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Interaction between Drugs and Water-Soluble Polymers. I.[1] Binding of Warfarin and 4-Hydroxycoumarin with Polyvinylpyrrolidone and Acrylamide-Vinylpyrrolidone Copolymer

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The interaction between drugs and water-soluble polymers was studied by an equilibrium dialysis method. Drugs used were warfarin (WF) and 4-hydroxycoumarin (HC). Polymers used were polyvinylpyrrolidone (PVP), polyacrylamide (PAA), acrylamide-vinylpyrrolidone copolymer (CAV), dextran (DX) and bovine serum albumin (BSA). No interaction of the drugs with PAA or DX was observed. The bindings of drugs with PVP and CAV were predominantly attributed to hydrophobic interaction, and the polymers had a large number of binding sites (n). BSA exhibited a specific hydrophobic interaction with the drugs at two binding sites. The standard increase of enthalpy (ΔH°) was estimated to be 16—20 kJ/mol from the temperature dependence of the binding constant. Phenylbutazone (PB) competed with WF for the binding to BSA, CAV and PVP.

Keywords——equilibrium dialysis; hydrophobic interaction; water-soluble synthetic polymer; polyvinylpyrrolidone; bovine serum albumin; warfarin; 4-hydroxycoumarin; phenylbutazone; competition

Introduction

It is well known that many drugs bind with proteins such as serum albumin, and the drug–protein binding influences the pharmacological action, distribution, metabolism, excretion, *etc.* It is important to elucidate the sites and characteristics of the drug–protein binding.[2] Some water-soluble synthetic polymers have also been used as medicines. In medical application of such a polymer, the side effects and binding with biological substances are important problems. For example, dextran (DX) has been practically used as a plasma substitute (expander) because it has no significant interaction with drugs and the body. Further, polyvinylpyrrolidone (PVP) has been used as a pharmaceutical ingredient, *etc.* Although the interaction between dyes and these water-soluble synthetic polymers has been studied by many workers,[3] little attention has been paid to the interaction between drugs and water-soluble synthetic polymers.

Therefore we started to study the interaction of drugs with water-soluble natural and synthetic polymers. In the present paper, binding of drugs to polymers was studied by an equilibrium dialysis method. Drugs used were warfarin (WF), an anticoagulant that binds strongly to protein, and 4-hydroxycoumarin (HC), a synthetic precursor of WF. Polymers used were PVP, polyacrylamide (PAA), acrylamide-vinylpyrrolidone copolymer (CAV), DX and bovine serum albumin (BSA). Furthermore, since it is known that the binding of phenylbutazone (PB) to protein increases the pharmacological action of WF, the competitive action of PB, an anti-inflammatory analgesic agent, was investigated.

Experimental

Materials——WF, HC and PB were of special reagent grade from Sigma and used without further purification. PVP was from Tokyo Kasei, DX and BSA from Wako. The average molecular weights of these polymers were 4.0×10^4, 4.0×10^4 and 6.9×10^4, respectively. α,α′-Azobisisobutyronitrile (AIBN), acrylamide (A) and vinylpyrrolidone (V) were commercial products and were purified by ordinary methods prior to use. Other reagents were from commercial sources and were used without further purification.

Preparation of Polymers——CAV was prepared by radical polymerization at 60 °C in tubes sealed under vacuum as follows. A mixture of monomers (A and V), initiator (AIBN) and solvent (methanol) in a tube was degassed by successive freeze-pump-thaw cycles and copolymerized until *ca.* 5% conversion. The reaction mixture was poured into a large excess of ether. The resulting precipitate of copolymer was filtered off, dried under reduced pressure, dissolved in water and ehtanol, filtered through an ultrafilter (Amicon 8050), and freeze-dried. PAA was prepared in similar manner.

