繊維の吸着特性の基礎的検討と繊維廃材による吸着剤の設計

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研究代表者　**金澤　等**
（福島大学教育学部 教授）

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　　　研究代表者　　金澤　等　（福島大学教育学部　教授）

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4．研究発表

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4．研究成果　（5頁より）

Adsorption of Dye onto
Silk and Wool Proteins

Hitoshi KANAZAWA and Yuriko MATSUSHIMA

Faculty of Education, Fukushima University,
Sugumichi, Asakawa, Matsukawa-machi,
Fukushima 960-12 Japan
(Received 25 October, 1996)

ABSTRACT

Adsorption of acid dyes onto wool keratin or silk fibroin was investigated using their fiber and powder at 30 ℃ and 70 ℃. Keratin fiber was dyed at 70 ℃ better than at 30 ℃, while fibroin fiber was dyed similarly at two temperatures. The adsorption of the dye on powder kerartin or fibroin seemed to occur mainly on the surface of the polymer. The amount of the adsorbed dye on the protein powder at 30 ℃ was more than that at 70 ℃. The adsorption was influenced by the molecular structure of the dye. The ratio of the role of the ionic bonding and that of intermolecular forces in the adsorption seemed to be changed by the structure of polymer and dyeing temperature.

1. INTRODUCTION

Adsorption of molecules onto polymer materials has been extensively used for removing impurities in water.[1-4] A bacteria (*Escherichia coli* IFO 12734) in water could be removed with the copolymer of several kinds of monomers containing amines.[5] Silk fibroin has the adsorptivity to a metallic ion.[6] Protein fibers such as silk and wool are easily dyed with many kinds of dyes; acid dyes, direct dyes, cationic dyes and vegetable dyes, etc. Dyeing with a mixture of several dyes is very useful for the identification of fibers.[7] When the dyeing is considered to be based on the molecular recognition between dyes and polymer, its essential investigation should give some information on the molecular structure of the polymer. In this paper, the adsorptivity of acid dyes onto proteins was investigated using fiber of wool or silk fibroin and powder of wool keratin or silk fibroin. The results were discussed with reference to the molecular structure of dyes.

2. EXPERIMENTAL

2. 1 Materials

Commercial reagent grade dyes were used after drying *in vacuo* without further purifications. Orange G (OG, C. I. Acid Orange 10 : C. I. 16230) was obtained from Wako Pure Chemical Co. Ltd. Bordeaux Red (BR, Acid Red 17 : C. I. 16180), Azophloxin (AZP, Acid Red 1 : C. I. 18050) and Acid Alizalin Blue B (C. I. Mordant Blue 13 : C. I. 16680) were obtained from Tokyo Kasei Kogyo Co. Ltd. and Crystal Scarlet (CS, Acid Red 44 : C. I. 16250) was from Aldrich Chem.

Co. Ltd. Usual commercial reagents were used without further purifications.

Silk fibroin fiber was prepared by degumming cocoon from Bombyx mori with an aqueous sodium carbonate solution. The fiber was washed with water and dried *in vacuo*. Wool fiber was prepared by washing plane weave fabrics (spurn yarns: 1/48 in warp and weft) of Kansai Iseikatsu Kenkyu-kai with toluene and methanol. Fibroin was dissolved in a saturated aqueous calcium chloride solution. The solution was purified by the dialysis in water using a cellophane tube (UC 27/32; Sanko Jyunyaku Co. Ltd.). Keratin powder was prepared by drying the precipitate obtained in the course of dialysis of carboxymethylated keratin in water.

2. 2 Dyeing

Water (buffer solution of pH 3.0), a dye and fiber or powder of keratin or fibroin was put in an Erlenmeyer flask or a test tube. The dyeing mixture was set in an incubator at 70 °C for 4 h or at 30 °C for 24 h with a shaking rate, 45 times/min. The dye concentration in the residual solution was determined by the spectroscopic measurement. The buffer solution was made with citric acid and sodium hydroxide.

2. 3 Spectroscopy

Visible (VIS) spectra of dye solutions were recorded by a Shimadzu UV 2200 spectrophotometer.

