Experimental Estimation of Hydropathy Scales of Apolar Amino Acid Residues by Measuring Langmuir Monolayer Behaviors of Amino Acid Derivative Polymers

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We described here a novel method for experimental estimation of hydropathy scales of amino acid by measuring Langmuir monolayer behaviors of the amino acid derivative polymers. The collapse pressure of the monolayers had a good correlation with the hydropathy scales.

It is well known that non-covalent bondings working in polypeptides, such as hydrogen-bonding[1,2] and hydrophobic interaction,[3] are important factors for a formation of a special conformation of protein. Especially, hydrophobic interaction between apolar side chains induces the folding of the secondary structure of polypeptide.[4–6] Most of the hydrophobicity of amino acids have been determined thermodynamically from the water–organic solvent partition coefficients, K_d, of amino acid molecules or analogues.[7–9] However, the hydrophobicity determined by the method does not consider the molecular environment in actual proteins. In this situation, Kyte and Doolittle proposed hydropathy scales[10] to modify the hydrophobicity scales by considering the position of the amino acid in a known protein structure.

On the other hand, Langmuir–Blodgett monolayer and multilayers are known to offer molecular environments similar to that in biomembrane.[11] Through the previous studies on a series of polyacrylamide monolayers and LB films, we showed that the polyacrylamide structure is suitable for the formation of high quality monolayer where hydrogen bonding of the amide groups plays an important role for self-assembly.[12–14] In this work, we prepared the monolayer of polyacrylamide modified by various amino acid derivatives (Figure 1) and measured the monolayer behaviors. Depending on the property of the amino acid in the polymer monolayer, whether the amino acid tends to participate in the water phase or hydrophobic phase, the properties of the polymer monolayers were changed. We found that the change in hydropathy scales of the amino acids can be estimated experimentally from the change in the monolayer behaviors of the amino acid derivative acrylamide polymers.

We employed poly(long-alkylacrylamide) as a base polymer and various amino acid residues (glycine (Gly), L-alanine (Ala), L-valine (Val), L-leucine (Leu), L-isoleucine (Ile), L-methionine (Met), L-phenylalanine (Phe), and L-tryptophan (Trp)) were introduced chemically into the polymers (P-(Amino acid)-Ac, Figure 1).[15]

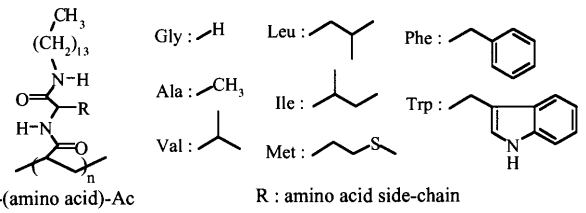

Figure 1. Chemical structures of P-(amino acid)-Ac and the side-chain.

The amino acid derivative polymer, for example, poly[*N*-{1-(tetradecylcarbamoyl)ethyl}acrylamide] (P-Ala-Ac), was prepared by usual radical polymerization of the corresponding monomer, N^{α}-acryloyl-*N*-tetradecylalanine amide. The molecular weights and dispersions of the polymers determined by a gel permeation chromatography are given in Table 1. The polymers were spread from a chloroform solution (10^{-3} M) onto the water surface to measure the surface pressure (π)–area (A) isotherms at 25 °C (Figure 2(A)). The steep rise in surface pressure and high collapse pressure in the isotherms indicate the formation of condensed monolayers. Despite only the amino acid side-chain (R) being different in the chemical structure of the polymers, the isotherms are drastically varied with the amino acid derivatives. Extrapolation of the steeply rising part of the π–A curve to zero surface pressure gives the average molecular occupied surface area per repeating unit (A_{LA}) in the monolayer (Table 1). The A_{LA} values varied with the amino acids, depending on the molecular size of the side chain. This result indicates that the amino acid side-chain places at the monolayer with its own surface area on the water surface.

