An Estimate of the Enthalpic Contribution to the Interaction between Dextran and Albumin*

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It is demonstrated that exclusion phenomena appear to dominate the interaction of dextran with albumin in aqueous solution. The enthalpic contribution to the interaction coefficient describing dextran/albumin mixtures is small, although its determination was subject to considerable error. These results support the earlier assumptions of the type of interaction between the two polymers. The conclusions are primarily based on the interpretation of the temperature-dependence of the interaction coefficient, as measured by light-scattering in the temperature range 6–33°C. Enthalpy of dilution measurements of dextran/albumin mixtures by microcalorimetry were in qualitative agreement with the light-scattering data.

The mutual-exclusion phenomenon has been proposed to explain many of the interactions of polysaccharides with other polymers. Studies in this direction have been performed on a variety of polysaccharide/polymer systems: e.g. hyaluronate–albumin interaction (Ogston & Phelps, 1961; Laurent & Ogston, 1963; Laurent, 1964; Preston *et al.*, 1965; Ogston & Preston, 1966); dextran–poly(ethylene glycol) interaction (Edmond & Ogston, 1968); and intervertebral-disc proteoglycan–gelatin–gel interaction (Meyer *et al.*, 1971). The exclusion concept has led to an understanding of processes of more practical nature such as chromatographic separation (Laurent & Killander, 1964), the distribution and deposition of macromolecular components in connective tissue as influenced by polysaccharides (Comper & Laurent, 1978) and the stabilization of ordered structures such as DNA (Laurent *et al.*, 1974).

It has been assumed that any interaction involving binding or other factors that produce changes in enthalpy were small in these systems and that the main interaction could be described in terms of an entropic exclusion process. The experiments described in this present paper were designed to test the validity of this assumption. We have studied the enthalpy of interaction for dextran/albumin mixtures. This system is typical for the polysaccharide–polymer interaction studies mentioned above and has direct application in gel-chromatographic processes. Two techniques have been used in this study: (a) tempera-

ture-dependence of light-scattering; (b) enthalpy of dilution of the mixture by microcalorimetry.

A part of this work has been presented previously (Comper, 1977).

Experimental

Materials

Human albumin (lot no. 50305) was obtained from AB Kabi (Stockholm, Sweden). Albumin constituted more than 98% of the total protein content according to the manufacturer. Dextran (FDR 7783) had a weight-average mol.wt. of 158000 and a number-average mol.wt. of 120000 according to the manufacturers, AB Pharmacia, Uppsala, Sweden. The moisture contents of the albumin and dextran preparations were 3.7 and 12.2% respectively with drying performed at 60°C and a pressure of 10 Pa (measurements kindly performed by Dr. G. Hesselius, Chemistry Institute, The Swedish University of Agricultural Sciences, Uppsala, Sweden).

Light-scattering

The intensity of the scattered unpolarized light was measured with a Sofica PGD 42000 M photometer at a wavelength of 436 nm. The polymer solutions showed no appreciable dissymmetric scattering, so the measurements were confined to 90°. Stock solutions of polymer, made by weight, were dialysed thoroughly against 0.2 M-NaCl/0.12 M-NaHCO₃, pH 6.7 ± 0.1, with changes in concentration of the polymer on dialysis being noted. Dilutions were performed by weight with the diffusate as solvent. Solutions were studied in the concentration range of 1–30 g/l. Solutions of dextran, albumin and a

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dextran/albumin mixture (with approximately equal weights of each polymer) were examined at five different concentrations.

All measurements were carried out in glass cells, as described by Dandliker & Kraut (1956). To clear the solutions from dust, the cells were centrifuged at 20000 rev./min for 1 h in a Spinco model L preparative ultracentrifuge, with rotor SW-25.1. The temperature-dependence of scattered intensity of a particular polymer solution was measured with the same solution in the same cell. Under these conditions any relative error of measurement, at the various temperatures studied, is minimized.

