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Utilization of synergetic effect of weak interactions in the design of polymeric sorbents with high sorption selectivity

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Abstract

Cystine and tyrosine were used as model sorbates to illustrate the design of sorbents with high sorption selectivity using two types of weak interactions that act synergistically. When two types of weak interactions are the driving forces in a sorption and they act synergistically, the second interaction would be effectively intramolecular. The entropy lost for the second interaction should be lower than that for the same interaction that occurs alone, and thus a significant enhancement of sorption should result. We designed an *N*-acetyl aminomethyl polystyrene resin (*N*-acetyl HC-D309), which was expected to sorb tyrosine through hydrophobic interaction and hydrogen bonding but not cystine. The chromatographic results for tyrosine and cystine indicate that the separation efficiencies on the *N*-acetyl HC-D309 column are higher than those on a styrene–divinylbenzene copolymer column, on which sorption should be driven by hydrophobic interaction only, and on an acrylamide-*N*,*N*′-methylene bisacrylamide copolymer column, on which sorption should be driven by hydrogen bonding only. Tyrosine as well as cystine had no retention at all on the acrylamide-*N*,*N*′-methylene bisacrylamide copolymer column, indicating the hydrogen bonding had little contribution to the sorption when it acted alone. The above results further indicate that hydrophobic interaction and hydrogen bonding contributed to the sorption of tyrosine on *N*-acetyl HC-D309 and they also acted synergistically. One of the conclusions of this paper is that some weak interactions which contribute little to the sorption when they act alone may contribute to the sorption when they act synergistically with other interactions.

Keywords: Synergetic effect; Polymeric sorbents; Hydrogen bonding; Hydrophobic interaction; Molecular recognition

1. Introduction

Polymeric sorbents have been widely used in separation and purification of chemical mixtures in the chemical, biological and environmental industries. In recent years growing attention has been paid to separation and purification of pharmaceutical and/or natural products using polymeric sorbents [1–6]. The processes may be carried out by batch separations in stirred tanks or packed bed adsorption. Most common polymeric sorbents are based on styrene–divinylbenzene copolymers (for example Amberlite XAD-2, XAD-4). They adsorb organic compounds from aqueous solutions through hydrophobic interactions due to van der Waals forces between the highly hydrophobic surface of the sorbents and the hydrophobic sites of sorbates. Hydrogen-bonding interaction has also been used as a driving force in sorption in nonpolar media [7–9].

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An example of such sorbents is Amberlite XAD-7 (crosslinked poly(methyl methacrylate)), and the sorbate may be oxygenated or nitrogenated compounds. Although the wide variations in functionality, surface area, and porosity available for polymeric resins present the possibility of customizing resins for the removal of specific organic species, the adsorption selectivity of these resins is generally low. This is because the sorption of sorbates on these resins is usually driven by a single type of weak interactions. At the other extreme are biospecific affinity-based separation systems, which are highly selective [10–12]. Biospecific affinity is usually the interaction between a ligand and a biological receptor. The high selectivity is due to the cooperation of a large number of weak non-covalent interactions and steric matching (complementarity) between the ligand and the receptor. However, the biospecific-affinity ligands, such as antibodies, substrates and coenzymes, have the disadvantage of being extremely expensive, chemically and enzymatically unstable, and may not be obtained at all for most of compounds of interest.

A biospecific affinity sorption mimic is molecularly imprinted polymer (MIP) based separation [13–16]. A molecularly imprinted polymer is synthesized by assembly of monomers around template molecules and subsequent polymerization using a suitable crosslinker, giving a rigid and robust material. Subsequent removal of template molecules provides a polymer with recognition sites (cavities) allowing specific rebinding of the template molecules. The recognition is due to shape and non-covalent interactions such as hydrogen bonding, ionic interactions and hydrophobic interactions. The high recognition selectivity has led to the application of MIPs to several areas of analytical chemistry including immunoassays, sensors and separation media. The low binding capacity of MIP-based stationary phases and low yields of high-fidelity sites, however, limit their application for preparation purposes.

In this paper, we mimic the multiple weak interactions of biospecific affinity using tyrosine, phenylalanine and cystine as model sorbates to illustrate the design of sorbents with high sorption capacity and selectivity. This design idea may be especially useful in the synthesis of sorbents with highly selective adsorption in the purification or separation of natural products.