The collapse surface pressures (π_{col}) of the monolayers were also changed by the amino acid derivatives. The glycine derivative shows the highest and the lowest pressure is for the isoleucine derivative. The π_{col} values decrease with the amino acids in the order of Gly > Trp > Ala > Met > Phe > Leu > Val

Table 1. The properties of P-(amino acid)-Ac monolayers

polymer	$M_w/10^3$ (M_w/M_n)	A_{LA} / nm^2	π_{col} / mN/m	hydrophobicity[a]	hydropathy[b]
P-Gly-Ac	6.91 (1.65)	0.291	56.1	0.00	- 0.4
P-Ala-Ac	5.60 (1.46)	0.434	45.1	0.67	1.8
P-Val-Ac	4.50 (1.67)	0.507	30.7	2.18	4.2
P-Leu-Ac	4.99 (1.40)	0.488	33.2	3.02	3.8
P-Ile-Ac	4.55 (1.34)	0.508	25.3	3.02	4.5
P-Met-Ac	5.48 (1.60)	0.477	43.0	1.30	1.9
P-Phe-Ac	4.99 (1.73)	0.511	41.3	3.24	2.8
P-Trp-Ac	5.40 (1.21)	0.531	48.5	2.86	- 0.9

[a]Hydrophobicity (Reference 7). [b]Hydropathy (Reference 10).

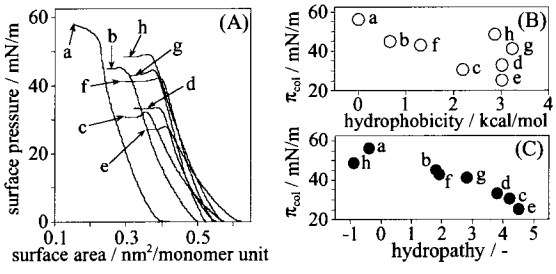

Figure 2. (A) Surface pressure - area isotherms of P-(amino acid)-Ac at 25 °C. (B) Correlation of the π_{col} value of P-(amino acid)-Ac with hydrophobicity. (C) Correlation of the π_{col} value of P-(amino acid)-Ac with hydropathy. P-Gly-Ac (a), P-Ala-Ac (b), P-Val-Ac (c), P-Leu-Ac (d), P-Ile-Ac (e), P-Met-Ac (f), P-Phe-Ac (g), P-Trp-Ac (h).

> Ile (Table 1). The order in the π_{col} value cannot be apparently explained by steric hindrance of the side chains, because P-Trp-Ac having imdolyl substituent and P-Ala-Ac having methyl one show similar π_{col} values. We previously reported that the π_{col} of poly(*N*-dodecylacrylamide) monolayer has nearly constant value if the molecular weights are larger than thousands.[16] Therefore it is considered that the effect of molecular weight of polymer on the π_{col} value should be also small. It is well known that monolayer property strongly depends on a hydrophobic–hydrophilic balance of the compound.[17] The π_{col} values are expected to correlate with the hydrophobicities of the amino acids, which are determined by a partition coefficient, K_d, of amino acid derivatives between water and various organic solvents. The π_{col} value is plotted as a function of the hydrophobicity[7] in Figure 2(B). Apparently, no correlation between them is obtained. Next, the π_{col} value was plotted against the hydropathy scale of amino acids in Figure 2(C). The hydropathy scales are determined by computer calculations based on known protein structures in which the amino acid residue is found to be in a water phase or in a protein-membrane phase.[11] A good correlation between the π_{col} value and the hydropathy is observed (Figure 2(C)).