The interpretation of the light-scattering data was based on the lack of dissymmetry afforded by both dextran and albumin. The measurements of the excess scattering intensities of solution over that of solvent were then confined to the 90° angle (see Table 1). Conventional extrapolation of the scattering data to infinite dilution at 20°C and use of values of the refractive increments (v) for dextran of 0.153 ml/g (Edmond & Ogston, 1968) and for human serum albumin of 0.193 ml/g (Perlman & Longworth, 1948) gave molecular weights for dextran of 149000 and for albumin of 96000. The high molecular weight obtained for albumin is equated with the presence of stable dimers and higher aggregates (Laurent & Öbrink, 1972). These molecular weights were used to obtain the refractive indices at various temperatures from:

$$(v)^{-2} = K^* (c/R_{90})_{c=0} M \quad (1)$$

where

$$K^* = 2\pi^2 n^2 \lambda_0^{-4} N^{-1}$$

M is the molecular weight, n is the refractive index of the solvent, c is concentration, R_{90} is Raleigh scattering intensity at an angle of 90°, λ_0 the wavelength of light in vacuum and N is Avogadro's number. With the temperatures used, the change in v^2 was no more than 1.6%.

The molecular components are designated as the following: solvent (NaCl+water), component 1, albumin, component 2; dextran, component 3. The second virial coefficient of albumin is thus $(A_2)_2$ and of dextran $(A_2)_3$. The interaction coefficient between the two components is A_{23} . The third virial coefficient of albumin is $(A_3)_2$. The molecular weight of component i is defined as the M_i ($i=2,3$).

The second virial coefficient was obtained by the method of Lechner & Schulz (1973) with least-squares analysis. The evaluation of the interaction coefficient, A_{23} , describing albumin-dextran interaction in aqueous solutions from light-scattering was made by using the following relation for multicomponent systems (Hyde, 1972):

$$\frac{K^*C}{R_{90}} = \frac{1}{M^*} + \frac{2C}{(M^*)^2} [(A_2)_2 M_2^2 v_2^2 f_2^2 + 2A_{23} M_2 M_3 f_2 f_3 \cdot v_2 v_3 + (A_2)_3 M_3^2 v_3^2 f_3^2] \quad (2)$$

where

$$M^* = M_2 v_2^2 f_2 + M_3 v_3^2 f_3$$

and

$$C = c_2 + c_3, \quad f_2 = c_2/C, \quad f_3 = c_3/C$$

When the experimental values of C/R_{90} were plotted versus C , linear relations were obtained, as expected from eqn. (2). The values of A_{23} were then obtained from the slopes of such plots with the knowledge of the second virial coefficients.

The nature of the value of A_{23} clearly differentiates between two basic types of interaction as has been discussed previously (Meyer *et al.*, 1971). One is exemplified by polyanion/polycation mixtures displaying electrostatic interactions which give rise to complex coacervation. For such reactions the interaction coefficient A_{23} would have a negative value. The other type of interaction, which is purely entropic and described by a positive value of A_{23} , is that which gives rise to 'excluded volume' effects as observed in the polysaccharide/polymer systems described above. In this case, the second virial coefficients and the interaction coefficient A_{23} may be related to the volumes mutually excluded by neighbouring molecules.

Calorimetry

The calorimetry experiments were performed in a LKB (Stockholm, Sseden) isothermal twin-batch calorimeter (type 2102) equipped with two glass reaction vessels. The potential difference signal of the thermopiles was amplified, recorded and integrated by means of a disc integrator. The experiments were performed at 25.2°C. Highest recording sensitivity used was approximately 0.4 mJ. The reproducibility of the instrument, as determined by a series of calibration measurements utilizing the calibration heaters, was within a mean error of $\pm 4.5\%$.

The polymer solutions of various concentrations were made on a weight basis, then dialysed against 0.2 M-NaCl/0.04 M-phosphate buffer, pH 6.8. Changes in concentration were noted by weighing.

Measurements of the enthalpy of mixing of a solution with its diffusate was performed in the following way. A weighed solution of 1.9–2.0 g was placed in one compartment of one of the reaction vessels. An approximately equal weight of the diffusate was placed in the other compartment of the same vessel. Both compartments in the second reaction vessel were filled with diffusate only. The time for temperature equilibration of the solutions in the calorimeter was 2–4 h.

On mixing the dextran-containing solutions with their diffusate two effects were seen to occur, namely the registration of a rapid apparent heat of reaction together with a particularly slow heat of reaction,

which resulted in a marked shift of the recording baseline subsequent to mixing. Calculations of the apparent heats of mixing were based on the recording baseline subsequent to mixing. For dextran solutions in the concentration range of 20–100 g/l the apparent heat of the reaction (dilution by approx. one-half) was concentration-independent and gave a value of 4.2 ± 1.1 mJ. Similarly, dextran/albumin mixtures (with values of f_2, f_3 as in light-scattering studies) with C varying from 80 to 200 g/l gave apparent heats of dilution 7.9 ± 4.8 mJ. We interpret these apparent endothermic heats as being due to adsorption of dextran on to the walls of the mixing vessel and conclude that concentration-dependent heats of dilution of the dextran samples are not observed under these conditions.