3. RESULTS AND DISCUSSION

Molecular structures of the dyes are given in Fig. 1.

Relation between dyeing time and amount of dye on fiber was examined in the dyeing of fiber of wool or fibroin at 30 °C and 70 °C. The results suggested that the amount of the adsorbed dye on the fiber was almost constant after 15 h at 30 °C and after 2 h at 70 °C. Thus, the dyeing was carried out for 24 h at 30 °C and for 3 h at 70 °C.
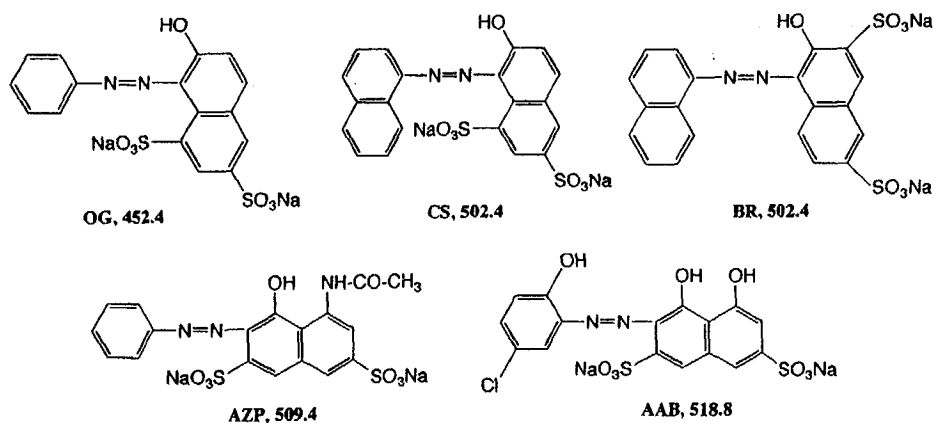

Fig. 1 Structure of dye (numbers represent molecular weight).

Fig. 2 gives the amount of each dye adsorbed on fiber of wool or fibroin. The adsorption of acid dyes on the fiber is considered to be made through the ionic bond and intermolecular force between the dye and the fiber. The ionic bonding does not largely depend on temperature, while the bonding through intermolecular force should be promoted at higher temperature. In the course of dyeing of wool fiber, scales should prevent the diffusion of dyes into the inner part of the fiber.[8,9] In the present result, the amount of each dye adsorbed on wool at 70 °C is more than that at 30 °C. This should be caused by the effect of scales. The binding of BR to wool is extremely dependent on the temperature, though the difference in molecular structure between BR and CS is only the position of one -SO3 group. The BR molecule seems to be too bulky to diffuse through scales of wool at 30 °C. The other bulky dye, AAB indicated a similar result to BR. On the other hand, the difference in the binding of each dye on fibroin between 30 °C and 70 °C is rather small. This is due to the absence of scales. The amount of OG and CS adsorbed onto the fiber is still less than that of the other dyes; their molecular structures are almost similar to each other. Two -SO3 groups on the naphthalene ring in BR, AZP and AAB are at the same positions to each other, which seems to be preferable for the ionic bonding between -SO3 and cations in the fiber.
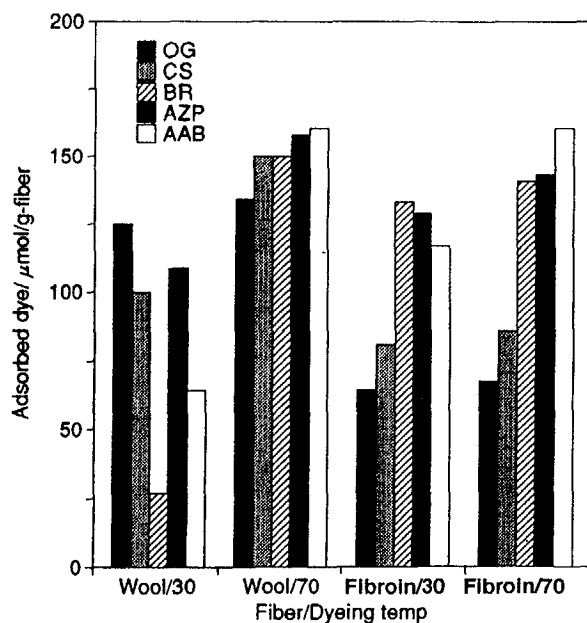

