Adsorption of Dipeptides on Activated Carbon

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The adsorption isotherms of some dipeptides composed of un-ionizable side chains were measured at 25°C in a single component system, and all of them obeyed the Langmuir equation. The parameters obtained at pH 7 could be roughly correlated with the hydrophobic and steric parameters of constituent amino acids. To find a method for predicting the amount of dipeptide adsorbed under various conditions, the applicability of the Polanyi adsorption potential theory was examined. The amount of dipeptide adsorbed could be roughly estimated from the adsorption isotherms of the amino acids, although the estimation lacked strictness in a physical sense.

Keywords: adsorption, dipeptide, activated carbon

Attention has been paid to the utilization of protein hydrolysate as a food seasoning (Ueno et al., 1990) and foaming agent (Nagatomo et al., 1990). In these applications, a separation process is incorporated to obtain a peptide mixture with a specific functionality. An infusion solution rich in branched amino acids and poor in aromatic ones is useful as a nutrient for patients with liver diseases (Fischer et al., 1976). A peptide mixture with such an amino acid composition would be more promising than an amino acid mixture because of its faster absorption in the intestine and lower osmotic pressure. We reported the preparation of a peptide mixture with a high Fischer ratio, which is the ratio of branched amino acids to aromatic ones, from the enzymatic hydrolysate of casein using gel chromatographic resin (Adachi et al., 1991) or activated carbon (Adachi et al., 1993). The preparation of a peptide mixture rich in aromatic amino acids from a protein hydrolysate was also attempted (Li et al., 1985). Thus, peptides with a definite composition would be useful as foods.

To reasonably design an adsorptive separation of peptides, knowledge of their adsorption equilibria and rate processes is required. A protein hydrolysate includes many kinds of peptides. It would be difficult to predict the adsorption isotherm of each peptide onto an adsorbent in a multi-component system. As the first step in the adsorptive separation design of peptides from protein hydrolysate, we measured the adsorption isotherms of some dipeptides composed of amino acids having un-ionizable side chains onto an activated carbon in a single component system, and the methods to predict them are discussed.

Materials and Methods

Materials Glycyl-L-isoleucine (Gly-Ile), glycyl-L-leucine (Gly-Leu), glycyl-L-phenylalanine (Gly-Phe), glycyl-L-tryptophan (Gly-Trp), glycyl-L-tyrosine (Gly-Tyr), L-leucyl-L-leucine (Leu-Leu), L-leucyl-L-tyrosine (Leu-Tyr) and L-phenylalanyl-L-leucine (Phe-Leu) were obtained from Sigma, St. Louis, MO. L-Methionyl-L-leucine (Met-Leu), L-methionyl-L-methionine (Met-Met) and L-seryl-L-leucine (Ser-Leu) were obtained from Kokusan Chemical Works, Osaka. Glycylglycine (Gly-Gly) and most of the amino acids used were purchased from Wako Pure Chemical Industries, Osaka. L-Tryptophan and L-tyrosine were products of the Peptide Institute, Osaka. Other chemicals were purchased from Nacalai Tesque or Wako Pure Chemical Industries and were of analytical grade.

The activated carbon was spherical beads called Kureha Beads (Kureha Chemicals, Tokyo). The properties of the beads, which were provided by the manufacturer, were as follows. The diameter of the beads ranged from 0.25 mm to 0.60 mm, the B.E.T. surface area was 420 m²/g, and the volume of the micropores was 0.18 cm³/g.

Measurement of adsorption isotherm A peptide or amino acid was dissolved in 100 mol/m³ phosphate buffer, pH 7, at a final concentration of 0.5 to 5 mol/m³. One milliliter (V) of adsorbate solution of concentration C₀ was pipetted into an Eppendorf microtube, and about 3 mg (w) of activated carbon was then added. The tube was kept at 25°C with shaking for 88 h. The solution phase was sampled, and the equilibrium concentration of the adsorbate C was determined. The amount adsorbed q was evaluated by

\[ q = \frac{V(C₀ - C)}{w} \]  

The amount of adsorbed peptide was varied between 0.5 and 10 mg depending on the adsorbate. The measurement of q was made at four or five different adsorbate concentrations.

The adsorption isotherms of the dipeptides at pH 2 were measured using 100 mol/m³ HCl-KCl buffer. To measure the adsorption isotherms of Gly-Phe and Gly-Ile at various pHs, 100 mol/m³ acetate buffer (pH 3, 4 and 5) and 100 mol/m³ phosphate buffer (pH 6) were used.

Solubility of dipeptides A large amount of a peptide was put into a buffer solution and shaken for 72 h under the given conditions. A portion of the mixture was carefully sampled so as not to take any undissolved peptide, and its concentration was determined.