No such adsorption effects were seen to occur with albumin solutions alone, where the shift in baseline was less than 2% of the peak value.

Results and Discussion

Light-scattering

The experimental light-scattering values are given in Table 1. They were used to calculate the second virial coefficients for albumin and dextran and their interaction coefficient (eqn. 2) over the temperature range 6–33°C. The result is shown in Fig. 1. The error analysis has been performed in terms of the standard deviation of the difference of the virial coefficients as a function of temperature by taking the lowest temperature measurements as reference. The

virial coefficient error is then a function of instrument reproducibility but not of concentration, as the same polymer solutions were used at the different temperatures.

The interaction coefficient values obtained from light-scattering are in reasonable agreement with the studies of Ogston & Silpananta (1970) on the interaction of albumin with Sephadex beads, where values of A_{23} of 1.7×10^{-4} – 2.0×10^{-4} ml·mol⁻¹·g⁻² at 25°C were obtained.

The interpretation of the temperature-dependence of the virial coefficients is based on the procedure of Schulz *et al.* (1960). The second virial coefficient is related to the chemical potential, excess of the solvent ($\Delta\mu_1^E$) over that of the solution, i.e. to the chemical potential in excess of that of an ideal solution, by:

$$A_2 = -\Delta\mu_1^E/RTc^2\bar{V}_1 \quad (3)$$

where R is the universal gas constant, T is the temperature and $\bar{V}_1$ is the partial molar volume of the solvent. The excess chemical potential, and therefore A_2 , can be separated into an enthalpic and an entropic part so that:

$$A_2 = A_{2,H} + A_{2,S} \quad (4)$$

in which, for dilute solutions

$$A_{2,H} = -\Delta\bar{H}_1/RTc^2\bar{V}_1 \quad (5)$$

and

$$A_{2,S} = \Delta\bar{S}_1/Rc^2\bar{V}_1 \quad (6)$$

where $\Delta\bar{H}_1$ and $\Delta\bar{S}_1$ are partial molar quantities.

Table 1. *Light-scattering data for polymer solutions*

Polymer	Concn. (g/l)	c/R_{90} (g/cm ²)		
	Temperature ...	7.3°C	19.0°C	32.9°C
Dextran	13.84	49.57	49.08	48.79
	9.78	39.24	38.16	38.57
	6.38	29.46	29.20	29.35
	3.15	23.07	23.46	23.02
	0.96	19.14	19.29	19.55
	Temperature ...	7.3°C	22.2°C	32.1°C
Human serum albumin	13.70	25.50	25.36	25.10
	10.77	24.06	23.82	23.79
	6.85	20.91	20.95	20.59
	3.29	18.90	19.18	19.27
	1.14	18.11	18.18	18.37
	Temperature ...	5.9°C	17.8°C	32.6°C
Dextran+albumin ($f_2=0.507, f_3=0.493$)	32.00	53.97	54.31	55.02
	21.70	42.48	41.95	42.38
	15.97	36.81	37.07	37.27
	7.61	27.20	27.59	27.74
	2.41	22.78	22.83	23.33
	Temperature ...	5.9°C	17.8°C	32.6°C

Fig. 1. Second virial coefficients of albumin (a) $[(A_2)_2]$ and dextran (b) $[(A_2)_3]$ solutions and the interaction coefficient describing albumin/dextran mixtures (c) (A_{23}) plotted versus temperature

The values were obtained by light-scattering as described in the text. For the dextran/albumin mixture $f_2=0.507$ and $f_3=0.493$.

By determining the temperature-dependence of A_2 at constant pressure the values of $A_{2,H}$ and $A_{2,S}$ can be calculated by differentiating eqn. (3) with respect to T :

$$A_{2,H} = T\alpha^*A_2 - T\left(\frac{\delta A_2}{\delta T}\right)_p \quad (7)$$

$$A_{2,S} = A_2(1 - \alpha^*T) + T\left(\frac{\delta A_2}{\delta T}\right)_p \quad (8)$$

where α^* denotes the thermal expansion coefficient of the solvent.

