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Adsorption of Neutral α-Amino Acids at the Mercury–Aqueous Solution Interface and Hydrophobicity Scales of Amino Acid Side Chains at the Electrified Interfaces

Takashi KAKIUCHI and Mitsugi SENDA*

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University,
Sakyo-ku, Kyoto 606

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The adsorption of glycine, DL-serine, L-threonine, L-asparagine, L-glutamine, L-valine, L-leucine, L-methionine, and L-tryptophan at the mercury-aqueous sodium fluoride solution interface has been studied by measuring differential capacities, potentials of zero charge, pzc, and surface tensions at the pzc. The standard adsorption free energies for these amino acids and for the amino acids previously studied have been used to deduce hydrophobicity scales of amino acid side chains at positively charged, negatively charged, and uncharged interfaces. The hydrophobicity scale at the uncharged interface was basically similar to that of Nozaki and Tanford. When the surface was charged positively, the positions of serine and alanine, and of threonine and α-aminobutyric acid, in the scale were reversed. This reversion was primarily ascribed to the difference in the positions of maximum adsorption potentials between the amino acids with an aliphatic side chain and those with a hydroxyl group in the side chain.

In previous papers we have reported the adsorption properties of four α-amino acids with normal hydrocarbon side chains[1] and of phenylalanines[2] at the mercury–aqueous solution interface. This paper is concerned with the adsorption behavior of nine neutral amino acids, *i.e.*, glycine, DL-serine, L-threonine, L-asparagine, L-glutamine, L-valine, L-leucine, L-methionine, and L-tryptophan, at the mercury–aqueous sodium fluoride solution interface.

The primary interest of this study is to find a hydrophobicity scale of amino acid side chains at the electrified interface. In recent years the electrochemical properties of proteins and enzymes at electrode interfaces have been a subject of intense studies.[3] Increasing attention has also been paid to the behavior of proteins and enzymes at the interfaces of two immiscible liquids. Since every interface is invariably electrified due to electric charges and/or oriented dipoles at the interface and the physicochemical properties of the interface largely depend on its electrical state, the hydrophobicity of amino acid side chains at electrified interface is expected to give important information for understanding electrochemical behavior of proteins and enzymes at such interfaces.

Nozaki and Tanford[5] have proposed a hydrophobicity scale of amino acid side chains based on the solubility measurements of amino acids in water and aqueous organic solvents. Aboderin[6] used the relative mobilities of amino acid derivatives in paper chromatography to estimate the hydrophobicity of amino acid side chains. These hydrophobicity scales resort to the physicochemical properties of amino acids in homogeneous bulk phases. On the other hand, the depression of the surface tension of the air–aqueous solution interface caused by the adsorption of amino acids was used by Bull and Breese[7] as a measure of the hydrophobicity. They found fairly good agreement between their hydrophobicity scale and that of Nozaki and Tanford. In the case of air–solution interfaces, however, it is not feasible to control its electrical state. In contrast, the mercury–aqueous solution interface behaves in a certain range of electrode potential as an ideally polarized electrode having an additional degree of freedom for an electrical variable,[8] which enables us to specify and control accurately its electrical state. In addition, the mercury in contact with aqueous solution provides an electrode surface of hydrophobic nature.[9,10] Therefore, the transfer free energies of amino acids from an aqueous bulk phase to the mercury–aqueous solution interface are expected to serve for a measure to estimate the hydrophobicity of amino acid side chains at electrified interfaces.

Experimental

Reagent grade glycine, DL-serine, L-threonine, L-asparagine, L-glutamine, L-valine, L-leucine, L-methionine, and L-tryptophan were obtained from Nakarai Chem. Co., Japan and were twice recrystallized from aqueous ethanol (for glycine, serine, and threonine) or from triple distilled water (for other amino acids). Sodium fluoride, a standard-reagent-for-analysis grade from Hashimoto Chem. Co., Japan, was used as the base electrolyte without further purification.

