{n^2(\omega)-1}{n^2(\omega)+2} = (c\bar{v})\frac{n_1^2(\omega)-1}{n_1^2(\omega)+2} + (1-c\bar{v})\frac{n_2^2(\omega)-1}{n_2^2(\omega)+2} \quad (1)$$

in which $n(\omega)$ is the refractive index of the solution at the angular frequency of incident light, ω, n_1 and n_2 are refractive indices of the solute and solvent, $\bar{v}$ is the partial specific volume of the protein and c is its concentration in g/cm^3 (see ref 11 for a discussion of this equation and ref 3 for a discussion of the assumptions made about the densities of the components of the solution). For a solution of known composition the refractive index of the solute can be calculated from the measured values of refractive indices of the solution and solvent. When refractive indices are available only in the visible range, a one-term dispersion equation must be used:

$$\frac{\tilde{n}_p^2(\omega)-1}{\tilde{n}_p^2(\omega)+2} = \frac{4\pi N}{3}\tilde{\alpha}(\omega) \quad (2)$$

where $\tilde{n}_p = (n + ik)$ is the complex refractive index of the pure substance, e.g. the protein solute, $\tilde{\alpha}(\omega)$ is its polariza-

bility which is a function of the angular frequency ω, and N is the number of molecules per unit volume of pure substance. Neglecting the imaginary part of $\tilde{n}$ in equation (2) and relating $\tilde{\alpha}(\omega)$ to the dispersion equation coefficient C and characteristic frequency[1,3] ω_c gives[12,13]:

$$\frac{n_p^2(\omega)-1}{n_p^2(\omega)+2}=\frac{C[1-(\omega/\omega_c)^2]}{[1-(\omega/\omega_c^2)]^2+(\gamma\omega/\omega_c^2)^2} \qquad (3)$$

which is reduced[14,15] to:

$$\frac{n_p^2-1}{n_p^2+2}=\frac{C}{1-(\omega/\omega_c)^2} \qquad (4)$$

when damping coefficient γ can be neglected.

Neglect of the imaginary part was considered in ref 13, in which it was shown that if absorption is weak so that $k \leqslant 0.05$ (which is the case at the wavelengths at which measurements were made in this work), k can be neglected in the dispersion equation.

The van der Waals' parameters, i.e. the parameters required in calculations of van der Waals' interactions, are the dispersion equation coefficient C and characteristic frequency ω_c[16]. In calculating C and ω_c we have employed both equation (3) and equation (4) in procedures which involve linear least-square calculations. The methods of extraction of van der Waals' parameters from refractive indices of pure liquids are discussed in refs 12, 13 and 15. Our method of extraction of parameters from refractive indices of solutions is discussed in refs 1 and 3. The isotropic static polarizability of the protein is calculated from the equation:

$$\alpha \equiv \alpha(0) = 3C/4\pi N \qquad (5)$$

where values of N are calculated from the known values of densities and molecular weights.

Van der Waals' dispersion force F is expressed in terms of the dispersion equation parameters, ω and C. For example, the force per unit area between two planar surfaces, each composed of one substance, separated by a distance d, is given by the equation[3]:

$$F = -A/6\pi d^3 \qquad (6)$$

in which A, the Hamaker constant[17], is given by the equation[3,16]:

$$A = A_{12} + A_{00} - A_{10} - A_{20} \qquad (7)$$

and

$$Aik = \frac{27}{32}\hbar\frac{C_iC_k\omega_i\omega_k}{\omega_i\omega_k} \qquad (8)$$

where $\hbar$ = Planck's constant divided by 2π, subscripts 1,2, and 0 refer to the left-hand surface, the right-hand surface and the volume between the surfaces, respectively, and C_i (or C_k) and ω_i (or ω_k) are the dispersion equation coefficient and characteristic frequency of the substance of which surface i (or k) is composed.