2. Experimental

2.1. Materials

HC-D309 (macroporous styrene–divinylbenzene copolymer containing aminomethyl groups, 4.5 mequiv. $-NH_2$/g, 40–60 mesh), HC-ADS-5 (macroporous styrene–divinylbenzene copolymer, 40–60 mesh), and macroporous chloromethyl polystyrene resin (5.2 mequiv. Cl/g, 40–60 mesh), the precursor of HC-D309, were obtained from Hecheng (Tianjin, China). Acetic anhydride (analytical grade) and triethylamine (analytical grade) were purchased from Tianjin First Chemical Plant (Tianjin, China). Cystine (biochemical reagent-grade), tyrosine (biochemical reagent-grade) and phenylalanine (biochemical reagent-grade) were purchased from Shanghai Changjiang Biochemical Plant (Shanghai, China). All other reagents were analytical grade. Acrylamide-*N*,*N*′-methylene bisacrylamide copolymer was synthesized as described previously [17].

2.2. Synthesis of N-acetyl HC-D309

HC-D309 was treated with 1 N NaOH to transfer the hydrochloride salt to the free amine form. Then the dry free amine resin (30 g, 135 mmol $-NH_2$) was suspended in DMF (100 ml) and the mixture was stirred for 30 min. To this, acetic anhydride (48.2 g, 473 mmol) and triethylamine (40 ml) were added and the mixture was heated to 60 °C and stirred until a negative or nearly negative ninhydrin test was obtained (about 6 h). The resulting resin was washed thoroughly with DMF, water and ethanol, and dried in vacuum. The resin has a strong IR absorption at 1661 cm^{-1}, indicating it contains amide groups.

2.3. Synthesis of acetyloxymethyl polystyrene resin

Macroporous chloromethyl polystyrene (30 g, 156 mmol Cl) was suspended in DMF (270 ml) and the mixture was stirred for 30 min. To this, potassium acetate (53.6 g, 546 mmol) was added and the mixture was heated to 85 °C and stirred for 48 h. The resulting resin was washed thoroughly with DMF, water and ethanol, and dried in vacuum. The residual chloride content of the resin was 0.6 mequiv./g. The

strong IR adsorption at 1738 cm^{-1} of the resin confirms its ester structure.

2.4. Chromatographic separation of amino acid mixtures

The resin was packed in a glass column (250×10 mm, I.D.) with the slurry method. Then the column was equilibrated with the eluant; amino acid mixture dissolved in 1 ml of 2 N HCl was loaded on the column and then the column was eluted. The effluent was collected in a fraction collector; 10 μl of the each collected sample was taken and applied to a piece of chromatography paper and the paper was developed with *n*-butanol/ethanol/acetic acid/water (4/1/2/2, v/v) as mobile phase. The developed paper was dried, sprayed with ninhydrin solution (1% in acetone), and heated between 100 and 110 °C for 10 min to reveal the characteristic spots of the amino acid. Each spot was cut and suspended in 4 ml of 0.1% $CuSO_4$/75% ethanol (1/19, v/v). The absorbance of the colored solution at 570 nm was measured. The blank measurement was done with the same size blank paper as the spot. The amount of the amino acid was obtained through the measurements and a standard curve constructed with known amounts of the same amino acid. The void volume of all columns used in this paper was approximately considered to be the same. It was determined to be 17.5 ml using glycine, which was supposed not to be adsorbed in styrene–divinylbenzene copolymer, as solute in an HC-ADS-5 column.

3. Results and discussion

A mixture of tyrosine and cystine are difficult to separate using common physical methods, as both of these two amino acids have very low solubilities in water (0.045 and 0.011 g/100 ml, at 25 °C, for tyrosine and cystine, respectively [18]) and their isoelectric points (5.66 and 5.05 for tyrosine and cystine, respectively [18]) are close. We used these two amino acids as model sorbates to design a polymeric sorbent with highly selective adsorption. Tyrosine contains a phenyl group, while cystine does not. Therefore, the former may be sorbed by hydrophobic sorbents. Fig. 1 shows the chromatograms of a mixture of tyrosine and cystine on the column packed with HC-ADS-5 (styrene–divinylbenzene copolymer). It can be seen that the sorption of tyrosine is indeed stronger than that of cystine. The retention volume of cystine is almost the same with the void volume, indicating little cystine is adsorbed on styrene–divinylbenzene copolymer.