Analysis of Polymers——The composition of CAV was calculated from the areas obtained by integration of proton nuclear magnetic resonance (^{1}H-NMR) peaks (in D_2O, 400 MHz, JEOL GX-400). The molecular weights of CAV and PAA were determined by gel permeation chromatography (GPC) as follows. Standard samples, poly(ethylene oxide) from Toyo Soda ($M = 1.8 \times 10^4$—9.9×10^5, $M_w/M_n = 1.02$—1.10, $V_R = 26.7$—36.9 ml, $W_h = 1.4$—1.8 ml); samples, CAV-1 ($M = 4.4 \times 10^5$, $V_R = 28.4$ ml, $W_h = 5.3$ ml) and CAV-2 ($M = 2.4 \times 10^5$, $V_R = 29.8$ ml, $W_h = 5.1$ ml); column, TSK Gel G6000PW + G3000PW (7.5 mm i.d. × 60 cm × 2); carrier solution, 0.1 M phosphate buffer; flow rate, 0.5 ml/min; temperature, 40 °C; detector, Showa Denko SE-31 refractometer.

Equilibrium Dialysis——Dialysis cellulose membrane (0.09 mm in thickness) supplied by Visking Company was boiled four times for 5 min each, and interposed between two parts of a dialysis cell made of polymethylmethacrylate.[4] The polymer solution or phosphate buffer solution (0.1 M, pH = 7, for the control experiment) was injected into one side of the cell, and the drug solution into the other side, the volume of each side being 0.8 ml. After the cell had been shaken in a thermostat at a given temperature for 24 h, the absorbance of the drug solution without polymer was measured on a ultraviolet (UV) spectrophotometer (Shimadzu, UV-3000). UV λ_{max}^{pH7} nm (ε): WF, 308 (14000); HC, 285 (13500); PB, 264 (19900). pK_a: WF, 4.8; HC, 3.9; PB, 4.2.

Results

The Binding of WF and HC to BSA

The binding of an anticoagulant (WF) and its precursor (HC) to BSA was examined by an equilibrium dialysis method at 30 °C. The free drug concentration (D_f) was determined from the residual drug concentration and the number of mol of drug binding to one mol of the polymer (r) was estimated from the decrease of drug concentration. The plot of $1/D_f$ *vs.* $1/r$ gave a linear relationship as shown in Fig. 1 and satisfied Eq. 1 proposed by Klotz *et al.*[5]

$$\frac{1}{r} = \frac{1}{nKD_f} + \frac{1}{n} \quad (1)$$

where n is the number of binding sites per mol of the polymer and K is the binding constant between the drug and the polymer. As the intercept of the line on the ordinate is $1/n$ and the slope is $1/nK$, n- and K-values can be calculated (Table I).

Similar experiments were run at 5—40 °C. Klotz's equation (Eq. 1) was applied at each temperature and the same n-value ($n = 2$) was obtained. The plots $\ln K$ *vs.* the reciprocal absolute temperature ($1/T$) gave a linear relationship, as shown in Fig. 2. The standard increase

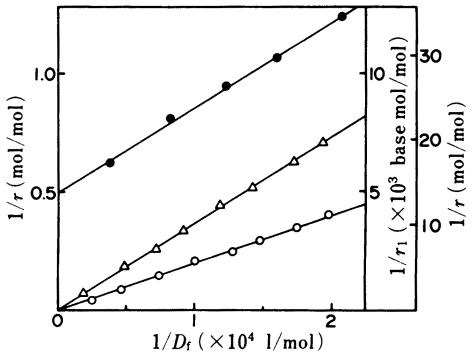

Fig. 1. Klotz Plots for the Binding of Drugs and Polymers in 0.1 M Phosphate Buffer (pH 7) at 30 °C

○, WF/PVP; ●, WF/BSA; △, HC/PVP. [PVP]= 1.25×10^{-4} M, [BSA]= 7.25×10^{-5} M.