Expressions for forces between multilayer, multicomponent systems for many different geometries are given in refs 2 and 3. Equations which take into account many-body interactions, the orientation effects which also contribute to van der Waals' forces and retardation effects are also given and discussed in refs 2 and 3. These expressions were used in calculating long-range van der Waals' interaction energies and forces between cell surfaces from values of van der Waals' parameters of components of the cell surface.

RESULTS

The values found for the refractive indices of the proteins are given in *Table 1*. Measurements were made for several concentrations of each protein but, for brevity, results are given for one concentration only, except for ribonuclease A.

The values of polarizabilities calculated from equation (5) and van der Waals' parameters, i.e. C and ω_c are given in *Table 2*. We have also included the results for bovine albumin, which has been extensively studied by a reflectance method both in solutions[1] and as a dry film[18] in the ultraviolet and visible regions.

There are very little refractive index data recorded for most of the proteins. For this reason we have included the values of refractive indices extrapolated to pure proteins even though the errors in these values are relatively large, ten-fold, compared with those determined from direct measurements on the pure substances. The extrapolated values we have found for albumin at visible wavelengths are in close agreement with those found by Inagaki *et al.*[18] by measurement on the pure substance.

The present results on solutions of bovine serum albumin support the conclusions of previous studies[12,13] indicating that the C value which results from the use of visible data only and the employment of equation (3) is close to the sum of C values obtained from many-term dispersion equations based on both visible and ultra-violet data. In addition the contribution of any individual absorption band to the sum of C values has been shown to be relatively small[1,3,13]. Hence, for the purpose of calculations of van der Waals' energies, or for the determination of polarizabilities, the C values obtained from visible data alone are sufficient. The value of the calculated characteristic frequency is mostly sensitive to refractive index data in the ultra-violet range[3] and, therefore, the results for ω_c presented here or in other studies in which ultra-violet data were not included[15] are relatively uncertain, even if least-square calculations provide them with a small uncertainty. However, while reflectance measurements in the ultra-violet are needed in order to obtain a more reliable estimate for the characteristic frequency, the experimental errors involved in such measurements[1,18] are at present an order of magnitude larger than those in measurements with an Abbé refractometer in the visible range. Fortunately, van der Waals' interaction energies are less sensitive to the value of characteristic frequency, which appears to the first power[19], and more sensitive to the values of C, which appear to the second power[16], in the expressions for interaction energies.

The results of the present study on all of the proteins are also in agreement with those of previous studies on solutions of bovine serum albumin[1], sugars, phospholipids and cholesterol[2], which indicated that the static isotropic polarizabilities of the solute were relatively independent of concentration used. Results given in *Table 2* for ribonuclease A further illustrate this independence of concentration. The above results support the assumptions made in using equations (1)–(4) in these calculations. It may be worthwhile to add that the resulting polarizabilities of pure substances

Table 1 Refractive indices of proteins

Solution*	Wavelength (Å)	Refractive index of solution	Refractive index of solute†
3.78% Actin 27°C	4358.3	1.3514	1.584
	5460.7	1.3454	1.565
	5892.6	1.3438	1.563
4.36 % Spectrin 28°C	4358.3	1.3515	1.576
	5460.7	1.3455	1.566
	5892.6	1.3440	1.561
7.44% Ribonuclease A 25°C	4358.3	1.3520	1.600
	5460.7	1.3461	1.588
	5892.6	1.3445	1.584
14.20% Ribonuclease A 25°C	4358.3	1.3619	1.597
	5460.7	1.3555	1.581
	5892.6	1.3541	1.580
18.59% Ribonuclease A 25°C	4358.3	1.3685	1.602
	5460.7	1.3619	1.586
	5892.6	1.3602	1.583
7.40% Fibrinogen 26.9°C	4358.3	1.3505	1.573
	5460.7	1.3446	1.563
	5892.6	1.3429	1.557
3.85% Haemoglobin ‡ 26.7°C	5460.7	1.3398	1.566 ± 0.009
	5892.6	1.3383	1.564 ± 0.009
4.67% Myoglobin ‡ 25°C	5460.7	1.3408	1.549 ± 0.009
	5892.6	1.3394	1.548 ± 0.009
15.45% Bovine serum albumin 25°C	4358.3	1.3625	1.582
	5460.7	1.3562	1.570
	5892.6	1.3545	1.565
3.22% Calf serum ptotein 25°C	4358.3	1.3443	1.554
	5460.7	1.3386	1.548
	5892.6	1.3371	1.547