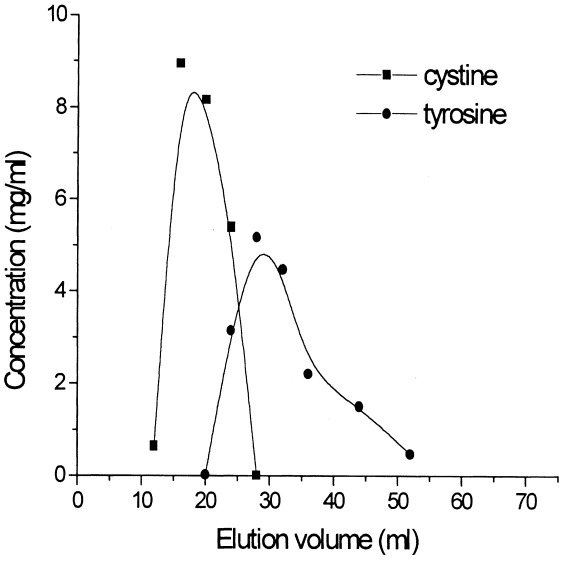

Fig. 1. Chromatograms of tyrosine and cystine on HC-ADS-5 column (250×10 mm, I.D.). Eluent: 0.1 N HCl; injection: 100 mg of each amino acid in 1 ml of 2 N HCl; flow-rate: 0.25 ml/min.

In a chromatographic process, the partition of a solute between the stationary phase and the mobile phase is usually considered as equilibrium distribution. The partition ratio, *K*, is defined by the equation:

$$K = C_S / C_M \quad (1)$$

where C_S and C_M are the concentrations of the solute in the stationary phase and the mobile phase, respectively. The partition ratio can be related to the retention volume (V_R) of the solute, the void volume (V_M) and the stationary phase volume (V_S) ($V_M + V_S = V_B$, where V_B is the bed volume of the column) by Eq. (2) [19]:

$$K = (V_R - V_M)/V_S \quad (2)$$

The relationship of the partition ratio and the transfer free energy, ΔG^0, for the solute going from the mobile phase to the stationary phase can be expressed as [19]:

$$\Delta G^0 = -RT \ln K \quad (3)$$

where R is gas constant; T is absolute temperature.

Substituting for K from Eq. (2) to Eq. (3):

$$\Delta G^0 = -RT \ln [(V_R - V_M)/V_S] \quad (4)$$

The transfer free energies of cystine and tyrosine on the HC-ADS-5 column were calculated using the data from Fig. 1 and Eq. (4) and are listed in Table 1.

In addition to the phenyl group, tyrosine also contains a phenolic hydroxyl group. Thus, we prepared a polystyrene resin containing an amide group (*N*-acetyl aminomethyl polystyrene resin, *N*-acetyl HC-D309). The driving forces of sorption of tyrosine on *N*-acetyl HC-D309 are expected to include hydrophobic interaction between a phenyl ring of the resin and a phenyl ring of tyrosine, and hydrogen bonding between an amide group of the resin and a phenolic hydroxyl group of tyrosine. The two interactions may occur simultaneously with the same tyrosine molecule (chelating effect). If the two interactions occur synergistically, the total interaction should be much stronger than the sum of the two interactions that occur individually. This is because the second interaction would be effectively intramolecular. The entropy lost for the second interaction should be lower than that for the same interaction that occurs individually, and thus a significant enhancement of sorption should result (intramolecular interactions can be accelerated by up to 10^9 relative to their intermolecular counterparts [20]). Fig. 2 shows the chromatograms of a mixture of tyrosine and cystine on a column packed with *N*-acetyl HC-D309. It can be seen that the retention volume of tyrosine on the *N*-acetyl HC-D309 column is much higher than that on the HC-ADS-5 column, while the retention volumes of cystine on both of the columns are the same. The transfer free energies of cystine and tyrosine on the *N*-acetyl HC-D309 column were calculated using Eq. (4) and are listed in Table 1. As the hydrophobicity of *N*-acetyl HC-D309 should be lower than that of HC-ADS-5, the former should have weaker hydrophobic adsorption for tyrosine than the latter, and thus there are other interactions in addition to the hydrophobic interaction between tyrosine and *N*-acetyl HC-D309. By exclusion, the interactions should be hydrogen bonding.