Test solutions were deaerated by bubbling purified nitrogen gas through the solution for 30 min and kept under nitrogen atmosphere during the measurement. Electrode potential was measured against a saturated calomel electrode (SCE). Procedures for measuring the potential of zero charge, surface tension at this potential, and double layer capacity for the amino acids at the dropping mercury electrode–aqueous solution interface are described elsewhere.[2] All measurements were made at 25±0.2 °C.

Results

The potentials of zero charge(pzc) and surface tensions at this potential for glycine, DL-serine, L-threonine, L-asparagine, L-glutamine, L-valine, L-leucine, L-methionine, and L-tryptophan in 0.500 mol l^{-1} sodium fluoride are summarized in Tables 1 and 2. The relative surface excesses of the amino acids at the pzc were calculated from the surface tension *vs.* logarithm of the amino acid concentration curves by using the basic electrocapillary equation.[11] Differentiation involved in the calculation was made numerically.[12] We assumed that the activity of sodi-

TABLE 1. POTENTIALS OF ZERO CHARGE, E_{pzc}, OF MERCURY IN CONTACT WITH AQUEOUS 0.5 mol l^{-1} SODIUM FLUORIDE SOLUTION CONTAINING AMINO ACID AT 25 °C

c / mol l^{-1}	E_{pzc}/V(*vs.* SCE)								
	Gly	L-Try	DL-Ser	L-Thr	L-Asn	L-Gln	L-Val	L-Leu	L-Met
0.005	—	0.596	—	—	—	—	—	—	—
0.008	—	—	—	—	—	—	—	—	0.449
0.010	—	0.608	0.430	—	0.431	0.432	0.430	0.432	0.450
0.012	—	0.615	—	—	—	—	—	—	—
0.015	—	0.615	—	—	—	—	0.430	0.433	0.452
0.020	—	0.622	0.430	0.431	0.431	0.434	0.430	0.432	0.455
0.025	—	0.620	—	—	—	—	—	0.433	—
0.030	—	0.622	—	—	0.433	0.434	0.428	0.431	0.457
0.040	—	—	0.431	0.431	0.433	0.435	0.431	0.433	0.462
0.050	0.432	—	—	—	—	—	—	0.432	—
0.060	—	—	0.432	0.432	0.435	0.437	0.427	0.432	0.465
0.080	—	—	—	—	—	—	—	—	0.467
0.100	0.432	—	0.433	0.432	0.437	0.439	0.429	—	0.469
0.120	—	—	—	—	0.439	0.440	—	—	0.470
0.150	0.433	—	0.434	0.434	0.442	0.442	0.426	—	0.472
0.200	0.433	—	0.436	0.435	—	0.445	0.423	—	0.473
0.250	—	—	—	—	—	—	0.420	—	—
0.300	0.433	—	0.438	0.437	—	—	0.417	—	—
0.400	0.435	—	0.440	—	—	—	—	—	—
0.500	—	—	—	0.438	—	—	—	—	—
0.600	0.435	—	—	—	—	—	—	—	—
0.700	—	—	—	0.440	—	—	—	—	—
0.800	0.437	—	—	—	—	—	—	—	—
1.000	0.440	—	—	—	—	—	—	—	—

TABLE 2. SURFACE TENSIONS OF MERCURY AT THE POTENTIAL OF ZERO CHARGE, γ_{pzc}, IN CONTACT WITH AQUEOUS 0.5 mol l^{-1} SODIUM FLUORIDE CONTAINING AMINO ACID AT 25 °C