Fig. 2 Amount of dye adsorbed on wool fiber or fibroin fiber in the dyeing for 24 h at 30 °C and for 4 h at 70 °C. [Dye] = 0.331 mmol / l, pH = 3.0 and liquor ratio = 1 / 500.

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Wool keratin powder and silk fibroin powder were prepared to see directly the interaction between dyes and protein molecules. Fig. 3 gives the result. The values in Fig. 3 are still smaller than those in Fig. 2. This suggests that the keratin or fibroin molecules are in the aggregate or crystalline states in the powder, which should make difficult for dyes to diffuse into intermolecular spaces. The amount of adsorbed dye at 30 ℃ is more than that at 70 ℃ in the adsorption of each dye on keratin. This suggests that the ionic bonding plays an important role for the binding between the dye and keratin molecules. The amount of BR adsorbed on keratin powder at 30 ℃ is almost the same as that on wool fiber. This fact suggests that the dye is considered to be adsorbed mainly on the surface of the protein powder.

OG and AZP adsorbed on the fibroin powder more than the other dyes both at 30 ℃ and 70 ℃ (see Fig. 3). On the other hand, OG does not adsorb well on fibroin fiber (see Fig. 2). Both of them do not have two naphthalene rings or a bulky benzene ring with chlorine. The structure is preferable for the ionic bonding between the dye and fibroin molecules. Apparently, large molecular size and the position of $-SO_3^-$ groups in dyes are important for the binding of the dye to fibroin fiber but smaller size is preferable for the binding of dyes to fibroin powder.

Thus, many differences were observed between the adsorprion of dyes on protein fiber and powder.
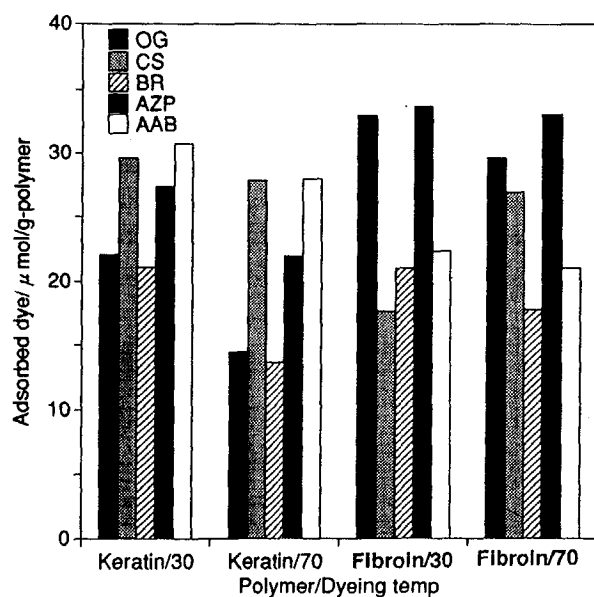

Fig. 3 Amount of dye adsorbed on wool keratin powder
or fibroin powder in the dyeing for 24 h at 30 ℃
and for 4 h at 70 ℃. [Dye] = 0.0486 mmol / l,
pH =3.0 and liquor ratio = 1 / 500.

4. CONCLUSION

The adsorption of acid dyes onto wool fiber is seen to be affected by scales on the fiber, which makes preferable for the dyeing at high temperature. Fibroin fiber showed a good dyeability at 30 ℃ and 70 ℃. In the dyeing of keratin powder, each dye adsorbed on the polymer at 30 ℃ was more than 70 ℃. On the other hand, large differences were not observed between at 30 ℃ and at 70 ℃ in the adsorption of dyes onto fibroin powder. The ratio of the role of the ionic bonding and that of intermolecular forces seemed to be changed by the polymer structure and dyeing temperature. Differences between the adsorptivity of dyes between keratin powder and fibroin powder should be influenced by the molecular interaction between them. In addition, the molecular conformation of polypeptides might influence the binding of dyes. Investigation with polypeptides of simple structure should be necessary to reveal the molecular interaction.