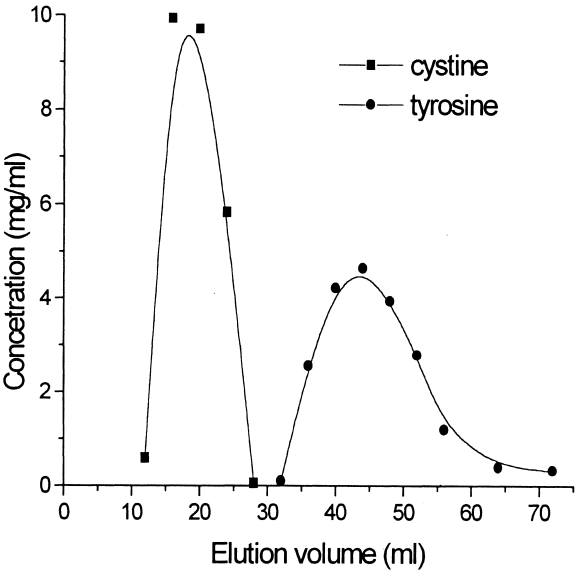

Fig. 2. Chromatograms of tyrosine and cystine on *N*-acetyl-HC-D309 column. The other conditions are the same as in Fig. 1.

The above results show only that hydrophobic interaction and hydrogen bonding might contribute to the sorption of tyrosine. Whether any synergetic effect exists between them is still unclear. In order to settle this problem, the chromatographic separation of tyrosine and cystine on a packing with amide group but without hydrophobic site, acrylamide-*N*,*N*′-methylene bisacrylamide copolymer, was done.

Table 1
The transfer free energies (ΔG^0, kJ mol^{-1})

	HC-ADS-5	*N*-acetyl HC-D309	Crosslinked polyacrylamide	Acetyloxymethyl polystyrene	HC-D309
Cystine	$\gg 0$	$\gg 0$	$\gg 0$	$\gg 0$	$\gg 0$
Tyrosine	−4.0	−6.3	$\gg 0$	−2.4	−1.9
Phenylalanine	−5.0	−3.2			

The chromatographic result (data not shown) showed that both tyrosine and cystine had no retention (the retention volumes were the same as the void volume) on the column packed with acrylamide-*N*,*N*′-methylene bisacrylamide copolymer, indicating that hydrogen bonding has little contribution to the interaction between polyacrylamide resin and the amino acids. The transfer free energies are high positive values according to Eq. (4). As acrylamide-*N*,*N*′-methylene bisacrylamide copolymer does not contain a hydrophobic site, hydrogen-bonding interaction, if any, may be the only interaction between the sorbent and the amino acids. This result showed that hydrogen-bonding interaction has little contribution to the sorption when the hydrogen bonding is the only interaction. This may be explained by the strong hydrogen bonding characteristics of water. The favorable enthalpy gain could not offset the entropy loss in the binding. As mentioned above, however, hydrogen bonding does contribute to the interaction between tyrosine and *N*-acetyl HC-D309. A reasonable explanation is that when tyrosine is sorbed on *N*-acetyl HC-D309 through hydrophobic interaction, forming a hydrogen bond between the amide group and the phenolic hydroxyl group would be effectively intramolecular. These results indicate that both hydrophobic interactions and hydrogen bonding contribute to the sorption of tyrosine on *N*-acetyl HC-D309, and they act synergistically.

