Binding of Warfarin with Quaternized Poly(4-vinylpyridinium bromide)s[1)]

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The interaction between warfarin (WF) and ionic synthetic polymer was investigated using an equilibrium dialysis method. Polymers used were quaternized poly(4-vinylpyridinium bromide)s (QPV), in which N-alkyl groups were butyl (QBu), methyl (QMe) and benzyl (QBz), and degree of quaternization of polymer was shown in parentheses as a percentage. The nature of bindings depended on both the hydrophilic property of the pyridinium part and the hydrophobic property of the alkyl and pyridine parts. The bindings of WF to QBu(100) and QMe(100) were assumed to be due to hydrophilic interaction. The binding capacity of WF to QBu decreased with increase of the degree of quaternization of QBu. The binding of WF to QBu(24) was attributed to both hydrophobic and hydrophilic parts. The binding of WF to QBz(80) was due to hydrophobic interaction because of the hydrophobic property of the benzyl group. *N*-Butyl-4-ethylpyridinium bromide (BEP) monomer and WF formed an equimolar complex. Phenylbutazone (PB) competed with WF for the hydrophobic binding to QBz(80).

Keywords quaternized poly(4-vinylpyridinium bromide); warfarin; hydrophilic/hydrophobic interaction; equilibrium dialysis

In a series of studies concerning the interaction between drugs and water-soluble polymers in an earlier paper,[2)] the bindings of warfarin (WF) with water-soluble synthetic polymers (polyvinylpyrrolidone (PVP) and acrylamide–vinylpyrrolidone copolymer (CAV)) were investigated by equilibrium dialysis, and their binding mechanisms were found to be mainly hydrophobic. In subsequent papers,[3-5)] the interactions between drugs (phenylbutazone (PB), ibuprofen (IB) and azathioprine (AZ)) and bovine serum albumin (BSA) were studied and it was shown that the bindings of drugs to BSA were hydrophobic. Furthermore, the binding positions of drugs to BSA were elucidated by means of the NMR spin–spin relaxation rate ($1/T_2$) in a microscopic view. This method was most useful to study the binding position of a drug to protein, but difference in the chemical shift was useless because of its slight variation in the presence of protein. Hosono *et al.*[6)] studied the interaction between ajmaline and PVP by ^{1}H-NMR solvent shift. Furthermore, Otagiri *et al.*[7)] studied the binding position of WF to PVP on the basis of a slight difference of chemical shift on ^{13}C-NMR. Though we examined the interaction between WF and PVP by NMR, this method could not be applied for the following reasons: (1) the ^{1}H-NMR spectrum of WF gave the overlapped multiplet; (2) measurement of ^{13}C-NMR relaxation time required a very long period when the magnetic field drift was not negligible.

In the present paper, the interaction between a drug (WF) and ionic synthetic polymer was investigated. Polymers used were quaternized poly(4-vinylpyridinium bromide)s (QPV), in which N-alkyl groups were butyl (QBu), methyl (QMe) and benzyl (QBz), and the degree of quaternization of polymer is shown in parentheses as a percentage (Table I). The binding of WF to QPV was found to be controlled by competition between hydrophilic property of the pyridinium part and hydrophobic properties of the N-alkyl groups and the pyridine part: QBz(80), hydrophobic; QBu(24—100), hydrophobic–hydrophilic; QMe(100), hydrophilic. It was also elucidated by UV spectrum method that *N*-butyl-4-ethylpyridinium bromide (BEP) monomer and WF formed an equimolar complex. PB competed with WF for the hydrophobic binding to QBz(80).

Experimental

Materials WF and PB were of special reagent grade from Sigma and used without further purification. Poly(4-vinylpyridine) (PV) was from Koei Chemical Industry and its average molecular weight was estimated to be 1.35×10^5 by gel permeation chromatography. Other reagents were from commercial sources and were used without further purification.

Preparation of QPV QPV was prepared by the method reported by Ando *et al.*[8)] The degree of quaternization of QPV was calculated from the areas obtained by integration of ^{1}H-NMR peaks (D_2O, 400 MHz, JEOL GX-400). The molecular weight of QPV was calculated from the degree of quaternization.