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Convenient Method for the Estimation of
Vapor Pressure of
Some Organic Compounds

Hitoshi KANAZAWA

Faculty of Education, Fukushima University,
Sugumichi, Matsukawa-machi, Asakawa,
Fukushima 960-12, Japan
(Received 11 April, 1997)

Abstract

Relation between temperature and vapor pressure for organic compounds was investigated and the curve fitting for the data was carried out. The vapor pressure (P/mmHg) fitted well to the polynomials of the fifth degree in temperature (t/℃). The values of vapor pressure estimated from the fitted equation coincide with those estimated from the Antoine's equation. The present fitting method is convenient to estimate the vapor pressure of organic compounds of which coefficients in the Antoine's equation are unknown.

1. Introduction

The authors studied the sorption of volatile organic compounds onto chemical and natural fibers and found that the fibers were identified by the GC analysis of the adsorbed compounds.[1,2] Vapor pressure of organic compounds is considered to be one of the important factors for the discussion of the sorption property. But, vapor pressure of an organic compound at a definite temperature is not always obtained from literatures. Though the Antoine's equation is useful for this purpose, the coefficients in this equation are not given for all of the organic compound. On the other hand, we observed the fading of azo dyes in the reaction with sodium hypochlorite and estimated the equation between the absorbance of the reaction mixture and reaction time by the curve fitting. The initial reaction rate was estimated very easily from the fitted equation.[3] In the present study, the relation between vapor pressure and temperature of organic compounds was investigated using the values in the references and those obtained experimentally to find a convenient method to estimate the vapor pressure.

2. Experimental

2. 1 Reagents

Commercial guaranteed grade of N, N-dimethylformamide (DMF), decane, o-dichlorobenzene (o-DCB), ethanol (EtOH), dioxane, methyl salicylate and dichloro acetic acid (DCA) were refluxed over usual drying agents and fractionally distilled.

2. 2 Vapor Pressure and Data Analysis

Almost values of vapor pressure were obtained from the references.[4-5] Several data were obtained by the measurement of the boiling point at reduced pressure by the Ramsay-Young's method.[6]

Regression analysis of the data was carried out by a computer, Power Macintosh 8100/100 AV using a software.[7]

3. Results and Discussion

In general, vapor pressure of an organic compound at a given temperature can be estimated from the following Antoine's equation,

$$\log(P/\text{mmHg}) = A - \frac{B}{C + t \, (^\circ C)} \tag{1}$$

where A, B and C are constants inherent for each compound. But, we can see these constants for only 50 % of organic compounds in the reference, Kagaku Binran.[4] Therefore, we investigated the relation between vapor pressure and temperature for organic compounds in order to know another estimation method for the vapor pressure.

First, vapor pressures were plotted against temperatures for each of five compounds, EtOH, DMF, o-DCB, dioxane and decane as given in Figures 1-5, where plots are indicated with circles. The curve fitting was examined for these data and it was found that the vapor pressure ($P/$ mmHg) was given by the following polynomial of the fifth degree in temperature ($t/^\circ C$),