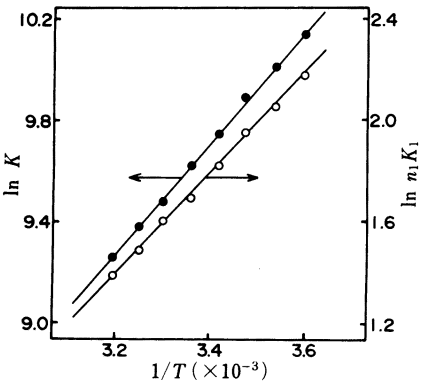

Fig. 2. Relationship between $\ln K$, $\ln n_1K_1$ and Reciprocal Absolute Temperature

●, WF/BSA; ○, WF/PVP.

TABLE I. Binding Parameter for the Interaction of Drugs with Polymers

Polym.	M_w ($\times 10^4$)	Mol. frac.		nK ($\times 10^3$ M^{-1})		n_1K_1 (l/base mol)	
		[AA]	[VP]	WF	HC	WF	HC
PVP	4.0	0	100	1.79	0.98	4.97	2.72
CAV-1	44.0	56	44	8.77	2.67	4.02	1.22
CAV-2	24.0	79	21	1.15		1.81	
PAA	20.0	100	0	0	0	0	0
BSA	6.9			27.8	11.5		
DX40	4.0			0	0		

[Polymer]=0.5%, pH=7, [Phos. Buf.]=0.1 M, 30 °C; BSA, $n=2$.

TABLE II. Thermodynamic Data for the Interaction of Drugs with Polymers

Drug	Polym.	$\Delta G'$ (kJ/base mol)	ΔH° (kJ/base mol)	$\Delta S'$ (J/base mol/K)
WF	PVP	−4.04	−16.74	−41.91
	CAV-1	−3.51	−20.42	−55.81
	BSA	−24.03[1)]	−21.76	7.49[2)]
HC	PVP	−2.52	−16.32	−45.54
	CAV-1	−0.51	−18.66	−59.90
	BSA	−21.81[1)]	−21.30	1.68[2)]

1) ΔG (kJ/mol), 2) ΔS° (J/mol/K). [Polymer]=0.5%, pH=7, [Phos. Buf.]=0.1 M, 30 °C.

of enthalpy (ΔH°) in the drug–polymer binding was determined from the slope of this line. Standard increases of free energy (ΔG°) and entropy (ΔS°) were calculated from Eqs. 2 as shown in Table II.

$$\Delta G^\circ = -RT \ln K$$
$$T\Delta S^\circ = \Delta H^\circ - \Delta G^\circ \qquad (2)$$

The Interaction between WF and PVP

The binding of WF to PVP was measured by the same method at 30 °C. As in the WF–BSA system, the plot of $1/D_f$ *vs.* $1/r$ gave a linear relationship (Fig. 1). Since the intercept was nearly zero, n was numerically large and could not be estimated accurately. Such a phenomenon has been observed in many cases of binding to synthetic polymer (PVP). Takagishi and Kuroki[6] have evaluated the magnitude of binding in terms of nK-value rather than K-value estimated from the uncertain n-value. Thus, the nK-value (1.79×10^3 M^{-1}) was estimated as shown in Table I.

On the other hand, Ando *et al.*[7] analyzed the binding of dye to PVP from the number of mol (r_1) of dye bound to one base mol of V residue. Therefore the plot of $1/D_f$ *vs.* $1/r_1$ again gave a linear relationship according to Eq. 3, as shown in Fig. 1,

$$\frac{1}{r_1} = \frac{1}{n_1 K_1 D_f} + \frac{1}{n_1} \tag{3}$$

where n_1 is the number of binding sites of drug per base mol of V, and K_1 is the binding constant between the drug and V.