In the above discussion, it was concluded that hydrogen bonding contributes to the sorption of tyrosine on *N*-acetyl HC-D309 in a chelating manner with hydrophobic interaction and assumed that the hydrogen bond was formed between the amide group of resin and the phenolic hydroxyl group of tyrosine. The resin contains only one type of hydrogen bond-forming group, the amide group. For tyrosine, in addition to the phenolic hydroxyl group, however, the carboxyl group and the amino group are also hydrogen bond-forming groups. To examine which of the groups, the phenolic hydroxyl group, the carboxyl group and the amino group, forms hydrogen bond with the resin, chromatograms of phenylalanine and tyrosine on the columns packed with HC-ADS-5 and *N*-acetyl HC-D309 were made, as shown in Figs. 3 and 4. The corresponding transfer free energies are listed in Table 1. As expected, phenylalanine, which contains one less hydroxyl

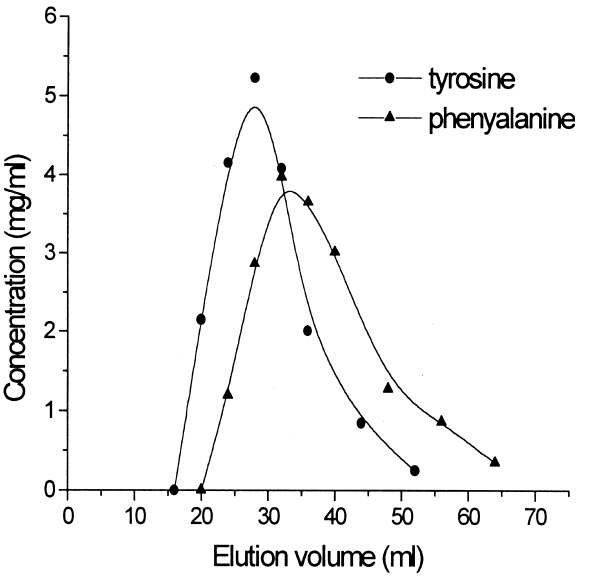

Fig. 3. Chromatograms of tyrosine and phenylalanine on HC-ADS-5 column. The other conditions are the same as in Fig. 1.

group and thus is more hydrophobic, was sorbed more strongly than tyrosine on HC-ADS-5 (Fig. 3 and Table 1). However, the former was sorbed less strongly than tyrosine on *N*-acetyl HC-D309 (Fig. 4

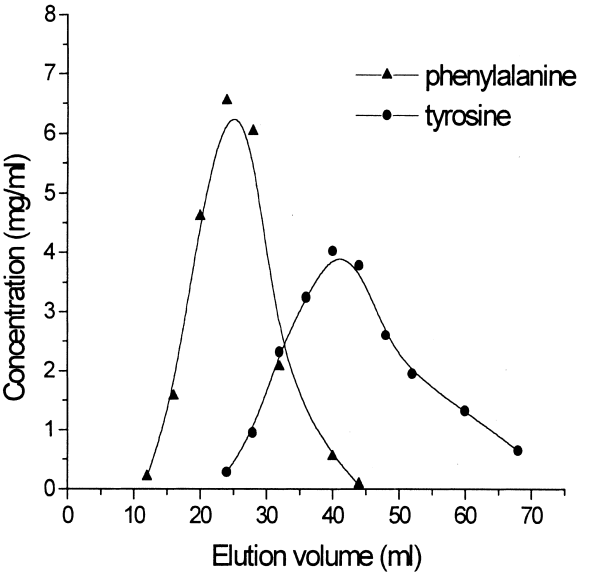

Fig. 4. Chromatograms of tyrosine and phenylalanine on *N*-acetyl-HC-D309 column. The other conditions are the same as in Fig. 1.

and Table 1). The rational explanation is that, in addition to hydrophobic interaction, hydrogen bonding contributed to the sorption of tyrosine on *N*-acetyl HC-D309 but not to that of phenylalanine. Thus the above assumption is correct, i.e. the phenolic hydroxyl group but not the carboxyl group or amino group of tyrosine formed a hydrogen bond with the amide group of the resin.