Equilibrium Dialysis The equilibrium dialysis method used was the same as described previously.[2)] Temperatures were regulated within 0.2 °C during all experiments. The drug concentration was determined by absorbance on a Shimadzu UV-190 spectrometer connected to an Iwatsu VOAC-7513 digital multimeter and a NEC PC-9801E microcomputer.

Results and Discussion

Complex of WF with Monomer (BEP) The interaction between WF and BEP, which corresponded to a residue unit of QBu(100), was investigated by means of the continuous variation method on UV spectra. The relationship between absorbances at 267 and 330 nm and the

TABLE I. Property of Quaternized Poly(4-vinylpyridinium bromide)s

Polymer	Substituent group (R)	Degree of quaternization (%)	Yield (%)	M_w[a)] ($\times 10^5$)
QBu (24)	Butyl	23.5	87.7	1.52
QBu (54)	Butyl	53.5	97.5	1.74
QBu (76)	Butyl	76.0	92.0	1.91
QBu (100)	Butyl	100.0	96.5	2.08
QMe (100)	Methyl	100.0	94.0	1.54
QBz (80)	Benzyl	80.2	95.2	2.65

a) Calculated value.

mol fraction of WF at the constant total concentration gave maxima at the center (Fig. 1), although the maximum wavelength of WF (308 nm) remained constant. The complex of WF and BEP was considered to be an ionic complex of WF anion and BEP cation rather than a charge-transfer complex.

The Interaction between WF and QBu The binding of WF to QBu(100) was examined by equilibrium dialysis at 20—40 °C. The free drug concentration (Df) was determined from the residual drug concentration, and the number of mol of the drug binding to 1 mol of QBu(100) (r) was estimated from the decrease in drug concentration.

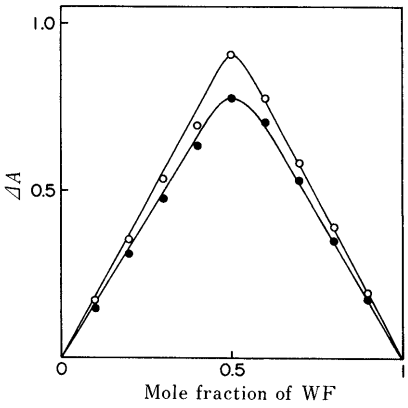

Fig. 1. Continuous Variation Method in UV Spectra for WF/BEP System

○, 330 nm; ●, 267 nm. [WF]+[BEP]=1 mM.

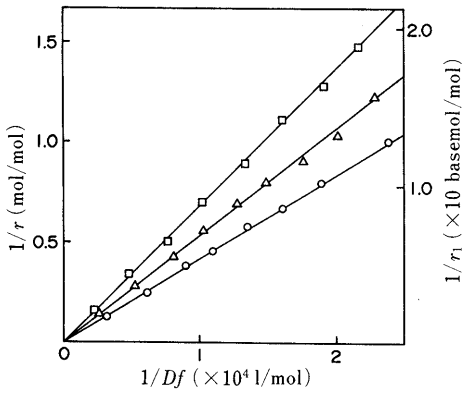

Fig. 2. Klotz Plots for the Binding of WF and QBu(100) in 0.1 M Phosphate Buffer (pH 7) at 20 °C (○), 30 °C (△) and 40 °C (□)

The plot of $1/Df$ *vs.* $1/r$ produced a linear relationship, as shown in Fig. 2, and satisfied Eq. 1 proposed by Klotz *et al.*[9]:

$$\frac{1}{r}=\frac{1}{nKDf}+\frac{1}{n} \tag{1}$$

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. Since the intercept of the line on the ordinate is $1/n$ and the slope is $1/nK$, n- and K-values can be calculated. However, n-value was numerically large and could not be estimated accurately because the intercept was nearly zero. Takagishi and Kuroki[10] evaluated the magnitude of binding in terms of nK-value (binding capacity) rather than K-value estimated from the uncertain n-value. Thus, the nK-values with S.D. were estimated as shown in Table II.

