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RELATIONSHIP BETWEEN THE RELATIVE HYDROPHOBICITY OF MACROMOLECULES AND THE HYDROPHOBIC CHARACTER OF THEIR AQUEOUS SOLUTIONS

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SUMMARY

The effect of poly(vinylpyrrolidones) (PVPs) of various molecular weights on the hydrophobic character of the polymers' aqueous solutions at pH 7.4 was studied. The results obtained are compared with estimates of the relative hydrophobicity of the PVP fractions reported previously. The relationship between the relative hydrophobicity of different biological and synthetic polymers and the relative hydrophobic character of their solutions in isotonic saline (pH 7.4) is established. The physical meaning of this relationship and possible ways of its application are discussed.

INTRODUCTION

The relative hydrophobicity of a number of synthetic and biological polymers was estimated previously[1–4] by a partition technique[5]. The relative hydrophobicity of a solute is a measure of the affinity of the solute for an aqueous environment, *i.e.*, of the strength of the solute–aqueous solvent interactions.

Previously[6–9] we have suggested and developed an approach to estimate the effect of macromolecules on the relative hydrophobic character of an aqueous solution. The hydrophobic character of a medium has been defined[6] as a measure of the affinity of the medium for a CH_2 group, and is characterized[6–10] by the free energy of transfer of a CH_2 group from pure water or from an organic solvent chosen as a reference to the medium. In order to estimate the free energy of transfer of a CH_2 group from water to an aqueous polymer solution, partitioning of a homologous series of solutes in *n*-octanol–aqueous polymer solution and in *n*-octanol–water as a reference system can be studied[6–9].

It has been found[6–9] that an increase of the concentration of the polymer in an aqueous solution is usually accompanied by an alteration in the relative hydrophobic

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character of the solution, up to a limit specific to the polymer under study. This limiting value can be considered as a measure of the effect of the polymer on the hydrophobic character of the solution. The effect in question is clearly the result of the polymer–solvent interactions. Thus, it seems likely that there would be a relationship between the relative hydrophobicity of the polymer and the effect of the polymer on the relative hydrophobic character of its aqueous solution. In order to establish this relationship we decided to study the effect of poly(vinylpyrrolidones) (PVPs) of different molecular weights on the hydrophobic character of their aqueous solutions.

MATERIALS AND METHODS

Materials

Poly(vinylpyrrolidone) fractions with molecular weight, M_w, $5 \cdot 10^3$, $12 \cdot 10^3$, $17 \cdot 10^3$, $50 \cdot 10^3$ and $180 \cdot 10^3$ were kindly provided by Dr. Yu. Kirsch (Institute for Technology of Blood Substitutes and Hormones' Preparations, Moscow).

Sodium salts of dinitrophenylated (DNP-)glycine, alanine, norvaline, norleucine and 2-amino-*n*-octanoic acid were prepared as described previously[5]. 1-Octanol and other chemicals were of analytical reagent grade and were used without further purification.

Methods

Biphasic systems were prepared by mixing 1–2 ml of octanol with an equal volume of a polymeric solution containing sodium phosphate buffer, NaCl, poly(vinyl pyrrolidone) (PVP) and DNP-amino acid at appropriate concentrations. The aqueous phase comprised 0.15 *M* NaCl and 0.01 *M* sodium phosphate buffer, pH 7.4. The reagents were mixed with a Vortex mixer, incubated at 25°C for 30–45 min and centrifuged for 20–30 min at 4400 *g* to speed phase settling. Then aliquots of both phases were analyzed for the DNP-amino acid concentration by measuring the optical density against a correspondingly diluted phase blank at 360 nm (aqueous phase) and at 350 nm (octanol phase) after appropriate dilution in water (or octanol).

The absence of the polymer examined in the octanol phase was tested in separate experiments by refraction measurements of the octanol phase.

The partition coefficient, *P*, is defined as the ratio of the solute concentration in the octanol phase to that in the aqueous phase. The partition coefficients were measured for each DNP-amino acid over approximately ten-fold concentrations ranges and were found to be independent of the solute concentration in the phase systems. For each solute, three to five measurements were made on three or four dilutions from each partition experiment carried out three or four times in a given biphasic system.