c / mol l^{-1}	γ_{pzc}/mN m^{-1}								
	Gly	L-Try	DL-Ser	L-Thr	L-Asn	L-Gln	L-Val	L-Leu	L-Met
0.005	—	395.3	—	—	—	—	—	—	—
0.008	—	—	—	—	—	—	—	—	424.2
0.010	—	392.4	427.3	—	427.2	426.9	426.8	426.5	423.6
0.012	—	391.6	—	—	—	—	—	—	—
0.015	—	389.8	—	—	—	—	426.7	426.5	422.5
0.020	—	388.4	427.2	427.0	427.2	426.7	426.7	426.3	421.4
0.025	—	387.0	—	—	—	—	—	426.2	—
0.030	—	385.8	—	—	427.1	426.4	426.5	425.8	419.7
0.040	—	—	427.1	426.9	426.9	426.2	426.3	425.7	418.4
0.050	427.1	—	—	—	—	—	—	425.2	—
0.060	—	—	427.1	426.8	426.7	425.7	426.0	425.0	416.2
0.080	—	—	—	—	—	—	—	—	414.5
0.100	427.1	—	427.0	426.7	426.2	424.9	425.5	—	412.9
0.120	—	—	—	—	426.0	424.5	—	—	411.8
0.150	427.2	—	427.0	426.4	425.7	424.0	424.7	—	410.0
0.200	427.1	—	426.9	426.3	—	423.1	423.7	—	407.7
0.250	—	—	—	—	—	—	423.3	—	—
0.300	427.1	—	426.8	426.0	—	—	422.7	—	—
0.400	427.0	—	426.7	—	—	—	—	—	—
0.500	—	—	—	425.3	—	—	—	—	—
0.600	427.0	—	—	—	—	—	—	—	—
0.700	—	—	—	424.5	—	—	—	—	—
0.800	427.0	—	—	—	—	—	—	—	—
1.000	427.0	—	—	—	—	—	—	—	—

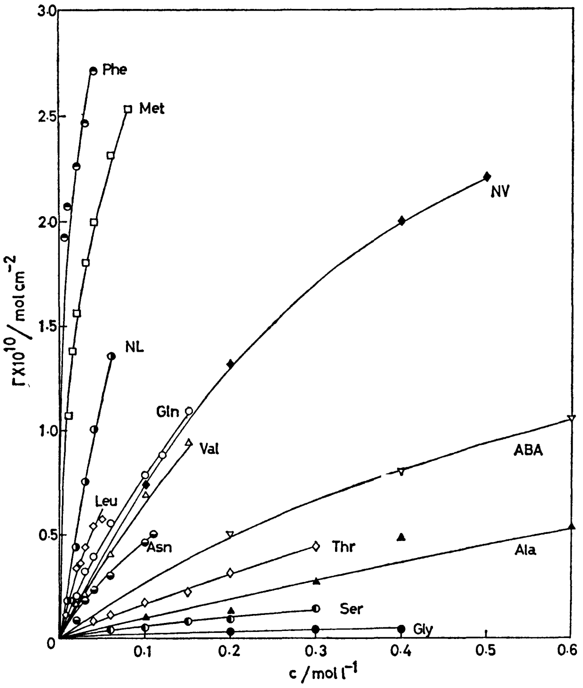

Fig. 1. Adsorption isotherms of amino acids at the mercury–aqueous 0.5 mol l^{-1} sodium fluoride solution interface at the potential of zero charge at 25 °C. See Table 3 for abbreviated name of the amino acids indicated by each line.

um fluoride and the activity coefficients of amino acids did not change over the experimental range of the amino acid concentration.[13] Figure 1 shows the relative surface excess of the amino acids, except tryptophan, plotted against their molar concentration. The relative surface excess of tryptophan seemed to reach a saturation value already at 0.005 mol l^{-1}, the lowest concentration studied; the surface tension *vs.* logarithm of tryptophan concentration plot was linear between 0.005 and 0.030 mol l^{-1}. The slope of this plot gave a relative surface excess of 2.4×10^{-10} mol cm^{-2} which was in agreement with the maximum surface concentration of 2.5×10^{-10} mol cm^{-2} calculated from a space filling model (CPK) of the tryptophan molecule adsorbed with its aromatic ring plane oriented parallel to the electrode surface. Figure 1 also includes the previous results for DL-alanine, DL-α-aminobutyric acid, DL-norvaline, DL-norleucine,[1] and L-phenylalanine.[2]