Now we discuss here why the hydropathy scale has a good correlation with the collapse pressure of the polymers rather than the hydrophobicity value. Most of the hydrophobicity values have been determined thermodynamically from the K_d of amino acid derivative compounds.[7–9] The hydrophobicity value varies by the amino acid derivatives employed for the measurement, that is, it is dependent on the chemical forms; zwitterionic form or neutral form. On the other hand, in the present polymers used in this paper, the amino acid side-chain moiety lies between the amide groups similarly to that in polypeptide and moreover, the polymer monolayer faces the water phase similarly to the actual proteins. The collapse of the monolayer means that the transfer of the amino acid moiety from the hydrophilic field (water–monolayer interfaces) into the hydrophobic field (tetradecyl substituent region) has occurred. The energy for the transfer of a hydrophilic side chain in a hydrophobic field is larger than that of a hydrophobic one. In other words, the π_{col} value of the monolayer having more hydrophobic side chain should be smaller than that of the hydrophilic ones. Here, taking that the hydropathy scale which is calculated from the structure of proteins indicates a tendency of transfer for the amino acid moiety into the inside of the protein into consideration, the meaning of π_{col} values should be identical with that of the hydropathy. Namely, the situation in the present polymer monolayer resembles the environment of a protein or lipid membrane. After all, the collapse pressure of the monolayer of amino acid derivative polymer has a good correlation with the hydropathy scale of the corresponding side chain. In this method, the hydropathy can be determined without getting an information on the exact protein structure. Therefore, it is valuable that this method is applicable to the determination of a hydropathy scale of an unnatural amino acid.

Next we examined the property of the monolayer at various temperatures (*t*) from the π–*A* isotherms. For example, the π–*A* isotherms of the monolayer of P-Ala-Ac are shown in Figure 3(A). Apparently the π_{col} value decreases with the increase in temperature whereas the A_{LA} is not changed, that is, the condensed monolayer is maintained. All of the monolayers, except

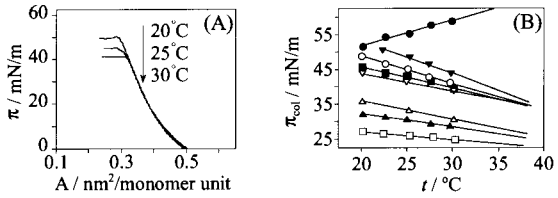

Figure 3. (A) Surface pressure - area isotherms for P-Ala-Ac at 20, 25, and 30 °C. (B) Dependence of the π_{col} values of the polymer monolayers on temperatures. P-Gly-Ac (●), P-Ala-Ac (○), P-Val-Ac (▲), P-Leu-Ac (△), P-Ile-Ac (□), P-Met-Ac (■), P-Phe-Ac (▽), P-Trp-Ac (▼).

that of P-Gly-Ac, decreased linearly with increasing temperatures (Figure 3(B)). P-Gly-Ac monolayer showed the reverse dependence on temperature. In the preceding section, we showed that the π_{col} values of the present monolayers are correlated with the hydropathy scale of the amino acid residues. The results in Figure 3(B) indicate that the hydropathy scale varies by temperature. The glycine residue becomes more hydrophilic with increasing temperature, whereas the alanine residue tends to avoid water, namely, it becomes more hydrophobic with increasing temperature. Moreover, it is noteworthy that the π_{col} values of P-Trp-Ac, P-Ala-Ac, P-Met-Ac, and P-Phe-Ac are gradually approaching the same value with increasing temperature. This result suggests that the hydropathy of amino acid side-chains might lose significant difference among them at the temperature of about 40 °C. Unfortunately, we cannot measure the isotherm at a hot water subphase. The phenomenon that the difference in the hydropathy values of the amino acids disappears at a certain temperature around 40 °C, may be related to thermal denaturation of protein.

Conclusively, it can be said that hydropathy scales for amino acids are determined experimentally by the measurement of π–*A* isotherms of the P-amino acid-Ac monolayers. Moreover, the change in the hydrophobic character of the amino acid with temperature can be monitored by the change in the π_{col} values of the monolayers. It is also suggested that there is a certain temperature where the difference in the hydrophobic properties among the amino acid residues disappears, which is related to the denaturation of protein.

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