RESULTS

A linear relationship is known to exist between the logarithm of the partition coefficient and the number of CH_2 and CH_3 groups in the aliphatic chain of solutes partitioned in a given biphasic system[5–10]

$$\ln P = C + En \tag{1}$$

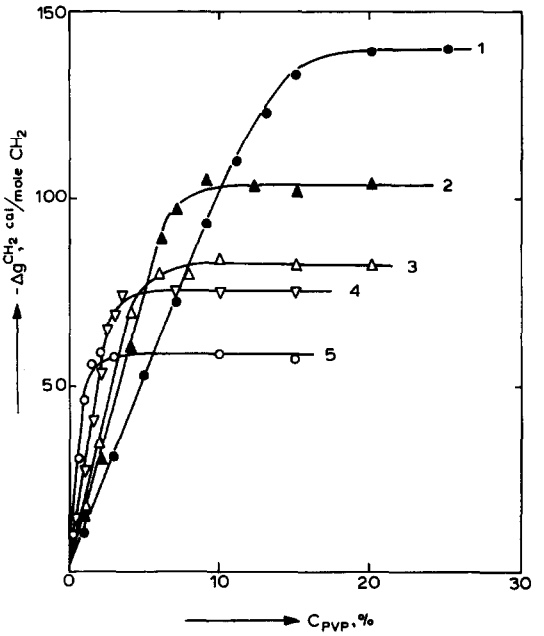

Fig. 1. Free energy of hypothetical transfer of a CH_2 group from 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.4 to a solution of poly(vinylpyrrolidone) (PVP) of the same ionic composition, Δg^{CH_2}, as a function of the polymer concentration, c_{PVP}. Molecular weight, M_w, of the polymers: 1, $5 \cdot 10^3$; 2, $12 \cdot 10^3$; 3, $17 \cdot 10^3$; 4, $50 \cdot 10^3$; 5, $180 \cdot 10^3$.

where n is the equivalent number of CH_2 groups in the aliphatic chain of the solute (amino acid side-chain in this case)[11], C is the increment of ln P per ionopolar fragment of the solute molecule (that of the sodium salt of DNP-glycine in this case) and E represents the average ln P increment per CH_2 group. It is clear that E is related to the free energy of transfer of a CH_2 group from one phase to the other in a biphasic system, Δg^{CH_2}, according to $\Delta g^{CH_2} = -RTE$. The Δg^{CH_2} value specific for the *n*-octanol–0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.4 is known[6–9] to be 618 cal per mole of CH_2. Hence, it is possible to calculate the free energy of transfer of a CH_2 group between the phases of the hypothetical biphasic system buffer–polymer solution, Δg^{CH_2}.

The Δg^{CH_2} values determined as described above are plotted as a function of the PVP sample concentration in Fig. 1.

DISCUSSION

The data presented in Fig. 1 indicate that the relative hydrophobic character of the aqueous solution of PVP at pH 7.4 depends on the molecular weight and on the concentration of the polymer. The curves shown have two main features. The initial linear range can be described as

$$\Delta g^{CH_2} = \beta c \tag{2}$$

TABLE I

CHARACTERISTICS OF THE EFFECT OF POLY(VINYLPYRROLIDONE) ON THE RELATIVE HYDROPHOBIC CHARACTER OF AN AQUEOUS MEDIUM CONTAINING 0.15 *M* NaCl IN 0.01 *M* SODIUM PHOSPHATE BUFFER, pH 7.4

M_w	$-\beta$ (cal per mole of CH_2/mole · l^{-1})	$-\delta(\Delta g^{CH_2})$ (cal per mole of CH_2)
$5 \cdot 10^3$	$5.17 \cdot 10^3$	139 ± 4
$12 \cdot 10^3$	$1.78 \cdot 10^4$	103 ± 3
$17 \cdot 10^3$	$2.95 \cdot 10^4$	82 ± 5
$50 \cdot 10^3$	$1.32 \cdot 10^5$	75 ± 4
$180 \cdot 10^3$	$8.33 \cdot 10^5$	58 ± 5

where c is the concentration of the polymer, Δg^{CH_2} is the free energy of the hypothetical transfer of a CH_2 group from the buffer to the polymer's solution in the buffer and β is a constant representing the effect of the polymer's concentration on the hydrophobic character of the solution. Values of β for all the fractions of PVP examined are given in Table I.