In order to obtain the surface excess of the amino acids at the electrically charged interfaces, differential capacities of the interface were measured for glycine, DL-serine, L-threonine, L-asparagine, L-glutamine, and L-methionine as functions of the electrode potential and amino acid concentration. Electrocapillary curves were obtained by numerical integration of the differential capacity *vs.* electrode potential curves and were used to calculate the surface excess of the amino acids at various electrode potentials. We chose for further analysis the electrode surfaces at −0.1 and −0.9 V (*vs.* SCE) as the representatives of positively charged and negatively charged interfaces, respectively. In 0.500 mol l^{-1} sodium fluoride base solution, the surface charge densities at −0.1 and −0.9 V were determined as 8.9 and −9.3 μC cm^{-2}, respectively. The relative surface excesses of the amino acids at −0.1 and −0.9 V are shown in Figs. 2 and 3 as a function of the amino acid concentration. These figures also include the previous results obtained for four aliphatic amino acids[1] and L-phenylalanine.[2]

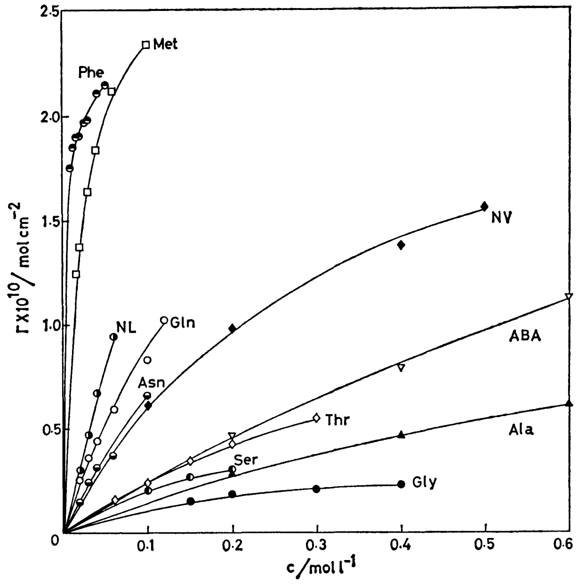

Fig. 2. Adsorption isotherms of amino acids at the positively charged mercury–aqueous 0.5 mol l^{-1} sodium fluoride solution interface (−0.1 V *vs.* SCE) at 25 °C.

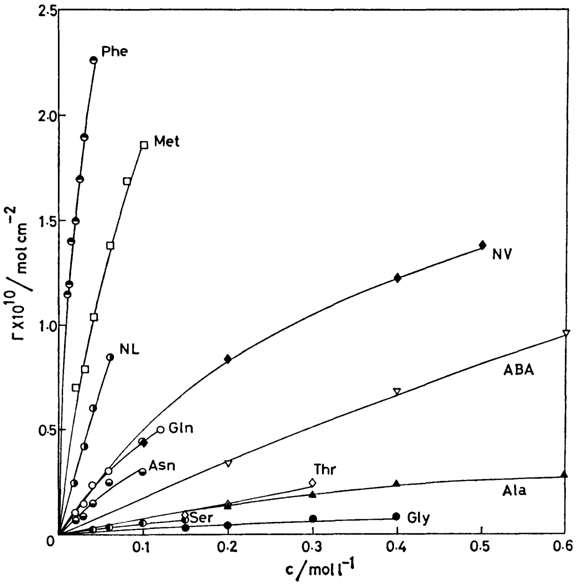

Fig. 3. Adsorption isotherms of amino acids at the negatively charged mercury–aqueous 0.5 mol l^{-1} sodium fluoride solution interface (−0.9 V *vs.* SCE) at 25 °C.

