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Thermodynamic Quantitative Structure–Activity Relationship Analysis for Enzyme–Ligand Interactions in Aqueous Phosphate Buffer and Organic Solvent

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Abstract—Thermodynamic quantitative structure–activity relationships (QSAR) for chymotrypsin–ligand binding is developed, and the results are compared for the effects of organic solvent on the substrate specificity of the enzymes to those in aqueous phosphate buffer. This is the first of such analysis utilizing thermodynamic QSAR. A possible explanation for the difference describing the effects of organic solvent for the binding of substituted phenyl esters of *N*-benzoyl L-alanine analogues [PhCONHCH(Me)$COOC_6H_4$-p-X, **I**] observed in both the classical and the thermodynamic QSAR is presented.

Introduction

The use of enzymes in organic solvents show considerable interest[1,2] not only for the enzyme-catalyzed synthesis, but also for identifying the ligand binding sites on proteins[3,4] and for protein–ligand interactions.

The enzymatic activity is influenced by the solvent and the amount of water used. The stability of enzyme is also influenced by the amount of water present. Therefore, the solvent used and the water content of the system is of great importance for enzymatic activity and stability[2] as well as for studies of ligand binding site identification or of protein–ligand interactions.

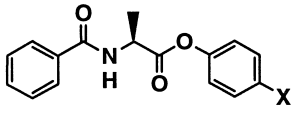
Ringe and her co-workers achieved significant accomplishments in the field of identifying ligand binding sites on proteins utilizing organic solvents.[3,4] On the other hand, there are few investigations described in the literature about the effects of organic solvent on the substrate specificity of enzymes. As far as the present author knows, there are only two such studies reported in the literature.[5,6] On the other hand, the thermodynamic aspects of the quantitative structure–activity relationships (QSAR) for the protein-ligand interactions in organic solvents are still not known.

The aim of this study is to describe the thermodynamic QSAR for a protein–ligand interaction and to compare the effects of an organic solvent on the substrate specificity of the enzyme to those in aqueous phosphate buffer solution. As an example, the biological data reported by Selassie et al.[5] on the specificity of chymotrypsin–ligand interactions in organic solvents are used in this study.

Results and Discussion

Classical QSAR of substituted phenyl esters of *N*-benzoyl L–alanines in phosphate buffer and pentanol

The binding constants (K_m) of substituted phenyl esters of *N*-benzoyl L-alanine analogues [PhCONHCH(Me)-$COOC_6H_4$-p-X, **I**] to chymotrypsin in phosphate buffer (pH 7.4) and in pentanol were reported by Selassie et al.[5] The experimentally determined values in log $1/K_m$ form and the physicochemical parameter values for the substituents of compound **I** are listed in Table 1. From the K_m values, Selassie et al. developed quantitative structure–activity relationships shown in eqs (1) and (2).



(**I**)

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Table 1. Observed and calculated log $1/K_m$ values by eqs (3)–(6) and physicochemical values used in the correlations