On the other hand, Ando *et al.*[11] analyzed the binding of dye to PVP from the number of mol (r_1) of dye bound to one base mol of monomer unit. In the present paper, the plot of $1/Df$ *vs.* $1/r_1$ again gave a linear relationship, as shown in Fig. 2, according to Eq. 2[2] instead of Eq. 1:

$$\frac{1}{r_1}=\frac{1}{n_1K_1Df}+\frac{1}{n_1} \tag{2}$$

where n_1 is the number of binding sites of drug per base mol of monomer unit, and K_1 is the binding constant between drug and monomer unit. Because of the formation of equimolar ionic complex of WF and BEP, the maximal number of binding sites for the WF–QBu(100) system might be equal to the polymerization degree of PV. However, it was considered that the number of binding sites of WF to QBu(100) was smaller than the polymerization degree of PV because of steric hindrance among WF and QBu(100) molecules.

Assuming that the number of binding sites (n) was independent of temperature, the standard increase of enthalpy ($\Delta H°$) was determined from the linear relationship between ln nK and the reciprocal absolute temperature ($1/T$) (Table II). The binding of WF to QBu(100) was considered to be advantageous for enthalpy because of the negative value of $\Delta H°$. It is difficult to discuss the binding mechanism on the basis of the apparent increases of free energy (ΔG) and entropy (ΔS) estimated from the nK and T values. It is also difficult to discuss the nature

Table II. Thermodynamic Data on the Interaction of WF with the Polymers

Polymer	Temp. (°C)	$nK\pm$S.D. ($\times 10^4$ M^{-1})	ΔG (kJ/mol)	$\Delta H°$ (kJ/mol)	ΔS (J mol^{-1} K^{-1})	n_1K_1 (l/basemol)
QBu (100)	20	2.37±0.018	−24.54	−18.19	21.67	18.45
	30	1.86±0.022	−24.77		21.72	14.48
	40	1.47±0.016	−24.97		21.66	11.44
					(Av.) 21.68	
QMe (100)	30	2.35±0.027	−25.35			
QBz (80)	20	14.77±0.088	−29.00	−23.07	20.24	115.0
	30	10.88±0.076	−29.22		20.30	84.7
	40	8.07±0.085	−29.40		20.22	62.8
					(Av.) 20.25	

[Polymer]=0.5%, pH=7, [Phos. buf.]=0.1 M.

Table III. Binding Parameter for the Interaction of WF with the Polymers

Polymer	[Phos. buf.] (M)	[MeOH] (%)	$nK \pm$S.D. ($\times 10^4$ M^{-1})
QBu (100)	0.05	0	2.44±0.024
	0.1	0	1.86±0.022
	0.1	5	1.88±0.020
QBu (24)	0.05	0	9.54±0.087
	0.1	0	8.56±0.080
	0.1	5	6.84±0.072
QMe (100)	0.05	0	2.78±0.034
	0.1	0	2.35±0.027
	0.1	5	2.49±0.031
QBz (80)	0.05	0	10.66±0.094
	0.1	0	10.88±0.076
	0.1	5	9.80±0.074

[Polymer]=0.5%, pH=7, 30 °C.

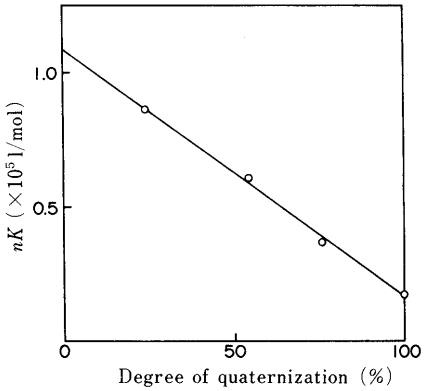

Fig. 3. Relationship between nK and Degree of Quaternization of QBu

of binding from almost no variation in the nK value with the addition of methanol (Table III). Since the nK-value decreased with increase of ionic strength, it was safely postulated that the binding of WF to QBu(100) was due to hydrophilic interaction.