Similar experiments were run at 5—40 °C. Klotz's equation (Eq. 3) was applied at each temperature, and $n_1 K_1$ could be calculated. The plots of $\ln n_1 K_1$ *vs.* the reciprocal absolute temperature gave a linear relationship as shown in Fig. 2. Assuming that the number of binding sites (n_1) was independent of temperature, the standard increase of enthalpy ($\Delta H^\circ = -16.74$ kJ/mol) at equilibrium binding between drug and polymer was determined from the slope of this line. Additionally, the apparent increases of free energy ($\Delta G'$) and entropy ($\Delta S'$) were tentatively estimated at 303 K by using Eqs. 4 (Table II).

$$\Delta G' = -RT \ln n_1 K_1$$
$$T\Delta S' = \Delta H^\circ - \Delta G' \tag{4}$$

The K_1-value increased when the concentration of phosphate buffer was varied from 0.05 to 0.2 M. The K_1-value obtained in 5% methanol solution was smaller than that in aqueous solution (Table III).

Copolymer of Acrylamide and Vinylpyrrolidone

CAV was prepared by the radical polymerization of A and V. The mol fraction of A in copolymer (0.6—0.95) was greater than that in the monomer feed (0.1—0.9), as shown in Fig. 3. Generally, the composition curve of copolymer is represented by Eq. 5[8]

TABLE III. Binding Parameter for the Interaction of Drugs with PVP

[Phos. Buf.] (M)	[MeOH] (%)	$n_1 K_1$ (l/base mol)	
		WF	HC
0.05	0	4.19	2.06
0.1	0	4.97	2.72
0.2	0	5.44	3.08
0.1	5	3.44	2.19

[Polymer]=0.5%, pH=7, 30 °C.

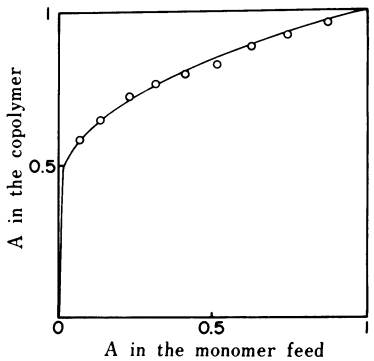

Fig. 3. Composition Curve for the Copolymerization of Acrylamide (A) and Vinylpyrrolidone (V) with α,α'-Azobisisobutyronitrile (5 mM) in Methanol at 60 °C

$$\frac{d[A]}{d[V]} = \frac{[A](r_1[A]+[V])}{[V]([A]+r_2[V])} \quad (5)$$

where the left-hand side is the molar ratio of A to V in the copolymer, and [A] and [V] in the right-hand side are the initial concentrations of the monomers. Monomer reactivity ratio (r_1) represents the ratio of the rate constant of A to that of V for the addition to the polymer radical with an A unit at the terminal position. Similarly, monomer reactivity ratio (r_2) is that of V to A for the addition to the growing chain having V as the terminal group. The r_1- and r_2-values were obtained by a curve-fitting treatment from Eq. 5 ($r_1=4.84$, $r_2=0.0014$). The copolymer (CAV-1) with the molar ratio of A/V=56/44 had a broader molecular weight distribution than standard poly(ethylene oxide) samples, and gave the average molecular weight of 4.4×10^5. The average molecular weight of CAV-2 (A/V=79/21) was similarly estimated to be 2.4×10^5.

The Interaction between WF and CAV

The bindings of WF to the copolymers (CAV-1, CAV-2) were investigated. The magnitudes of the n_1K_1-value calculated from Klotz's equation (Eq. 3) were in the order of PVP>CAV-1>CAV-2 (Table I). No interaction of WF with PAA and DX was observed. Since the interaction between WF and pyrrolidone residue in CAV may play an important role in the binding process, the binding constant (n_1K_1) of WF per V unit was estimated. The constant (n_1K_1) became close to zero with decrease in the mol fraction of V in the copolymer. The thermodynamic parameters (ΔH°, $\Delta G'$, $\Delta S'$) were determined from the temperature dependence of binding constants between WF and CAV-1 (Table II).

The Interaction between HC and Polymers (PVP, CAV, BSA)

The binding between HC, an analog of WF, and polymers (PVP, CAV, BSA) was examined by a method similar to that described above. The data in all cases satisfied Eq. 1 and Eq. 3 suggested by Klotz, and the nK- and n_1K_1-values estimated from the slopes were smaller than those in the case of WF–polymer binding (Table I). Further the absolute values of thermodynamic parameters (ΔH°, $\Delta G'$, $\Delta S'$) determined from the temperature dependence of the constants (n_1K_1) were smaller than those in the binding between WF and polymers (Table II).