Since the bulk concentrations of the amino acids used in this study are not high, as much as 1 mol l^{-1} for glycine at the highest, the relative surface excess of the amino acid may be regarded as the surface concentration of the adsorbed amino acid per unit area, Γ, without introducing significant error.[14] Then,

Table 3. Limiting slopes of adsorption isotherms, $B\Gamma_m$, and adsorption free energies of amino acids, ΔG_a°, at positively charged(−0.1 V), uncharged(pzc) and negatively charged(−0.9 V) mercury-aqueous 0.5 mol l^{-1} sodium fluoride solution interface at 25 °C

Amino acids	$B\Gamma_m\times10^{10}$/cm			$\Gamma_m\times10^{10}$ c)	ΔG_a°/kJ mol^{-1}		
	−0.1 V	pzc	−0.9 V	mol cm^{-2}	−0.1 V	pzc	−0.9 V
Glycine (Gly)	1.1	0.2	0.3	6.6	4.4	9.0	8.0
DL-Serine (Ser)	2.1	0.6	0.6	5.9	2.6	5.8	6.0
DL-Alanine (Ala)[a]	1.5	1.2	0.7	5.9	3.3	4.0	5.1
L-Threonine (Thr)	2.7	1.8	0.7	4.2	1.1	2.1	4.4
DL-α-Aminobutyric acid (ABA)[a]	2.4	2.7	1.7	5.0	1.8	1.5	2.6
L-Asparagine (Asn)	8.0	5.9	3.7	3.7	−1.9	1.2	0.0
L-Valine (Val)	—	9.8	—	4.3	—	−2.0	—
DL-Norvaline (NV)[a]	6.9	10.2	4.6	4.3[d]	−1.2	−2.1	−0.1
L-Glutamine (Gln)	13.0	13.0	5.6	3.2	−3.5	−3.5	−1.4
L-Leucine (Leu)	—	16.2	—	3.8	—	−3.6	—
DL-Norleucine (NL)[a]	17.5	22.0	15.6	3.8	−3.8	−4.3	−3.5
L-Methionine (Met)	91.5	127.5	36.0	3.7	−7.9	−8.8	−5.6
L-Phenylalanine (Phe)[b]	245	390	127	3.2	−10.7	−11.9	−9.1

a) Data from Ref. 1. b) Data from Ref. 2. c) Calculated from CPK model of amino acid. d) Experimental value from Ref. 1.

the relative surface excess *vs.* concentration curve shown in Figs. 1, 2, and 3 can be considered as the surface concentration *vs.* bulk concentration curves for the amino acids at the pzc, —0.1, and —0.9 V, respectively. In dilute concentration region the adsorption isotherm generally obeyes Henry's law[15)]

$$Bc = \Gamma/\Gamma_m, \qquad (1)$$

where B is the adsorption coefficient, c the bulk concentration of an adsorbate and Γ_m the maximum surface concentration of an adsorbate. The adsorption coefficient is related to the standard adsorption free energy, ΔG_a°, by

$$-\Delta G_a^\circ = RT\ln B, \qquad (2)$$

where R is the gas constant and T the absolute temperature. The standard adsorption free energy in Eq. 2 is defined as the free energy change accompanied with the transfer of an adsorbate from a bulk phase at infinite dilution to an adsorption phase at infinite dilution. In this definition the adsorption free energy of an enantiomer and that of the correponding racemate must be the same[2)] and, hence, will not be distinguished hereafter. We graphically evaluated the limiting slopes of the adsorption isotherms, $(\partial\Gamma/\partial c)_{c=0}$, in Figs. 1, 2, and 3 to obtain $B\Gamma_m$. The values are summarized in Table 3. The maximum surface concentrations of amino acids in the fifth column of Table 3 were calculated by using the CPK model of the amino acid molecules adsorbed with their side chains oriented parallel to the electrode surface. The ΔG_a° values evaluated from these data by using Eq. 2 are given in the last three columns in Table 3.