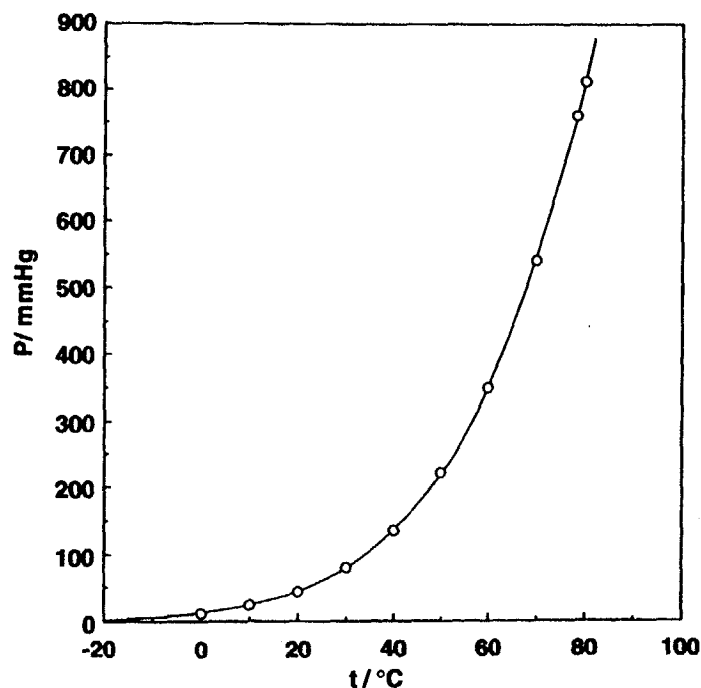$$P = a_0 + a_1 t + a_2 t^2 + a_3 t^3 + a_4 t^4 + a_5 t^5 \tag{2}$$

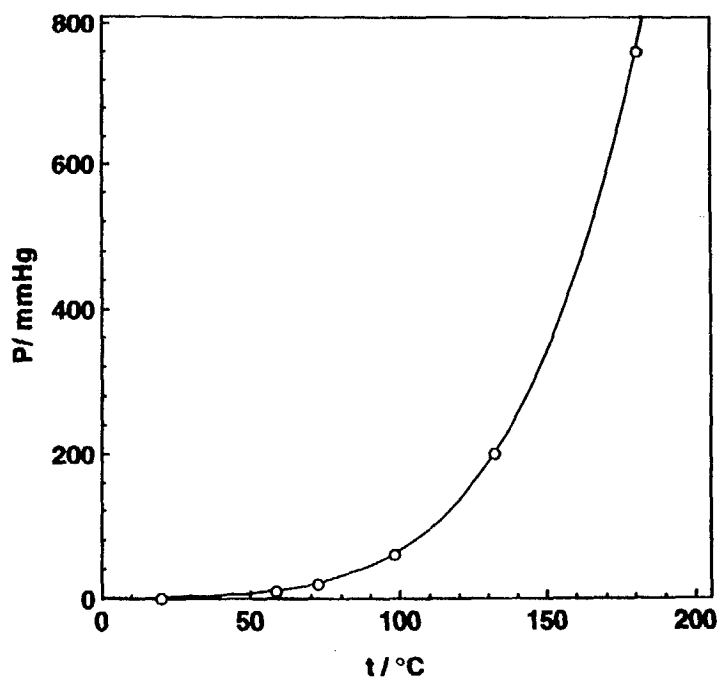Fig.1 Plot between vapor pressure and temperature and the fitted curve with the data for ethanol.

Fig.2 Plot between vapor pressure and temperature and the fitted curve
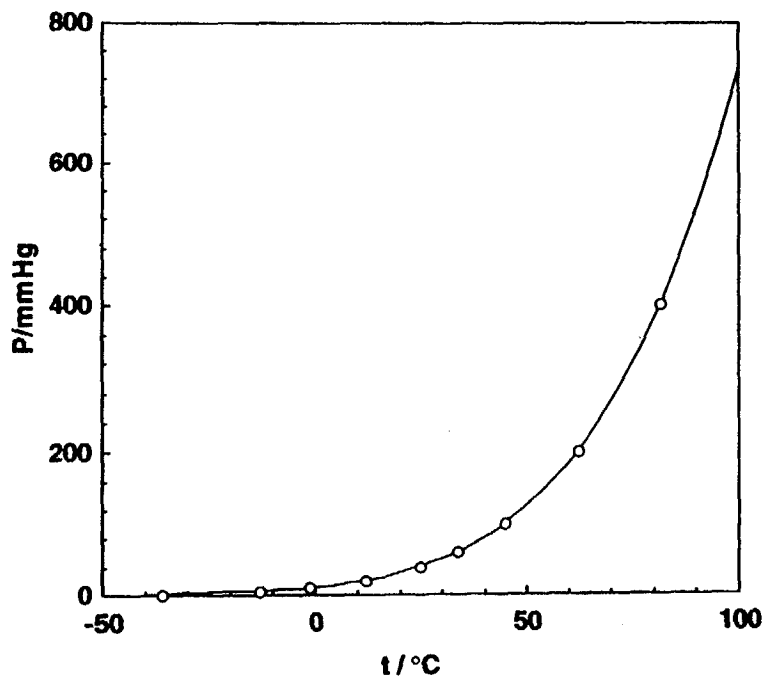with the data for DMF.