PB as a Competitor of WF Binding

Since PB binds strongly to protein, the possible competitive effect of PB against WF–polymer binding was investigated. In the absence (A) or presence (B) of 1 mM PB, Klotz's plots showed a linear relationship and the n_1K_1-value estimated from the slope was reduced in the presence of PB (Table IV). The degree of reduction were in the order of BSA>CAV-1>PVP.

TABLE IV. Binding Parameter for the Interaction of Warfarin with Polymer in the Presence and Absence of Phenylbutazone

Polymer	n_1K_1 (l/base mol)		(B/A)×100
	[PB]=0 mM (A)	[PB]=1 mM (B)	
PVP	4.97	4.16	83.8
CAV-1	4.02	2.20	54.8
BSA	13900[1)]	3150[1)]	22.6

1) K (M^{-1}), [Polymer]=0.5%, pH=7, [Phos. Buf.]=0.1 M, 30 °C.

Discussion

It is generally accepted that the intermolecular interaction is attributed to ionic force, dipole interaction, hydrogen bonding, charge–transfer complex formation, hydrophobic interaction, *etc.* The interaction between drugs and proteins, especially BSA and human serum albumin (HSA), has been investigated by many workers. It is usually considered that BSA has two classes of hydrophobic binding sites[9] and that the hydrophobic binding causes the destruction of water structure. In the case of drug–protein binding, Klotz's equation (Eq. 1) applies to the relationships between free drug concentrations (D_f) and bound concentrations (r) in many cases.

In the present paper, Eq. 1 was also valid when the binding of an anticoagulant (WF) and its synthetic precursor (HC) with BSA was examined by an equilibrium dialysis method. The following results were obtained. (1) The number of binding sites (n) on BSA is two. (2) The binding constant (K) is large (Table I). (3) The standard increase of enthalpy (ΔH°) is large and negative (Table II). (4) The standard increase of entropy (ΔS°) is positive. These results agree with those in the previous paper.[10] Therefore, the interaction of WF and HC with BSA is primarily attributable to hydrophobic interaction.

The interactions between the water-soluble synthetic polymers (PVP, PAA, CAV, DX) and the drugs (WF, HC) were investigated. While no interaction of drugs with PAA or DX was observed, the drugs did bind with PVP and CAV, and Klotz's equation (Eqs. 1 and 3) was also valid. However, since the numerical values of the intercepts on the ordinate were nearly zero, differently from the drug–BSA system, the numbers (n and n_1) of binding sites on the polymer were uncertain. Since the binding constants (K and K_1) could not be obtained accurately, the products (nK and n_1K_1) were evaluated from the slope (Table I). Standard increase of enthalpy (ΔH°) was determined from the relationship between $\ln n_1K_1$ and reciprocal absolute temperature ($1/T$), assuming a constant n_1-value independent of temperature (Table II). The values of ΔH° were negative and large, and the absolute values of ΔH° were smaller than those for the interaction with BSA. Consequently, the bindings between drugs (WF, HC) and polymers (PVP, CAV) may be stable enthalpically, though the bindings are unstable in comparison with the bindings between drugs and BSA. The apparent parameters ($\Delta G'$, $\Delta S'$) calculated by using Eqs. 4 were negative and large (Table II). Since the intercept ($1/n_1$) in Fig. 1 seems to be smaller than 140, the K_1 value for the WF–PVP binding will not exceed $710\,\mathrm{M}^{-1}$ and the ΔS° value calculated by Eqs. 2 will be lower than -0.66 J/mol/K. Therefore, the interaction between WF and PVP is disadvantageous entropically because of the negative value of ΔS°. It is considered that the copolymers, CAV-1 and CAV-2, may have sequences such as $(-\mathrm{AV}-)_m$ and $(-\mathrm{AAAV}-)_m$, respectively, where A and V represent acrylamide and vinylpyrrolidone, respectively. WF did not bind to polyamide (PAA), $(-\mathrm{A}-)_m$, but bound to PVP, $(-\mathrm{V}-)_m$. It can therefore be presumed that the hydrophobic interaction between the methylene group of pyrrolidone residue in CAV and the phenyl group of WF plays a predominant role in the binding process. Furthermore, the binding constant (n_1K_1) of WF per mol of V residue decreased with reduction of the molar ratio of V in CAV, especially below 20% V. Consequently, it is considered that WF could not bind to the polymer when isolated V units exist in long sequences of $(-\mathrm{A}-)_m$, and that several V units arrange hydrophobically around a molecule of WF when V units are separated by 1—4 units of A (Chart 1).