The solubility of the amino acids was cited from a
handbook (Narita, 1979).

Analysis The peptide concentration was measured using an HPLC (LC-6A, Shimadzu Seisakusho, Kyoto) with an ODS column (8 mmφ×300 mm, Nacalai Tesque) and a UV detector at 220 nm after the appropriate dilution. The eluent was a mixture of 10 mol/m³ phosphate buffer, pH 7, and acetonitrile (1:1 in volume).

Fig. 1. Adsorption isotherms of dipeptides onto activated carbon at 25°C and pH 7. The dipeptides used were Gly-Gly (△), Gly-Ile (▲), Gly-Leu (■), Gly-Phe (●), Gly-Trp (◇), Leu-Leu (○), Leu-Tyr (●), Met-Leu (□), Met-Met (■), Phe-Leu (◇) and Ser-Leu (■). The curves were drawn using the estimated K and qm values.

Analysis The peptide concentration was measured using an HPLC (LC-6A, Shimadzu Seisakusho, Kyoto) with an ODS column (8 mmφ×300 mm, Nacalai Tesque) and a UV detector at 220 nm after the appropriate dilution. The eluent was a mixture of 10 mol/m³ phosphate buffer, pH 7, and acetonitrile (1:1 in volume).

The concentrations of Trp, Tyr and Phe were spectrophotometrically measured at 260 nm. The concentrations of the other amino acids were determined using the ninhydrin method (Takahashi, 1978).

Results

Figures 1 and 2 show the adsorption isotherms of the dipeptides at pH 7 and 2, respectively. All the isotherms could be expressed by the Langmuir equation:

\[ q = \frac{q_m K C}{1 + K C} \]

where \( q \) is the amount adsorbed [mol/kg], \( C \) is the equilibrium concentration [mol/m³], \( q_m \) is the maximum amount adsorbed, and \( K \) is a constant [m³/mol]. The \( K \) and \( q_m \) values were, in the most cases, evaluated by the least squares method with the Taylor expansion of the equation (Sakoda & Hiromi, 1976). At pH 7, there was a tendency for the dipeptides including the aromatic amino acids to be the most adsorptive, followed by those including the branched ones. On the other hand, dipeptides including Met were the most adsorptive at pH 2, and those with aromatic amino acids were also adsorptive.

The isoelectric points of the tested dipeptides seemed to range between pH 5.2-5.7 (Akamatsu & Fujita, 1992). Because the charge of each peptide depends on pH, its adsorbatibility might be affected by pH. All of the adsorption isotherms of Gly-Phe and Gly-Ile, observed at different pHs and at 25°C, obeyed the Langmuir equation. The estimated \( K \) and \( q_m \) values were plotted against pH in Fig. 3. The \( K \) and \( q_m \) values for Gly-Phe became smaller and larger, respectively, at higher pH. The \( K \) and \( q_m \) values for Gly-Ile were small at a pH near its isoelectric point. We could not obtain a conclusive tendency of the pH dependency for the \( K \) and \( q_m \) values from these experiments.
Discussion

The hydrophobicity of an adsorbate could play an important role in its adsorption onto activated carbon. The logarithm of the partition ratio \( \log P' \) in 1-octanol-aqueous system is a measure of peptide hydrophobicity. An empirical equation correlating \( \log P' \) of a peptide at pH 7 to the sum of the hydrophobic parameter of the side chain substituents \( \Sigma \pi \) and the corrected Dubois steric parameter \( E_s^c \) has been reported (Akamatsu & Fujita, 1992). The \( \log P' \) values of the tested peptides were evaluated using this equation and are shown in Table I. The \( K \) and \( q_m \) values obtained at pH 7 are plotted versus the \( \log P' \) values in Fig. 4. Both the \( K \) and \( q_m \) values seem to be larger for peptides with larger \( \log P' \) values.

A correlation of the \( K \) and \( q_m \) values themselves with the \( \Sigma \pi \) and \( E_s^c \) values was also made and the following equations were obtained:

\[
\log K = 0.311 \Sigma \pi - 0.347 E_s^c(R_0) + 0.251 E_s^c(R_c) + 0.749
\]

(3)

\[
q_m = 0.187 \Sigma \pi + 0.0658 E_s^c(R_0) - 0.00664 E_s^c(R_c) + 0.322
\]

(4)

where \( E_s^c(R_0) \) and \( E_s^c(R_c) \) are the \( E_s^c \) for the side chains of the amino acids at the N- and C-termini, respectively. Figure 5 shows a comparison of the calculated \( K \) and \( q_m \) values with the observed ones. Although the correlations for the \( K \) and \( q_m \) values were not very precise, we could roughly predict the adsorption isotherm at 25°C and at pH 7 of any dipeptide using Eqs. (3) and (4).