For the hydrogen bonding between the amide group of the resin and the phenolic hydroxyl group of tyrosine, there may also be several possible forms. For example, the phenolic hydroxyl group may act as either proton donor or acceptor in the hydrogen bond. The amide group has two proton-accepting atoms (N and O atoms) and a proton donor (–NH). Fig. 5 shows the effect of acidity of the eluent on the retention of tyrosine and cystine on the column packed with *N*-acetyl HC-D309. The retention volume of tyrosine increased with the increasing acidity. In the acidity range studied, the form of tyrosine should change little, and the amount of protonated amide group should increase with the increasing acidity (pK_a value of protonated amide is -0.5 [21]). Therefore, the hydrogen bonding should include the protonated amide group. As the protonated amide group should be a very weak proton acceptor but a very strong proton donor for hydrogen bonding, it should act as proton donor. Thus the phenolic

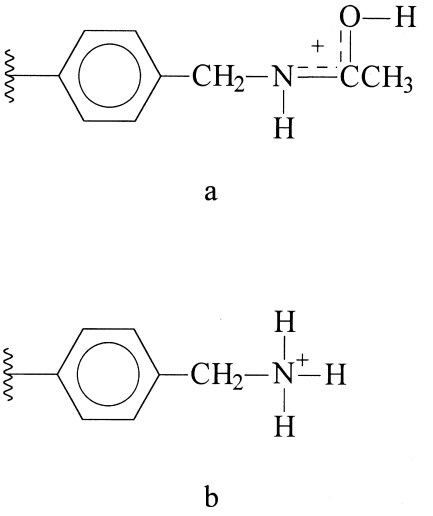

Fig. 6. Comparison of the structures of protonated *N*-acetyl HC-D309 (a) and protonated HC-D309 (b).

hydroxyl group should be a proton acceptor. In the hydrogen bonding, there are two possible proton donors, –OH and –NH (Fig. 6a). If the –NH group of protonated *N*-acetyl HC-D309 acts as proton donor, then protonated HC-D309 (Fig. 6b) would have the same or similar action. Fig. 7 shows the chromatographic separation of tyrosine and cystine on a column packed with HC-D309 using an acidic

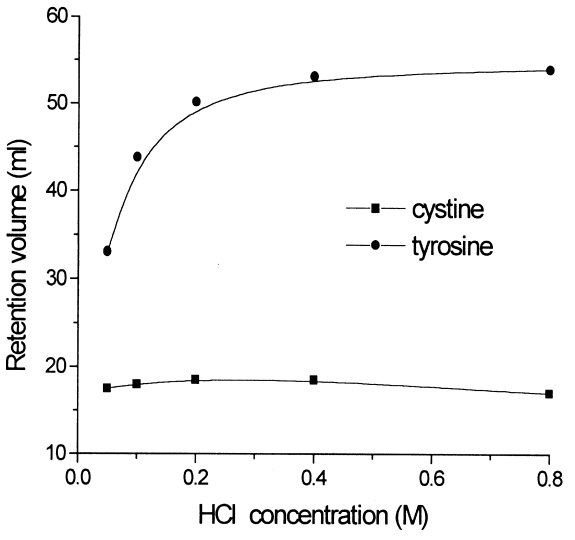

Fig. 5. The effect of acidity of the eluent on the retention of tyrosine and cystine on *N*-acetyl HC-D309 column. The chromatographic conditions are the same as in Fig. 1.

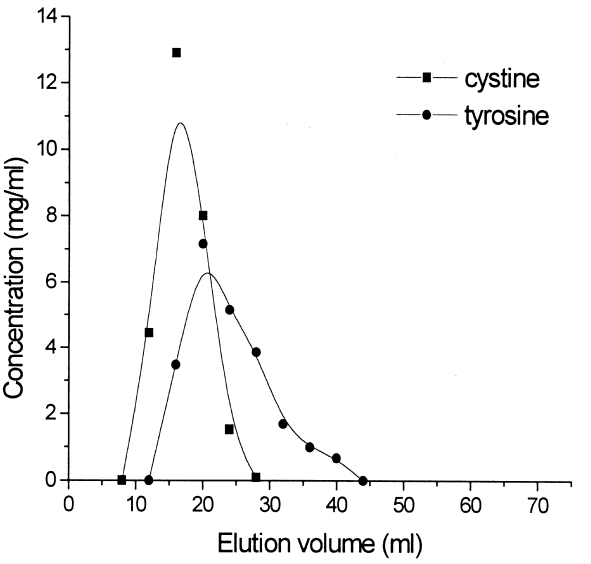

Fig. 7. Chromatograms of tyrosine and cystine on HC-D309 column. The other conditions are the same as in Fig. 1.