* The solvents used for the actin and spectrin solutions were aqueous SDS (sodium dodecylsulphate) solutions of about 5% concentration. The solvent used for the other proteins was distilled water. In this work solution is defined such that a 3.78% solution is one that contains 3.78 g solute per 100 ml solvent.

† Values of densities used in these calculations (see equation 1) were 1.3361 g/cm^3 (20°C) for actin[25], 1.37 g/cm^3 (25°C) for spectrin, 1.373 g/cm^3 for ribonuclease A[26], 1.3793 g/cm^3 (20°C) for fibrinogen[25], 1.3351 g/cm^3 (20°C) for haemoglobin, 1.3495 g/cm^3 (20°C) for myoglobin[25], 1.3642 g/cm^3 (20°C) for bovine serum albumin[25] and 1.36 g/cm^3 (25°C) for the calf serum protein. Densities of spectrin and the calf serum protein were obtained in this work. If we use a density correction of 0.001 g/cm^3 per degree to correct the densities of proteins to the temperature at which the measurements were made, the calculated values of refractive indices of the pure proteins become smaller by 0.001 to 0.004. The estimated error in the refractive index measurements is ±0.0001. The estimated error in the extrapolated values of refractive indices of the pure proteins is ±0.004 except for myoglobin and haemoglobin (±0.009). Values of the refractive indices of the solvent water at 25° C used in the calculations were 1.3325 at 5893 Å, 1.3339 at 5460 Å, and 1.3397 at 4358 Å. These values, which were measured in our work, are in agreement with those given in ref 27, which gives values for the total relevant range of temperatures. Values of refractive indices of the 5% SDS solution which was the solvent for spectrin were 1.3376 at 5893 Å, 1.3390 at 5460 Å and 1.3449 at 4358 Å.

‡ Measurements for haemoglobin and myoglobin were made at only two wavelengths because strong absorption at 4358 Å prohibited measurements from being made at this wavelength. Relatively large absorption at 5460 and 5893 Å introduced larger errors in the calculated values of refractive indices

are almost the same in their gaseous or liquid states[13,19–21]. Furthermore, the isotropic polarizability of bovine serum albumin has been shown[28] to agree within less than 3% with the value obtained by the addition of bond polarizabilities, as has also been found for a host of other organic molecules[2,22,23].

Inspection of *Table 2* indicates a wide range of polarizabilities of the proteins, roughly in proportion to their molecular weights, but a relatively narrow range, between 0.309 and 0.324, for the C values. The range of C values found for these proteins is lower than that for the sugars (0.317 to 0.332)[2] and higher than that for the phospholipids (0.27 to 0.28)[2], and calculations of van der Waals' forces between cell surfaces[2,3] show that the contribution of the proteins at a cell surface to the total long-range van der Waals forces is correspondingly intermediate between the contribution of sugars and that of phospholipids.

CONCLUSION

This work was begun in order to study the effect on cell adhesion properties of replacing one protein in the cell periphery by another[2,3]. The major conclusion of this work is that the van der Waals' parameters for proteins fall within a characteristic range. Because their van der Waals' parameters are very close in value the contributions of different proteins at the cell periphery to long-range van der Waals' forces acting between cells differ by a relatively small amount and the effect on the long-range van der Waals' forces of replacing one protein at the cell periphery by another is small. Larger differences in van der Waals' interactions may be expected by replacing proteins in the cell surface by water ($C = 0.21$, $\omega = 1.95 \times 10^{16}$ rad/sec) or, to a lesser extent, by phos-