							log $1/K_m$ (buffer)					log $1/K_m$ (pentanol)				
								eq (5)		eq (6)			eq (3)		eq (4)	
	X	π[a]	σ^-	MR_4[a]	π_H[b]	π_S[b]	Obs[a]	Calcd	Dev	Calcd	Dev	Obs[a]	Calcd	Dev	Calcd	Dev
1	4-*t*-Bu	1.98	−0.13	1.96	−0.601	2.156	4.34	4.38	−0.04	4.51	−0.17	4.63	4.69	−0.06	4.61	0.02
2	4-Me	0.56	−0.17	0.57	−0.089	0.306	3.71	3.85	−0.14	3.92	−0.21	3.99	4.22	−0.23	4.25	−0.26
3	4-OMe	−0.02	−0.25	0.79	−0.203	0.116	3.59	3.78	−0.19	3.84	−0.25	4.07	4.12	−0.05	4.08	−0.01
4	4-Br	0.86	0.25	0.89	0.738	0.032	4.25	4.42	−0.17	4.27	−0.02	4.34	4.37	−0.03	4.42	−0.08
5	4-Bu	2.13	−0.12	2.00	−0.587	2.176	4.34	4.41	−0.07	4.55	−0.21	4.88	4.70	0.18	4.65	0.23
6	4-H	0.00	0.00	0.10	0.000	0.000	3.65	3.79	−0.14	3.80	−0.15	4.32	4.17	0.15	4.15	0.17
7	4-I	1.12	0.27	1.40	0.864	0.144	4.68	4.63	0.05	4.45	0.23	4.25	4.43	−0.18	4.49	−0.24
8	4-SO_2NH_2	−1.82	0.94	1.23	−0.295	−1.193	4.15	4.26	−0.11	4.28	−0.13	3.73	3.93	−0.20	3.91	−0.18
9	4-Cl	0.71	0.19	0.60	0.506	0.071	4.26	4.23	0.03	4.14	0.12	4.44	4.33	0.11	4.37	0.07
10	4-F	0.14	−0.03	0.09	0.235	−0.222	3.82	3.82	−0.00	3.81	0.01	4.09	4.14	−0.05	4.17	−0.08
11	4-CN	−0.57	1.00	0.63	−0.245	−0.169	4.36	4.34	0.02	4.37	−0.01	4.27	4.26	0.01	4.24	0.03
12	4-NO_2	−0.28	1.27	0.74	−0.240	0.042	4.66	4.56	0.10	4.59	0.07	4.58	4.38	0.20	4.38	0.20
13	4-NH_2	−1.23	−0.15	0.54	−0.880	−0.399	3.64	3.42	0.22	3.63	0.01	3.86	3.85	0.01	3.80	0.06
14	4-NHAc	−0.97	−0.46	1.50	0.909	−1.685	3.93	3.94	−0.01	3.75	0.18	3.96	3.74	0.22	3.79	0.17
15	4-Et	1.02	−0.19	1.03	0.342	0.504	4.62	4.16	0.46	4.10	0.52	4.27	4.35	−0.08	4.36	−0.09

[a]Taken from ref 5.
[b]Taken from refs 7 and 8.

Binding of **I** in phosphate buffer:

$$\log 1/K_m = 0.28(\pm 0.11)\,\pi + 0.51(\pm 0.24)\,\sigma^- + 0.38(\pm 0.23)\,\mathrm{MR} + 3.70(\pm 0.24)$$
$$n = 16, \qquad r = 0.913, \qquad s = 0.198 \qquad (1)$$

Binding of **I** in pentanol:

$$\log 1/K_m = 0.25(\pm 0.09)\,\pi + 0.24(\pm 0.18)\,\sigma^- + 4.10(\pm 0.09)$$
$$n = 17, \qquad r = 0.873, \qquad s = 0.156 \qquad (2)$$

From the 18 compounds the authors originally studied, two compounds (X = *t*-Bu and X = OH) were not used in deriving eq (1) and one compound (X = $CONH_2$) in deriving eq (2) because of poor fit.

The binding constants (log $1/K_m$) obtained from the phosphate buffer correlate well with π, σ^-, and MR with the correlation coefficient of 0.913 and the standard deviation of 0.198. In this equation, π is the Hansch hydrophobic constant derived from octanol–water partition coefficients, σ^- is the Hammett electronic constant, and MR is the molar refractivity for the *para*-substituent X. Compared to the binding constants obtained from aqueous phosphate buffer, the binding constants obtained from pentanol show a dependence only on π and σ^-. The correlation coefficient and the standard deviation for eq (2) are 0.873 and 0.156, respectively.

Eq (1) indicates that the binding of compound **I** to the enzyme in the phosphate buffer is influenced by the hydrophobic, electronic, and steric effects of the *para*-substituent X. The positive coefficients of π and σ^- suggest that hydrophobic electron-withdrawing *para*-substituents increase the binding affinity of **I** to chymotrypsin. The small coefficient of π shows that the binding involves partial desolvation on the enzyme surface. It appears that the electron-withdrawing substituents help polarize the carbonyl bond and facilitate the nucleophilic attack by the serine hydroxyl group of the enzyme and thus facilitate the binding by stabilizing the oxyanion tetrahedral intermediate. The positive coefficient of MR suggests that a large size substituent enhances the binding.