The relationship between the degree of quaternization of QBu and the binding capacity (nK) was investigated. The plot of the degree of quaternization (m) *vs.* nK produced a linear relationship (Fig. 3), and an empirical formula (3) was obtained.

$$nk = 1.08 \times 10^5 - 9.22 \times 10^2 m \quad (3)$$

Since increase in the degree (m) of QBu resulted in decrease of the binding capacity, it was considered that the binding capacity was dependent on competition of the hydrophobic nature of pyridine (PV) with the hydrophilic nature of pyridinium (QPV). PV, which was insoluble in phosphate buffer (0.1 M, pH=7), was thought to have six times the binding capacity of QBu(100) from the extrapolated value ($nK = 1.08 \times 10^5$ M^{-1}). Since the nK value of the WF–QBu(24) system decreased with increase of ionic strength (Table III), this system showed a hydrophilic nature. On the other hand, since the nK value of the same system decreased with the addition of methanol (Table III), it showed a hydrophobic nature. These results suggested that the binding of WF to QBu(24) was controlled by not only the hydrophilic nature of the pyridinium portion but also the hydrophobic nature of the pyridine portion.

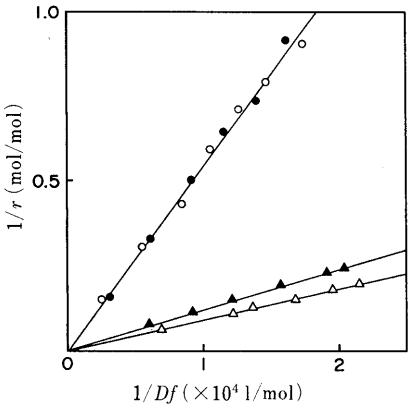

Fig. 4. Klotz Plots for the Binding of WF and Polymers in the Presence and Absence of PB (1 mM)

○, QBu(100); ●, QBu(100)/PB; △, QBz(80); ▲, QBz(80)/PB.

The Interaction between WF and QMe The binding of WF to QMe(100) was examined to determine the difference of quaternized alkyl substituents. Binding capacity of WF–QMe(100) increased in lower ionic strength and with the addition of methanol so that it was larger than that of WF–QBu(100) (Table III). These results were reasonable because the hydrophobic nature and steric hindrance of the methyl group were smaller than those of the butyl group, so that total hydrophilic nature of QMe(100) was larger than that of QBu(100).

The Interaction between WF and QBz Binding of WF to QBz(80) was similarly investigated, since QBz(100) was not available. The binding of WF to QBz(80) was larger than that of QBu(100). It was considered that the binding of WF to QBz(80) was advantageous for enthalpy because of the negative value of ΔH°. Since nK-value increased with increase of ionic strength and decreased with the addition of methanol (Table III), the binding of WF to QBz(80) was thought to be due to hydrophobic interaction. Therefore, the hydrophobic nature of the benzyl group was considered to exceed the hydrophilic nature of the quaternized pyridinium part.

Competition of PB with WF in the Interaction with QBz The competitive effect of PB against WF–polymer binding was investigated, since PB was well known to bind strongly to protein[1,2] and to stimulate the pharmacological action of WF. In the absence or presence of 1 mM PB, Klotz plots showed the same linear relationship in the WF–QBu(100) system (Fig. 4). However, the competitive effect of PB against the WF–QBz(80) system was observed, since the nK-value decreased in the presence of PB. In the hydrophobic binding of WF–PVP or WF–BSA, the competitive effect of PB was also observed.[2] Consequently, it was considered that WF competed with PB in the case of hydrophobic interaction rather than hydrophilic interaction.

References and Notes

1) This reports constitutes Part VI of the series entitled "Interaction between Drugs and Water-Soluble Polymers." Part of this work was presented at Meeting of the Chugoku-Shikoku Branch, Chemical Society of Japan, Okayama, Oct. 1989.

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