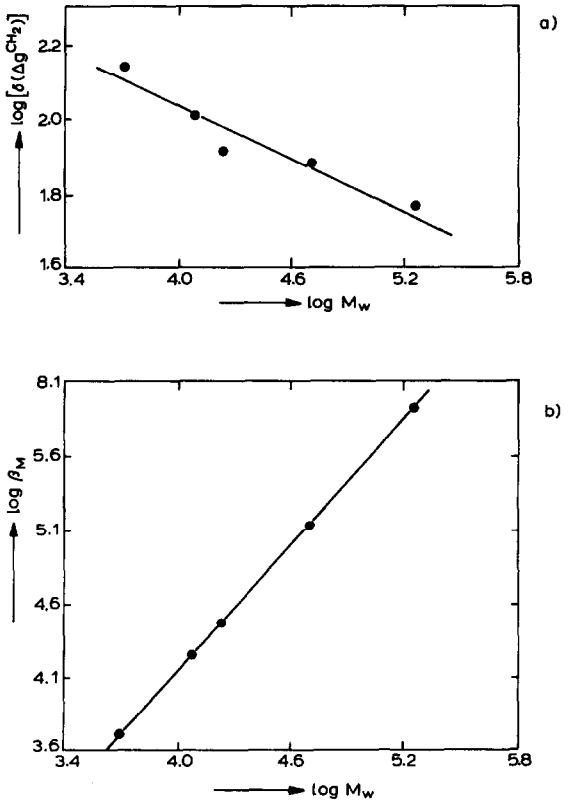

Fig. 2. $\delta(\Delta g^{CH_2})$ (a) and β (b) as a function of the molecular weight, M_w, of poly(vinylpyrrolidone).

The data presented in Fig. 1 indicate that the relative hydrophobic character of the PVP solution attains a limit, $\delta(\Delta g^{CH_2})$, specific to the given molecular weight of the polymer at a certain concentration of PVP. The $\delta(\Delta g^{CH_2})$ values determined are presented in Table I.

The data in Table I indicate that both β and $\delta(\Delta g^{CH_2})$, characterizing the effect of PVP on the relative hydrophobic character of the polymer's aqueous solution, depend upon the molecular weight of the polymer as shown in Fig. 2. The relationships plotted in Fig. 2 are described as

$$\beta = -0.030 M_w^{1.416} \qquad (r = 0.999) \tag{3}$$

and

$$\delta(\Delta g^{CH_2}) = -892.5 M_w^{-0.230} \qquad (r = 0.960) \tag{4}$$

where r is the correlation coefficient.

The results obtained are presented in Table II together with those reported earlier[7–9] on the relative hydrophobic character of aqueous solutions of different polymers and on the relative hydrophobicity of the polymers[1,2,4]. The $\delta(\Delta g^{CH_2})$ values are plotted in Fig. 3 *versus* the corresponding estimates of the relative hydrophobicity of the polymers examined[1,2,4]. The relative hydrophobicity of a polymer is expressed in terms of equivalent CH_2 groups[5]. In the case of the plant β-1,4-glucomannanes,

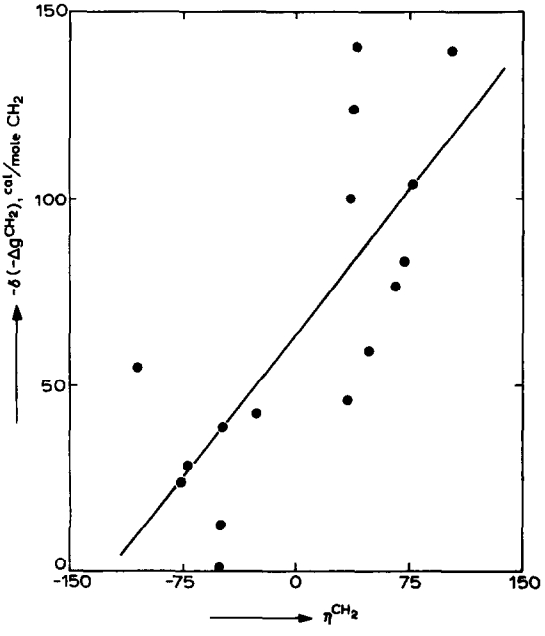

Fig. 3. Relationship between the relative hydrophobicity of polymers, n^{CH_2}, and the maximum value of the hydrophobic character of the polymers' aqueous solutions containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.4, $\delta(\Delta g^{CH_2})$.