Eq (2) suggests that the binding of compound **I** to the enzyme in pentanol is only influenced by the hydrophobic and electronic effects of the *para*-substituents X. The effects described by MR in eq (1) is no longer needed in eq (2). The coefficients of π and σ^- in eqs (1) and (2) are essentially identical considering their standard error of estimates indicating that the partial desolvation described by π and the electronic influence of the *para*-substituents remain unchanged both in phosphate buffer and in pentanol.

It is not well understood in these classical QSAR, however, why MR term is needed to describe the binding of compound **I** in aqueous phosphate buffer, whereas it is not needed for the binding in pentanol. It was speculated[5] that the pentanol competes with the enzyme for the buffer water resulting in desolvation of the enzyme surface and enhancing easier access of the ligand to the binding site. The binding in pentanol was enhanced by 3-fold over that in aqueous phosphate buffer (the intercept of 3.70 in phosphate buffer vs 4.10 in pentanol).

In order to compare these classical QSAR results with those of thermodynamic QSAR that will be discussed below, the analyses were performed again with only 15 compounds used in the thermodynamic QSAR. Two compounds could not be used in the thermodynamic QSAR because of missing parameter values (vide infra).

Eqs (3) and (4) show the results. The coefficients of all parameters as well as the intercepts are essentially the

same as those in eqs (1) and (2) considering their confidence intervals.

For the binding of **I** in aqueous phosphate buffer:

$$\log 1/K_m = 0.16(\pm 0.07)\ \pi + 0.53(\pm 0.13)\ \sigma^- + 0.24(\pm 0.12)\ \mathrm{MR} + 3.78(\pm 0.12)$$
$$n = 15, \qquad r = 0.842, \qquad s = 0.234 \tag{3}$$

For the binding of **I** in pentanol:

$$\log 1/K_m = 0.25(\pm 0.04)\ \pi + 0.24(\pm 0.09)\ \sigma^- + 4.15(\pm 0.05)$$
$$n = 15, \qquad r = 0.863, \qquad s = 0.168 \tag{4}$$

The statistical quality of eqs (3) and (4) is somewhat inferior to that of eqs (1) and (2). The correlation coefficients of eqs (3) and (4) are reduced slightly, and the corresponding standard deviations are increased slightly. On the other hand, the coefficients of each term in eqs (3) and (4) are more tightly defined than those in eqs (1) and (2).

In deriving eq (1), *t*-Bu analogue of compound **I** was excluded by the original authors because of a poor fit. However, this compound does not show any abnormal behavior in eq (3) and thus included in eq (3). In fact, the deviation of the calculated log $1/K_m$ value of this compound by eq (3) from the corresponding observed value is one of the smallest as can be seen in Table 1. Similar results were obtained in eq (5) (vide infra).

Thermodynamic QSAR analysis of substituted phenyl esters of *N*-benzoyl L-alanines in phosphate buffer and pentanol

Thermodynamic analyses of the substituted phenyl esters of *N*-benzoyl L-alanines in phosphate buffer and pentanol were performed using the data presented in Table 1. The thermodynamic parameters π_H and π_S defined by Fujiwara et al.[7,8] were employed in these analyses: π_H is the hydrophobic enthalpy parameter, and π_S is the hydrophobic entropy parameter. The same sets of data used in eqs (3) and (4) were used in this analysis. Two compounds (X = 4-$CONH_2$ and X = 4-SMe) included in eqs (1) and (2) could not be used in these analyses because of the unavailable thermodynamic parameter values (π_H and π_S).

Eqs (5) and (6) show the results. Two compounds (X = *t*-Bu and X = OH) were poorly fit and excluded by Selassie et al. in deriving eqs (1) and (2). However, one (X = *t*-Bu) of these two compounds was included in eq (5). As eq (3), this compound fits the QSAR well and does not show significantly large deviation from the observed log $1/K_m$ value. The other compound (X = OH) still shows a poor fit to eq (5) as eqs (1) and (2) and thus was not included in deriving eqs (5) and (6).