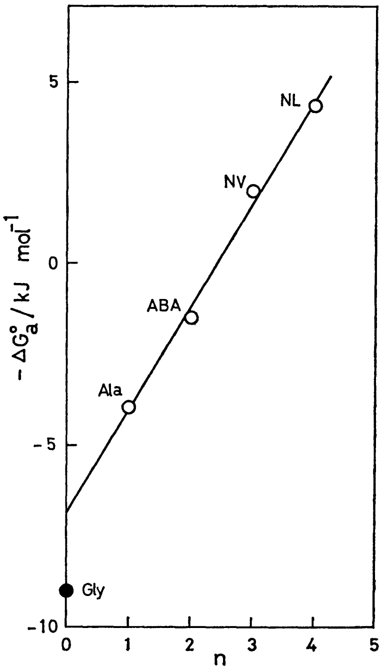

Fig. 4. Standard adsorption free energies of normal aliphatic α-amino acids and glycine as a function of the number of carbon atom, n, in the side chain.

Discussion

The standard adsorption free energies for glycine and a series of aliphatic α-amino acids, alanine, α-aminobutyric acid, norvaline, and norleucine, at the pzc are plotted in Fig. 4 against the number of carbon atom in the side chain, *n*. The linear plot in Fig. 4 provides an experimental basis for dividing the standard adsorption free energy of an α-amino acid into two contributions, a contribution from a zwitterion group and that from a side chain group. The decrement of ΔG_a° per one methylene group in the side chain was found to be 2.9 kJ mol^{-1}. This may be comparable with 3.3 kJ mol^{-1} [16)] obtained for the adsorption of normal aliphatic alcohols at the mercury–aqueous solution interface and 3.1 kJ mol^{-1} [17)] for the adsorption of normal aliphatic acids at the copper-aqueous solution interface.

Table 4. Transfer free energy of amino acid side chain, $\Delta G^\circ_{a,s}$, from aqueous bulk phase to the mercury-aqueous sodium sulfate solution interface at 25 °C as a hydrophobicity scale of amino acid side chains on positively charged(-0.1 V), uncharged(pzc), and negatively charged(-0.9 V) interfaces

Amino acid side chains	$-\Delta G^\circ_{a,s}$/kJ mol^{-1}		
	-0.1 V	pzc	-0.9 V
Serine	1.8	3.2	2.0
Alanine	1.1	5.0	2.9
Threonine	3.3	6.9	3.6
α-Aminobutyric acid	2.6	7.5	5.4
Asparagine	6.3	7.8	8.0
Valine	—	11.0	—
Norvaline	5.6	11.1	8.0
Glutamine	7.9	12.5	9.4
Leucine	—	12.6	—
Norleucine	8.2	13.3	11.5
Methionine	12.3	17.8	13.6
Phenylalanine	15.1	20.9	17.1

The transfer free energy of an amino acid side chain from aqueous bulk phase to the interface, $\Delta G^\circ_{a,s}$, was calculated by subtracting ΔG°_a for glycine from ΔG°_a for each amino acid. Table 4 summarizes the $\Delta G^\circ_{a,s}$ values thus estimated at the pzc, -0.1 V and -0.9 V (*vs.* SCE). These series of the transfer free energies of the side chains may be regarded as interfacial hydrophobicity scales at electrified interfaces. The major trend in the series of $\Delta G^\circ_{a,s}$ at the uncharged interface (at the pzc) is essentially similar to that found by Nozaki and Tanford,[5] as illustrated in Fig. 5 in which $\Delta G^\circ_{a,s}$'s at the pzc are plotted against the transfer free energies of amino acid side chains from water to organic solvents, ΔG°_{tr}.[5] As seen in this figure serine, threonine, alanine, valine, leucine, and norleucine fall around the straight line of unit slope.