By a similar procedure, we have derived (see the

Appendix) the enthalpic part of the interaction coefficient A_{23} so that:

$$A_{23,H} = \frac{1}{2f_2 \cdot f_3} \left\{ T\alpha^* [(A_2)_2 \cdot f_2^2 + (A_2)_3 \cdot f_3^2 + 2A_{23} \cdot f_2 \cdot f_3] - T \frac{\delta}{\delta T} [(A_2)_2 \cdot f_2^2 + (A_2)_3 \cdot f_3^2 + 2A_{23} \cdot f_2 \cdot f_3] - f_2^2 (A_2)_{2,H} - f_3^2 (A_2)_{3,H} \right\} \quad (9)$$

and

$$A_{23,S} = A_{23} - A_{23,H} \quad (10)$$

From the data in Fig. 1, the enthalpic and entropic contributions at 25°C to the virial coefficients were calculated and are displayed in Table 2. For albumin, $A_{2,H}$ dominates the second virial coefficient term. Its positive value indicates negative heats of dilution. Therefore solutions of albumin are exothermic, which agrees with calorimetry data described below. On the other hand, the values of the enthalpic contribution for both dextran and dextran/albumin systems appear to be relatively small. Their estimates are subject to large error owing to the sensitivity of the $\delta A_2 / \delta T$ term in eqn. (7) to small errors in the virial coefficient. The athermal character of dextran solutions has been noted by Vink (1971) in precise studies of the temperature-dependence of osmotic pressure at temperatures above 20°C. This agrees with our data, although it appears some exothermic character may be conferred on dextran solutions at lower temperatures, as demonstrated by the increase in $(A_2)_3$ (Fig. 1).

From our attempts to estimate the enthalpic contribution to A_{23} it is evident that $A_{23,H}$ is small (Table 2) but subject to error. Its measured value represents an endothermic-type interaction between the two polymers. The striking feature of the results is that the exclusion term, $A_{23,S}$, dominates the value of A_{23} . This demonstrates that the main interaction between dextran and albumin is entropic in nature. This supports the earlier assumptions on the interpretation of this interaction in terms of an exclusion phenomenon (see the introduction).

Calorimetry

As mentioned above, apparent heats of dilution for dextran-containing solutions were ascribed to adsorption effects due to their concentration-independence. We have calculated the maximum enthalpic virial coefficient from the error of such measurements at the highest concentrations used, by assuming that a real heat of dilution was involved. The values so obtained are given in Table 1 and are seen to confirm qualitatively the conclusions based

Table 2. Enthalpic and entropic contributions at 25°C to the virial coefficients in albumin, dextran and mixed albumin/dextran solutions

In calculation of $A_{2,H}$ from eqns. (7) and (9), α^* was taken as that for water with a value of $257.05 \times 10^{-6} \text{ deg}^{-1}$ (Kell, 1967). The values of the coefficients are given in $\text{ml} \cdot \text{mol} \cdot \text{g}^{-2}$, except for $A_{3,H}$, which has units of $\text{ml} \cdot \text{mol} \cdot \text{g}^{-3}$.

Polymer system	Virial coefficients					
	From light-scattering				From calorimetry	
	$10^4 \times A_2$	$10^6 \times \delta A_2 / \delta T$	$10^4 \times A_{2,S}$	$10^4 \times A_{2,H}$	$10^4 \times A_{2,H}$	$10^4 \times A_{3,H}$
Albumin	1.55	-0.61	-0.48	$1.93 (\pm 0.57)$	—	14.7
Dextran	3.23	-0.19	2.41	$0.82 (\pm 0.56)$	$0.0 (\pm 0.4)$	—
Albumin+dextran ($f_2=0.507$, $f_3=0.493$)	$10^4 \times A_{23}$ 2.54	$10^6 \times \delta A_{23} / \delta T$ 0.43	$10^4 \times A_{23,S}$ 3.65	$10^4 \times A_{23,H}$ $-1.11 (\pm 0.65)$	$10^4 \times A_{23,H}$ $0.0 (\pm 2.0)$	—

Fig. 2. Plot of the variation of $A_{2,H} + A_{3,H} \cdot c_2$ versus c_2 as obtained by enthalpy of dilution of albumin solutions in the calorimeter (●—●).