Fig.3 Plot between vapor pressure and temperature and the fitted curve
with the data for o-DCB.

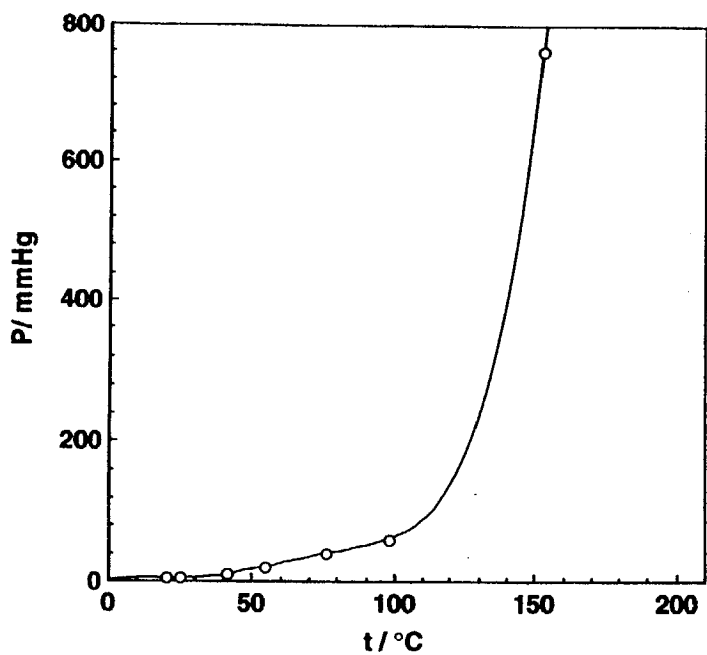Fig.4 Plot between vapor pressure and temperature and the fitted curve
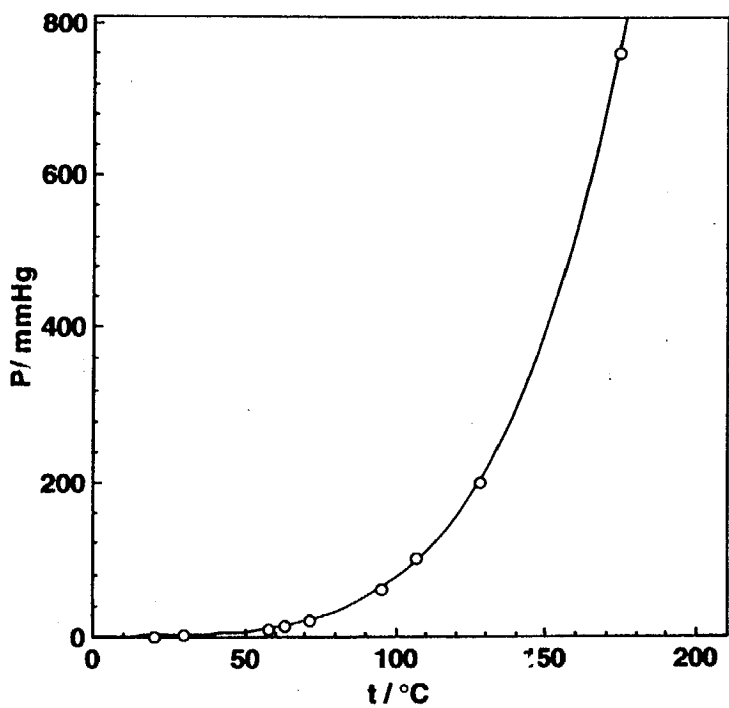with the data for dioxane.