Unfortunately, the \( \log P' \) values or \( \Sigma \pi \) and \( E_s^c \) values are available only at pH 7 and 25°C. Therefore, another method should be considered to predict the adsorption of dipeptides under other conditions. The Polanyi adsorption potential theory, which was originally proposed for gas phase adsorption, has been expanded to liquid phase adsorption (Manes & Hofer, 1969; Abe et al., 1979). The volume of adsorbate

![Fig. 4. Correlations between the \( K \) (○) or \( q_m \) (△) values observed at pH 7 and \( \log P' \).](image-url)

![Fig. 5. Comparison of the \( K \) and \( q_m \) values estimated using Eqs. (3) and (4) with those experimentally observed at pH 7.](image-url)

| Peptide   | Mw   | \( V_s \times 10^9 \) [m³/mol] | \( \Sigma \pi \) | \( E_s^c(R_0) \) | \( E_s^c(R_c) \) | \( \log P'^a \) | Solubility\(^a\) | Molar solubility [mol/m³] |
|-----------|------|-------------------------------|----------------|----------------|----------------|----------------|----------------|--------------------------|
| Gly-Gly   | 132.1| 1.38                          | 0.00           | 0.00           | 0.00           | 0.00           | 0.00           | 0.00                     |
| Gly-Ile   | 188.2| 2.27                          | 1.81           | 0.00           | -1.81          | -2.69          | 188            | 611                      |
| Gly-Leu   | 188.2| 2.27                          | 1.81           | 0.00           | -1.44          | -2.57          | 366            | 349                      |
| Gly-Phe   | 222.3| 2.49                          | 1.95           | 0.00           | -0.90          | -2.28          | 274            | 104                      |
| Gly-Trp   | 261.3| 2.83                          | 1.92           | 0.00           | -0.86          | -1.92          | 17.5           | 24.8                     |
| Leu-Leu   | 244.3| 3.16                          | 3.26           | -1.44          | -1.44          | -1.66          | 117            | 88.6                     |
| Leu-Tyr   | 294.4| 3.50                          | 3.01           | -1.44          | -0.90          | -1.93          | 13.5           | 23.4                     |
| Leu-Trp   | 262.4| 3.19                          | 2.42           | -1.03          | -1.44          | -1.91          | 77.0           | 103                      |
| Met-Leu   | 280.4| 3.23                          | 1.22           | -1.03          | -2.26          | 88.1           | 74.6           | 31.3                     |
| Met-Met   | 278.4| 3.38                          | 3.76           | -0.90          | -1.44          | -1.23          | 31.3           | 31.1                     |
| Phe-Leu   | 218.3| 2.61                          | 0.32           | -0.48          | -1.44          | -2.66          | 141            | 130                      |
| Ser-Leu   | 294.4| 3.50                          | 3.01           | -1.44          | -1.44          | -2.66          | 141            | 130                      |

\(^a\)log \( \log P' = 0.943 \Sigma \pi + 0.550 E_s^c(R_0) + 0.307 E_s^c(R_c) + 0.135 I_t + 0.375 I_s + 0.654 I_k + 1.584 I_t - 3.388 \) where \( I_t \), \( I_s \), and \( I_k \) are indicator variables, zero or unity, depending on the absence or presence, respectively, of tyrosine, tryptophan, methionine, and serine (Akamatsu & Fujita, 1992).

\(^a\)The solubilities of Gly-Ile and Gly-Phe at pH 3, 4, 5 and 6 were 1100, 1060, 1080 and 1060, and 199, 194, 195 and 168 mol/m³.
adsorbed $W$ is a function of $\varepsilon / V_s$, where $\varepsilon$ is the adsorption potential and $V_s$ is the molar volume of the adsorbate in the solid state on the adsorbent’s surface during liquid phase adsorption (Abe et al., 1979). The $W$ value can be related to the $q$ value by

$$W = qV_s$$

and the adsorption potential $\varepsilon$ can be estimated from the saturated solubility of the adsorbate $C_{sat}$:

$$\varepsilon = RT\ln(C_{sat} / C)$$

where $R$ is the gas constant and $T$ is the absolute temperature. Although this function depends on a combination of adsorbent and adsorbate, we examined the applicability of the function determined for amino acids at 25°C and pH 7 to adsorption isotherms of dipeptides under various conditions.