TABLE II

RELATIVE HYDROPHOBICITY AND THE EFFECT OF MACROMOLECULES ON THE RELATIVE HYDROPHOBIC CHARACTER OF AN AQUEOUS SOLUTION CONTAINING 0.15 *M* NaCl IN 0.01 *M* SODIUM PHOSPHATE BUFFER, pH 7.4

Polymer	M_w	n^{CH_2}*	$-\delta(\Delta g^{CH_2})$** *(cal per mole, CH_2)*
Poly(vinyl alcohol)	$2 \cdot 10^4$–$1 \cdot 10^5$		
Acetylation degree:		33.4	45.0
1%		34.8	100.0
6%		36.4	124.0
12%		38.2	140.0
18%			
Polyacrylamide	$1.06 \cdot 10^4$	−51.0	11.0
	$6.65 \cdot 10^4$	−76.4	24.0
	$4.54 \cdot 10^5$	−106.3	55.0
Poly(vinylpyrrolidone)	$5 \cdot 10^3$	102.5	139.0
	$12 \cdot 10^3$	75.8	103.0
	$17 \cdot 10^3$	71.0	82.0
	$50 \cdot 10^3$	64.8	75.0
	$180 \cdot 10^3$	49.3	58.0
β-1,4-Glucomannanes			
From *Eremurus comosus*	$60 \cdot 10^3$	−50.8	0.0
From *Eremurus fuscus*	$158 \cdot 10^3$	−72.7	27.0
From "toober-salep"***	$300 \cdot 10^3$	−49.3	38.0
From *Eremurus hissaricus*	$360 \cdot 10^3$	−27.2	42.0

* From refs. 1, 2 and 4.

** From refs. 7–9.

*** The pharmaceutical preparation from which the polysaccharide in question was isolated as described in ref. 7.

the relative hydrophobicity of which depends upon the ionic composition of the aqueous medium[4], the n^{CH_2} values given in Table II and plotted in Fig. 3 are those characterizing the relative affinity of the polysaccharides for an aqueous medium containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.4.

The relationship plotted in Fig. 3 is described as

$$\delta(\Delta g^{CH_2}) = -64.12 - 0.51 n^{CH_2} \qquad (5)$$
$$m = 16, \quad r = 0.746, \quad s = 30.63$$

where $\delta(\Delta g^{CH_2})$ is the maximum value of the relative hydrophobic character attainable for the aqueous solution of a given polymer in the presence of 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.4, n^{CH_2} is the relative hydrophobicity of the polymer in the aqueous medium, m is the number of samples of different polymers examined, r is the correlation coefficient and s is the standard deviation from the regression.

The correlation established between the affinity of a solute for an aqueous medium and the effect of the solute on this medium appears to be substantiated theoretically. The relative hydrophobicity of a solute by definition[12] is a measure of

the total free energy of the solute–aqueous solvent interactions. It seems likely that these interactions perturb the intermolecular hydrogen bonds in water, which due to a cooperative effect[13] results in an alteration of the thermodynamic state of the bulk aqueous medium. The most spectacular example of the relationship between the affinity of a solute for water and its effect on the water structure appears to be the relationship between the position of an ion in the Hofmeister lyotropic series and the relative hydrophilicity (hydrophobicity) of the ion[14].

The most important feature of the established relationship described by eqn. 5 seems to be that this equation can be used to estimate the hydrophobic character of aqueous solutions of biological macromolecules which cannot be experimentally determined. For example the hydrophobic character of the aqueous protein solutions cannot be estimated by the method used by us[6–9] as it is impossible to form an octanol–aqueous protein biphasic system. The relative hydrophobicity of proteins can be evaluated, however, by partitioning of proteins in the aqueous ficoll–dextran biphasic system[3], and the corresponding $\delta(\Delta g^{CH_2})$ values can be calculated using eqn. 5. This approach is now under investigation in our laboratory.

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