For the binding of **I** in aqueous phosphate buffer:

$$\log 1/K_m = 0.38(\pm 0.11)\ \pi_H + 0.19(\pm 0.07)\ \pi_S + 0.53(\pm 0.11)\ \sigma^- + 0.26(\pm 0.10)\ \mathrm{MR} + 3.77(\pm 0.11)$$
$$n = 15, \qquad r = 0.898, \qquad s = 0.200 \tag{5}$$

For the binding of **I** in pentanol:

$$\log 1/K_m = 0.21(\pm 0.08)\ \pi_H + 0.31(\pm 0.05)\ \pi_S + 0.20(\pm 0.08)\ \sigma^- + 4.16(\pm 0.04)$$
$$n = 15, \qquad r = 0.887, \qquad s = 0.160 \tag{6}$$

In eqs (5) and (6), the hydrophobic term π used in eqs (3) and (4) was replaced by the thermodynamic hydrophobic enthalpy term π_H and the hydrophobic entropy term π_S.

The hydrophobic enthalpy (π_H) and entropy (π_S) parameters were originally derived from the experimentally determined equilibrium thermodynamic parameters ΔG°, ΔH°, and ΔS° for benzoic acids in the octanol–water system. The standard free energy change in partition (ΔGp°) was calculated from the relationship, $\Delta Gp^\circ = -RT \ln P$, where P is the octanol–water partition coefficient. The free energy change was then divided into the enthalpy change (ΔHp°) and the entropy change (ΔSp°) as described in eq (7).

$$\log P = -\Delta Gp^\circ/(2.303\ RT) = -\Delta Hp^\circ/(2.303\ RT) + \Delta Sp^\circ/(2.303\ R) = \pi_H + \pi_S \tag{7}$$

The hydrophobic enthalpy π_H can be related to the heat evolved when a solute is transferred from water to the octanol phase, and the hydrophobic enthalpy π_S to the heat reflecting the change of randomness of mobility induced in solution when a solute is transferred from water to octanol phase. Fujiwara et al.[7,8] showed that the hydrophobic constant π values are not exclusively determined by either the enthalpy or the entropy term, but determined by the two terms cooperatively. Therefore, it is not surprising to see that both π_H and π_S terms are present in eqs (5) and (6) obtained in this study.

The statistical quality of eqs (5) and (6) is slightly improved over those of eqs (3) and (4) with a higher correlation coefficient and a smaller standard deviation. Considering an additional term included in eqs (5) and (6), however, the quality of both sets of correlations are considered similar. The coefficients of σ^- in eqs (5) and (6) are essentially the same as the corresponding values in eqs (1) and (2) or eqs (3) and (4). Even the corresponding intercepts are identical. These show that no unusual effects are introduced into the correlations by π_H and/or π_S.

A comparison of the QSAR for the binding constants of compound **I** to chymotrypsin determined in aqueous phosphate buffer and in pentanol suggests their similarities and differences in their mode of protein–ligand interactions. The hydrophobic and electronic effects of

the *para*-substituents are important in both cases. The coefficients of π or π_H and π_S and σ- remained essentially identical. The results indicate that the partial desolvation described by π and electronic influences of the *para*-substituents remain unchanged both in phosphate buffer and in pentanol.

One might ask why are hydrophobic and electronic descriptors similar in aqueous buffer and pentanol if the solvent properties such as polarity and dielectric constants between the phosphate buffer and pentanol solvent are clearly distinct.[9] The reason for this is because the hydrophobic and electronic effects of the *para*-substituents presented in eqs (1)–(6) are describing the substituent's effects on the enzyme–ligand interactions. Therefore, although the polarity and dielectric constants of the phosphate buffer and pentanol solvent are different, they may not be significantly different at the binding site where the ligand are interacting with the enzyme.

Based on the coefficients of π_H and π_S terms, the hydrophobic enthalpy term might be considered more important for the binding in phosphate buffer than the hydrophobic entropy term. On the other hand, for the binding in pentanol, the hydrophobic entropy term might be considered more important than the hydrophobic enthalpy term. Although the coefficients of the two terms are somewhat different, however, the relative contributions of hydrophobic enthalpy and hydrophobic entropy for the two experimental conditions are difficult to firmly conclude with the present results because of their large confident intervals associated with the coefficients.