The amounts adsorbed $q$ and equilibrium concentrations $C$ measured for amino acids at 25°C and pH 7 were converted to $W$ and $\varepsilon$ using Eqs. (5) and (6), respectively, and then the $W$ and $\varepsilon / V_s$ values are plotted in Fig. 6. The molar volumes of the amino acids at the boiling point estimated according to Kopp’s rule (Sato, 1975) were used as the $V_s$ values. The plots were roughly on a curve except for Pro. The function relating $W$ and $\varepsilon / V_s$ was empirically formulated as follows:

$$\log W = -4.79 - 1.38 \times 10^{-8} (\varepsilon / V_s > 9 \times 10^7)$$

and

$$\log W = 3.58 - 1.75 \times 10^{-3} (\varepsilon / V_s \leq 9 \times 10^7)$$

Applicability of the Polanyi adsorption potential theory to the adsorption of dipeptides under different conditions was examined by comparing the experimentally observed amount adsorbed $q$ with that calculated from the function prepared using amino acids. The saturated solubilities of the dipeptides, which are necessary for the calculation of $\varepsilon$ by Eq. (6), are listed in Table I. The $V_s$ values of the dipeptides were evaluated according to Kopp’s rule and are listed in Table I. The comparison is shown in Fig. 7. The plots include the data shown in Figs. 1, 2 and 3. There was a fairly good correlation between the $q$ values observed and those predicted. The standard deviation was 0.21. Therefore, the Polanyi adsorption potential theory would be applicable to predict the amount of any dipeptide adsorbed onto an activated carbon under any condition with rough precision using the adsorption data of amino acids onto the adsorbent, although there are some problems. The theory is, in principle, applicable to a multi-layer adsorption. On the other hand, the experimentally observed adsorption isotherm of each dipeptide obeyed the Langmuir equation which presupposes a mono-layer adsorption. Thus, the application lacks strictness in a physical sense. Furthermore, it is limited to a single component system. Much effort is required to fully understand the adsorption of a peptide onto activated carbon in a single-component system and to predict the adsorption isotherm in a multi-component system.

**References**

Abe, I., Hayashi, K., Kitagawa, M. and Urahata, T. (1979). Application of the Polanyi adsorption potential theory to adsorption of surfactants from aqueous solution on activated carbon. _Chem. Lett._, 1517-1520.

Adachi, S., Kimura, Y., Murakami, K., Matsuno, R. and Yokogoshi, H. (1991). Separation of peptide group with definite characteristics from enzymatic protein hydrolysate. _Agric. Biol. Chem._, 55, 925-932.

Adachi, S., Yamanaka, T., Hayashi, S., Kimura, Y., Matsuno, R. and
Yokogoshi, H. (1993). Preparation of peptide mixture with high Fischer ratio from protein hydrolysate by adsorption on activated carbon. Bioseparation, 3, 227-232.

Akamatsu, M. and Fujita, T. (1992). Quantitative analyses of hydrophobicity of di- to pentapeptides having un-ionizable side chains with substituent and structural parameters. J. Pharm. Sci., 81, 164-174.

Fischer, J.E., Rosen, H.M., Ebeid, A.M., James, J.H., Keane, J.M. and Soeters, P.B. (1976). The effects of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery, 80, 77-91.

Li, Y., Ye, L. and Sui, P. (1985). Study on the extraction of amino acids from the waste water of vermicelli production. Chin. J. Ind. Microbiol., 10(4), 20-22.

Manes, M. and Hofer, L.J.E. (1969). Application of the Polanyi adsorption potential theory to adsorption from solution on activated carbon. J. Phys. Chem., 73, 584-590.

Nagatomo, S., Hirano, K. and Naoki, H. (1990). Production of functional protein from soybean. In “Practical Bioreactors,” ed. by Research Association for Development of Bioreactor Systems for Food Industries, Shokuhin Kagaku Shinbunsha, Tokyo, pp. 148-165.

Narita, K. (1979). “Databook for Biochemistry I,” ed. by Japanese Society for Biochemistry, Tokyo Kagaku Dojin, Tokyo, pp. 31-32.

Sato, K. (1975). “Bussei Josu Suisanho (8th ed.),” Maruzen, p. 150.

Sakoda, M. and Hiromi, K. (1976). Determination of the best-fit values of kinetic parameters of the Michaelis-Menten equation by the method of least squares with Taylor expansion. J. Biochem., 80, 547-555.

Takahashi, S. (1978). Sodium borohydride as a reducing agent for preparing ninhydrin reagent for amino acid analysis. J. Biochem., 83, 57-60.

Ueno, S., Sakai, K. and Soga, T. (1990). Hydrolysis of fish protein. In “Practical Bioreactors,” ed. by Research Association for Development of Bioreactor Systems for Food Industries, Shokuhin Kagaku Shinbunsha, Tokyo, pp. 203-222.