eluent. By comparing Figs. 1, 2 and 7, it can be seen that cystine has almost the same retention volume on these three columns, while the retention volume of tyrosine on the column packed with HC-D309 is less than not only that with *N*-acetyl HC-D309 but also that with HC-ADS-5. The transfer free energy of tyrosine on the HC-D309 column is much less than those values on the HC-ADS-5 column and the *N*-acetyl HC-D309 column (Table 1). From these results it is concluded that there is no hydrogen bonding between tyrosine and HC-D309. Thus the hydroxyl group of the protonated amide group participates in the hydrogen bonding, as shown in Fig. 8. This kind of hydrogen bonding form seems uncommon. The participation in the hydrogen bonding of H^+ probably changes the orientation and/or the lengths of the related bonds so that the sorbate and the sorption site of the sorbent are more complementary. Based on the pK_a value mentioned above, the ratio of the protonated amide groups to the unprotonated ones should be very small. Thus the hydrogen bonding formation should stabilize the protonated amide group. As mentioned above, the hydrogen bonding could be considered as an intramolecular interaction and thus the proton forms an internal bridge (Fig. 8). Witt and Grutzmacher reported that formation of an internal proton bridge by protonation significantly increased the proton affinities of diamides [22].

If the –OH group of protonated *N*-acetyl HC-D309 acts as proton donor in hydrogen bonding in the adsorption, protonated acetyloxymethyl polystyrene resin should act similarly to the protonated *N*-acetyl HC-D309. However, the retention of tyrosine on an acetyloxymethyl polystyrene resin column (data not shown) in the same condition is less than that on not only the *N*-acetyl HC-D309 column but also the HC-ADS-5 column. The corresponding transfer free energies of cystine and tyrosine on the acetyloxymethyl polystyrene resin column are listed in Table 1. Thus no hydrogen bonding between tyrosine and the acetyloxymethyl polystyrene resin exists. This may be explained by the much weaker protonation tendency of the carbonyl group of esters than that of the amide groups (six orders of magnitude less [21]).

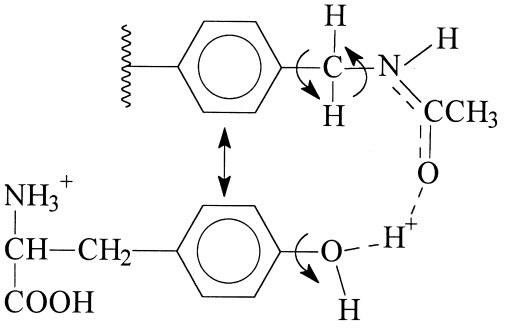

Fig. 8. Scheme for the interactions between *N*-acetyl-HC-D309 resin and tyrosine.

The hydrophobicities of *N*-acetyl HC-D309 and HC-D309 should be similar, therefore we assume that the hydrophobic affinities of tyrosine for *N*-acetyl HC-D309 and HC-D309 are the same. Thus the contribution of the hydrogen bonding to the transfer free energy in the interactions between tyrosine and *N*-acetyl HC-D309 was estimated to be –4.4 kJ mol^{-1} (−6.3 to −1.9 kJ mol^{-1}, see Table 1) with the assumption of no strain in the bound complex. The formation of the hydrogen bond restricts three internal rotations (Fig. 8). If each internal rotation restriction is adversely affected by 3.5–5 kJ mol^{-1} [23], the intrinsic free energy of the hydrogen bonding is −14.9 to −19.4 kJ mol^{-1} (−4.4–3×(3.5 to 5)) (the entropy loss of the bridge proton was not considered), in accord with the amide–amide hydrogen bonding free energies in the interactions between cell wall analogues and vancomycin group antibiotics (−18 to −21 kJ mol^{-1} [23]).

4. Conclusion

When two weak interactions occur in the sorption and these two interactions act synergistically, the sorption is much stronger than that driven by the same interactions that act individually. Some weak interactions that contribute little to the sorption when they act individually, e.g. hydrogen bonding in aqueous solution, may contribute to the sorption when they act synergistically with other interactions.

Acknowledgements

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