Table 2 Van der Waals parameters of proteins*

Component	Characteristic frequency (10^{16} rad/sec)	Dispersion coefficient, C	Static polarizability, α (0)‡ (10^{-21} cm^3)
Actin 27°C	1.7 ± 0.4	0.313 ± 0.003	6.0
Spectrin 28°C	2.1 ± 0.4	0.317 ± 0.003	
Ribonuclease A (extrapolated from 7.44% solution) 25°C	2.0 ± 0.2	0.326 ± 0.002	1.29
Ribonuclease A (extrapolated from 14.20% solution) 25°C	1.9 ± 0.2	0.323 ± 0.002	1.28
Ribonuclease A (extrapolated from 18.59% solution) 25°C	1.8 ± 0.2	0.324 ± 0.002	1.28
Fibrinogen 27°C	2.0 ± 0.4	0.316 ± 0.006	31.5
Haemoglobin § 25°C	2.7 ± 0.6	0.320 ± 0.007	6.2
Myoglobin § 25°C	2.0 ± 0.6	0.309 ± 0.008	1.5
Bovine serum albumin† 25°C	1.9 ± 0.2	0.317 ± 0.003	6.5
Calf serum protein 25°C	2.8 ± 0.6	0.313 ± 0.003	46.7

* These values are calculated from refractive indices given in *Table 1*. When the density correction mentioned in *Table 1* was used, the values of the coefficients were reduced by less than 0.001 and the values of the characteristic frequencies by less than 0.1

§ Because values of refractive indices were measured at only two wavelengths, there is an increased uncertainty in the values of coefficients and characteristic frequencies

† In previous work on bovine albumin we used the value $C = 0.295$, which was obtained from ultra-violet data which gave a range of values of 0.284 to 0.319 for several concentrations of solutions in water and 2-chloroethanol[1]

‡ Values of molecular weights used in calculations of α (0) were: actin, 57 900[25]; haemoglobin, 64 500[25]; myoglobin, 17 000; fibrinogen, 339 700[25]; albumin, 69 900; calf serum protein, 500 000; ribonuclease, 13 683[26]. The polarizability of spectrin is not given because of uncertainty in the value of its molecular weight

pholipids ($\omega \simeq 2.0 \times 10^{16}$ rad/sec)[2]. It should be emphasized that the above statements regarding van der Waals interactions are limited to the 'long-range' case, i.e. to distances of separation between cell surfaces of several tens of Ångstroms or above, where the macroscopic theories of van der Waals forces are applicable, and where specific interactions between atomic groups do not play an important role.

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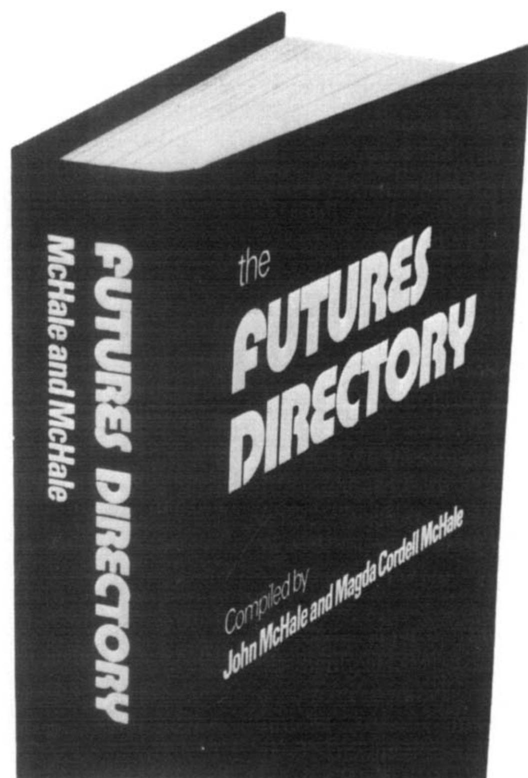

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