However, there is one clear, significant difference between the two sets of QSAR (from aqueous phosphate buffer and from pentanol). The MR term is not needed for the QSAR of the binding of compound **I** in pentanol, whereas it is important for the binding in phosphate buffer.

In order to understand what might be happening in the protein–ligand interactions in the presence of an organic solvent, pentanol, it would be helpful to remember the work of Ringe et al.[3] who utilized organic solvents as search probes for the binding site of a protein. By exchanging the mother liquor several times, the solvent inside and outside the protein crystal was equilibrated allowing the organic solvent molecules to interact with the surface and the favorable binding site. From such an experimental approach, they were able to find the binding site cleft. A binding site found in such an experiment is generally considered to be a region that contains bound water molecules that are not tightly held but which make specific interactions with the polar groups of the protein.

Determination of whether a specific protein–ligand interaction at equilibrium is enthalpy- and/or entropy-driven can be achieved by thermodynamic analysis. An enthalpy-driven process is usually associated with the formation of new bond such as hydrogen-bonds and van der Waals interaction. On the other hand, an entropy-driven process is usually characterized by the displacement of ordered water molecules coupled with the formation of new hydrophobic interactions.[10] The initial process in the receptor association is generally entropy-driven.

When chymotrypsin is in pentanol, it is likely that the organic solvent molecules displace one or more water molecules. This process of displacing the water molecules to form new hydrophobic interactions by the pentanol would be an entropy-driven process. When compound **I** is added to the protein in pentanol, the compound must now replace one or more pentanol molecules bound to the active site in order for the hydrolysis by the enzyme to occur. The binding of compound **I** replacing the pentanol occupied in the active site would be associated with the formation of new bond such as hydrogen-bonds and van der Waals interactions. Therefore, the binding of compound **I** to α-chymotrypsin in pentanol can be expected to be largely an enthalpy-driven process. On the other hand, the binding of compound **I** to α-chymotrypsin in phosphate buffer involves directly displacing the water molecules, which would be largely entropy-driven process.

Going back to the results of QSAR describing that MR term is not significant for the binding of Compound **I** in pentanol, it is recalled that MR term is usually considered to describe the nonspecific dispersion interaction in the polar space.[11] In view of the above-mentioned aspects, it is conceivable that MR term in eqs (1) and (3) (for aqueous phosphate buffer) may describe the process of displacing the ordered category two water molecules[3] (which is polar in nature) by compound **I**. When compound **I** was added to the α-chymotrypsin in pentanol, the substrate must now replace one or more pentanol molecules (which is nonpolar in nature) rather than the water molecules, and thus MR term may be no longer needed.[12]

It is worth to note that the binding in pentanol was enhanced by 3-fold (the intercept 4.1 in phosphate buffer vs 3.7 in pentanol). Replacing the pentanol molecules in the binding site by compound **I** produces tighter binding than replacing the water molecules in the binding site.

Selassie et al.[5] also studied the chymotrypsin binding of compound **I** in acetonitrile solvent. However, they have not yet reported the experimentally determined binding constant values. They described that octanol–water-derived π constants fail miserably to explain the enzyme–ligand interactions. It would be interesting to study the corresponding thermodynamic QSAR obtained from acetonitrile to see whether these hydrophobic enthalpy (π_H) and entropy (π_S) parameters provide any correlation.

Conclusion

This study described the thermodynamic QSAR for chymotrypsin–ligand binding and compared the effects

of organic solvent on the substrate specificity of the enzymes to those in aqueous phosphate buffer. This is the first of such analysis utilizing thermodynamic QSAR. A possible explanation for the differences in describing the effects of organic solvent for the binding of compound **I** observed both in the classical and in the thermodynamic QSAR was presented.

Experimental

All regression analyses were performed on a personal computer using the JUMP statistical program (version 4.0) of SAS institute.[13] All physicochemical parameter values used in this analysis were taken from the literature.[5,7,8]

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