$A_{2,H}$ (intercept on ordinate) was taken as $1.93 \times 10^{-4} \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$ (Table 2). The experimental points (●) are calculated from the data of Davalloor *et al.* (1972).

on light-scattering data that the enthalpic contribution is small, at least for dextran solutions. For albumin the data are significantly better. Measurable heats of dilution were obtained above concentrations of 5%. From eqn. (5) and by incorporating the third virial coefficient we represent the data in Fig. 2 as a linear plot of $A_{2,H} + A_{3,H}c_2$ versus c_2 , where $A_{3,H}$ represents the enthalpic third virial coefficient. We have set $A_{2,H}$ to $1.93 \times 10^{-4} \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$ as obtained from light-scattering. Under these circumstances, $A_{3,H}$ is evaluated to be $14.7 \times 10^4 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-3}$. We have

included the limited data of Davalloor *et al.* (1972) for comparison. The apparent differences that exist may be explained by the lack of dialysis used in their studies, since we have noted that a low-molecular-weight diffusible component present in our albumin sample contributes significantly to the heats of dilution of the albumin solution.

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APPENDIX

Eqn. (9) in the main paper (Comper & Laurent, 1978) was derived in the following way. Differentiating eqn. (3) of the main paper with respect to T at constant pressure (see also Schulz *et al.*, 1960, p. 404) gives:

$$-\left(\frac{\delta A_2}{\delta T}\right)_P = \frac{\delta}{\delta T} \left(\frac{\Delta \mu_1^E}{c^2 R T \bar{V}_1} \right)_P \quad (1)$$

where:

$$A_2 = (A_2)_2 \cdot f_2^2 + (A_2)_3 \cdot f_3^2 + 2A_{23} \cdot f_2 \cdot f_3 \quad (2)$$

Eqn. (1) may be expanded:

$$-\left(\frac{\delta A_2}{\delta T}\right)_P = \frac{1}{c^2 \bar{V}_1} \frac{\delta}{\delta T} \left(\frac{\Delta \mu_1^E}{R T} \right) + \frac{\Delta \mu_1^E}{c^2 R T \bar{V}_1^2} \left(\frac{\delta \bar{V}_1}{\delta T} \right) \quad (3)$$

If we introduce:

$$\frac{\delta}{\delta T} \left(\frac{\Delta \mu_1^E}{T} \right) = -\frac{\Delta \bar{H}_1}{T^2}$$

and

$$\alpha^* = \frac{1}{\bar{V}_1} \left(\frac{\delta \bar{V}_1}{\delta T} \right)_P$$

in eqn. (3) we obtain:

$$-\left(\frac{\delta A_2}{\delta T}\right)_P = \frac{\Delta \bar{H}_1}{R T^2 c^2 \bar{V}_1} + \frac{\Delta \mu_1^E}{c^2 R T \bar{V}_1} \quad (4)$$

but

$$(A_2)_H = -\frac{\Delta \bar{H}_1}{R T c^2 \bar{V}_1} \quad (\text{eqn. 5 in the text})$$

and

$$A_2 = -\frac{\Delta \mu_1^E}{c^2 R T \bar{V}_1} \quad (\text{eqn. 3 in the text})$$

Eqn. (4) can therefore be written:

$$-\left(\frac{\delta A_2}{\delta T}\right)_P = \frac{(A_2)_H}{T} - \alpha^* A_2$$

which can be rearranged to:

$$(A_2)_H = T \left[\alpha^* A_2 - \left(\frac{\delta A_2}{\delta T}\right)_P \right] \quad (5)$$

From eqn. (2) follows:

$$(A_{23})_H = \frac{1}{2f_2 \cdot f_3} [(A_2)_H - f_2^2 (A_2)_{2,H} - f_3^2 (A_2)_{3,H}] \quad (6)$$

Substituting eqn. (5) into eqn. (6) gives:

$$(A_{23})_H = \frac{1}{2f_2 \cdot f_3} \left\{ T \left[\alpha^* A - \left(\frac{\delta A_2}{\delta T}\right)_P - f_2^2 \cdot (A_2)_{2,H} - f_3^2 \cdot (A_2)_{3,H} \right] \right\} \quad (7)$$

Substituting eqn. (2) into eqn. (7) gives the final answer:

$$(A_{23})_H = \frac{1}{2f_2 \cdot f_3} \left\{ T \alpha^* [(A_2)_2 \cdot f_2^2 + (A_2)_3 \cdot f_3^2 + A_{23} \cdot 2 \cdot f_2 \cdot f_3] - T \frac{\delta}{\delta T} [(A_2)_2 f_2^2 + (A_2)_3 f_3^2 + A_{23} \cdot 2 \cdot f_2 \cdot f_3] - f_2^2 (A_2)_{2,H} - f_3^2 (A_2)_{3,H} \right\} \quad (8)$$

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