There are, however, a few facts in Table 4 and Fig. 5 which do not conform to the results by Nozaki and Tanford. In their scale, threonine is less hydrophobic than alanine whereas the converse is true in the interfacial hydrophobicity scale at the pzc. Introduction of a hydroxyl group into a hydrocarbon side chain decreases the hydrophobicity of the side chain by 1.8 kJ mol^{-1} (for an alanine and serine pair) and 0.6 kJ mol^{-1} (for an α-aminobutyric acid pair). These values are appreciably smaller than 3.4 kJ mol^{-1} estimated by Nozaki and Tanford[5] as a contribution of a hydroxyl group to the transfer free energy. At the interface the hydroxyl group of serine or threonine is probably only partially dehydrated because a part of the adsorbed molecule should be exposed to the aqueous phase, whereas on transfer of the molecule from water to nonaqueous solvent the hydroxyl group must completely be dehydrated and surrounded by nonaqueous medium. Table 4 also shows that glutamine is more hydrophobic than asparagine. This is contrary to the result obtained by Nozaki and Tanford who found that glutamine was apparently less hydrophobic than asparagine. They have suggested that the formation of an intramolecular hydrogen bond in asparagine molecule in nonaqueous phase is responsible to the abnormal position of asparagine with respect to glutamine in their hydrophobicity scale. In adsorption layer asparagine molecules will not form an intramolecular hydrogen bond but remain to hold hydrogen bonding with adjacent water molecules. Figure 5 shows that methionine and phenylalanine have higher values of $\Delta G^\circ_{a,s}$ than those expected from the line of unit slope. Since sulfur atom has strong affinity with mercury,[18] such a chemical interaction should be involved in the observed high surface activity of methionine. Phenylalanine interacts with mercury surface through not only the hydrophobic interaction but also the charge transfer interaction due to π-electron in the aromatic ring.[2] Specific interaction also seems to exist between the amide group in asparagine and the mercury surface. Among the amino acid side chains studied only asparagine side chain showed higher surface activity at the negatively charged surface than at the pzc.

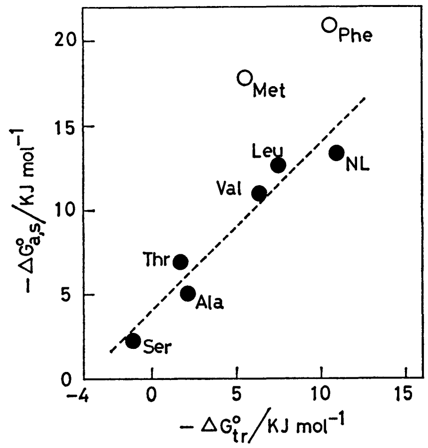

Fig. 5. Plot of the free energies of transfer of amino acid side chains from aqueous bulk phase to the uncharged mercury surface in contact with aqueous phase, $\Delta G^\circ_{a,s}$, against the free energies of transfer of amino acid side chain from aqueous bulk phase to pure organic solvents, ΔG°_{tr}, taken from Ref. 5.

Table 4 shows that when the mercury surface is charged either positively or negatively, $\Delta G^\circ_{a,s}$'s for all the amino acid side chains but asparagine side chain at -0.9 V are increased; their surface activity becomes lower when the surface comes to bear excess charges. The change of $\Delta G^\circ_{a,s}$ with the change of the electrical state of the interface is illustrated in Fig. 6 for ten amino acid side chains. The effect of electrode charge on $\Delta G^\circ_{a,s}$ for alanine, α-aminobutyric acid, norvaline, and norlecuine, which we refer as group I, is appreciably asymmetrical with respect to the pzc, but the effect is almost symmetrical for serine and threonine (group II). This difference in the dependence of $\Delta G^\circ_{a,s}$ on electrode charge between groups I and II causes the reversion of the mutual positions of serine and α-alanine, and of threonine and α-aminobutyric acid in their interfacial hydrophobicity scale at the positively charged electrode surface.