On the other hand, the contribution of dipole interaction to binding between WF and polymers may be small since no dipole interaction between WF and polyamide (PAA) is observed. A contribution of hydrogen bonding to the binding may also be ruled out since WF and the polymers (PVP, CAV, PAA) have no hydroxyl group and no interaction between WF and polysaccharide (DX) was observed.

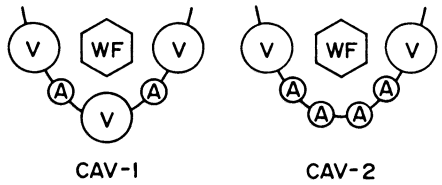

Chart 1. Hydrophobic Interaction between Warfarin (WF) and Acrylamide (A)–Vinylpyrrolidone (V) Copolymers (CAV)

As shown in Table III, the n_1K_1-value increases with increasing concentration of phosphate buffer and decreases on addition of methanol. It seems reasonable that PVP is a randomcoil chain molecule and that its structure may not transform in the presence of low concentrations of inorganic salt or methanol. Since WF is a weak acid (pK_a 4.8), the ionic form would be predominant at pH 7. However, as the amide group of PVP is a very weak base, the undissociated form only exists at pH 7. It is considered from these facts that the role of hydrophobic interaction must be more important than that of ionic interaction in the binding of WF and PVP. A charge–transfer complex can also be ruled out because no shift in the UV spectrum of WF was observed on addition of PVP.

The interaction between polymers and HC which has no substituent at the 3-position was investigated. The bindings of HC with PVP, CAV-1 and BSA were weaker than that of WF, and the nK- and n_1K_1-values for the HC–polymer system were smaller than those for the WF–polymer system. HC has a hydrophilic part, the 4-hydroxypyrone group, and a hydrophobic part, the benzene group, and it is considered that this benzene group binds to the hydrophobic part of polymers. The reason why WF binds to polymer more strongly than HC is presumably the contribution of hydrophobic interaction of the substituent at the 3-position.

It is well known that an anti-inflammatory analgesic agent, PB, binds strongly to protein, and that PB stimulates the pharmacological action of WF. Thus, the competitive action of PB on the bindings between WF and polymers (PVP, CAV, BSA) was investigated. It is considered that the competition of PB is more prominent in the case of WF–BSA because PB and WF competitively bind to the hydrophobic part of BSA. On the other hand, the weak competition of PB in the case of WF–PVP may be explained by the comparable nonspecific binding of WF and PB to PVP. As mentioned above, the binding constant (n_1K_1) of WF per pyrrolidone residue in CAV is smaller than that in PVP. It is therefore considered that PB competes more strongly with the binding in WF–CAV than in WF–PVP. A detailed discussion on the binding mode based on NMR studies will be presented elsewhere.

In conclusion, it is presumed that the bindings between drugs (WF, HC) and water-soluble polymers (PVP, CAV, BSA) are predominantly owing to hydrophobic interaction.

References and Notes

1) This work was presented at the Meeting of the Chugoku Shikoku Branch, Chemical Society of Japan, Matsuyama, Nov. 1985.
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