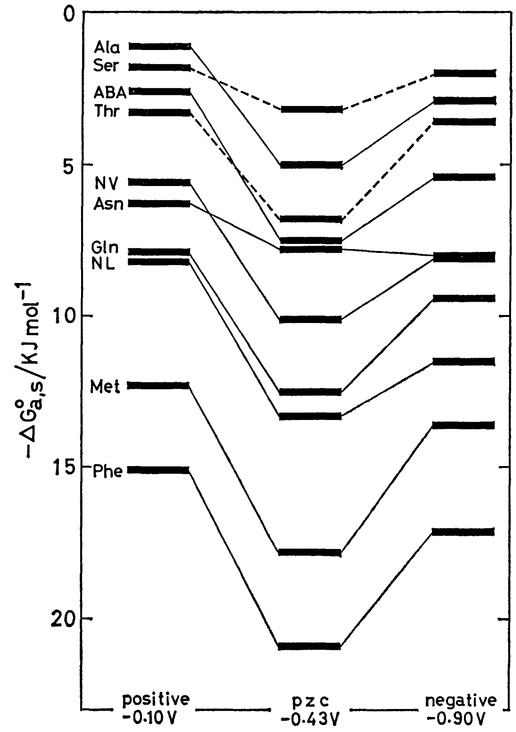

Fig. 6. Schematic representation of the change of the interfacial hydrophobicity of amino acid side chains with the change of the electrical state of the mercury–aqueous 0.5 mol l^{-1} sodium fluoride solution interface at 25 °C.

According to the theory of Frumkin[19] and Butler[20] on the adsorption of organic compounds at electrified interface, the dependence of the adsorption free energy, ΔG_a°, of an organic compound on electrode potential is described by

$$\Delta G_a^\circ = \Delta G_{a,max}^\circ + RT\alpha(E-E_{max})^2, \tag{3}$$

where $\Delta G_{a,max}^\circ$ is ΔG_a° at $E=E_{max}$, E the electrode potential, E_{max}, the electrode potential at which the adsorption maximum occurs, and α the constant represented by

$$\alpha = \frac{C_0-C_1}{2RT\Gamma_m}. \tag{4}$$

In Eq. 4 C_0 is the double layer capacity in the absence of adsorption ($\theta=0$) and C_1 is the double layer capacity at the full coverage of the electrode with adsorbate ($\theta=1$). The maximum adsorption potential in Eq. 3 is given by[21]

$$E_{max}-E_{pzc}^\circ = \frac{C_1(E_{pzc}^{\theta=1}-E_{pzc}^\circ)}{C_1-C_0}, \tag{5}$$

where $E_{pzc}^{\theta=1}$ and E_{pzc}° are the potentials of zero charge at $\theta=1$ and $\theta=0$, respectively.

In view of Eqs. 3, 4, and 5, the difference in the effect of the electrode potential on $\Delta G_{a,s}^\circ$ between groups I and II is probably due primarily to the different values of E_{max} for groups I and II. Group II seems to have E_{max} values around the pzc, whereas the E_{max} values for group I seem to be located in more negative potential region with respect to E_{pcz}°. The presumption of $E_{max}-E_{pzc}^\circ<0$ for group I implies[1] that water dipoles are oriented with their negative end pointing toward the electrode surface,[9,10] because in this group the effective dipole moment of the side chain should be small, if any. The more positive E_{max} for group II compared with that of group I indicates the orientation of the hydroxyl group dipole with its negative end pointing toward the electrode surface.

According to Eqs. 3 and 4, the change of $\Delta G_{a,s}^\circ$ with the change of the electrical state of the interface depends also on the magnitude of the double layer capacities of the interface. The double layer capacities in the presence and absence of the adsorption are generally a function of the electrical state of the interface due to the change in the orientation mode of adsorbed molecules as well as solvent molecules in the compact layer of the double layer with the change of the electrical state.[22] Therefore, the change of the capacities may partly contribute to the observed dependence of $\Delta G_{a,s}^\circ$ on the electrical state of the interface.

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