Fig.5 Plot between vapor pressure and temperature and the fitted curve
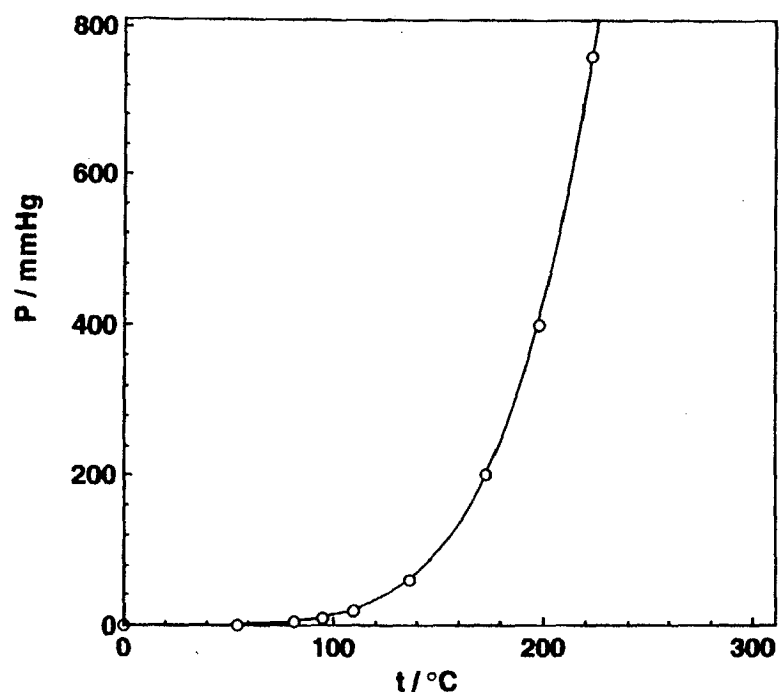with the data for decane.

Fig.6 Plot between vapor pressure and temperature and the fitted curve with the data for methyl salicyate.
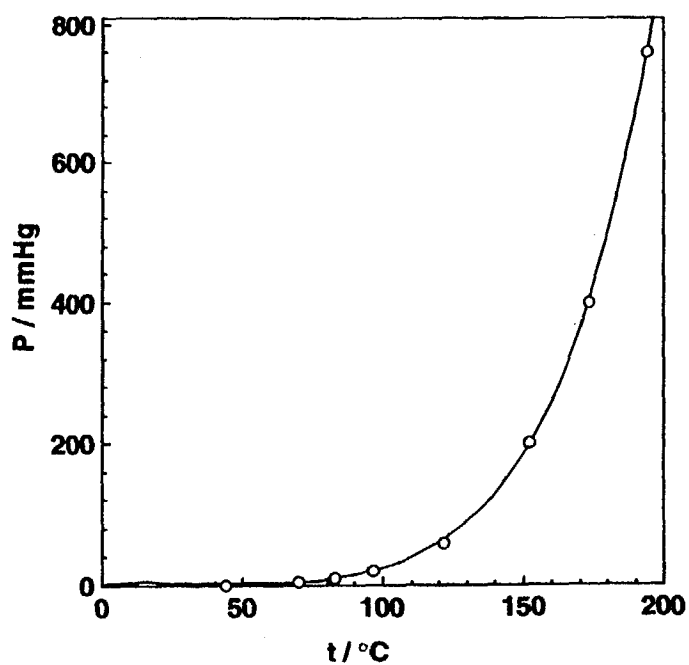

Fig.7 Plot between vapor pressure and temperature and the fitted curve with the data for DCA.

Table 1 Coefficients in fitted equations

Compound	a_0	a_1	$a_2 \times 10^2$	$a_3 \times 10^4$	$a_4 \times 10^6$	$a_5 \times 10^8$	R^2
EtOH	12.02	0.8903	2.389	5.094	4.614	3.853	1.000
DMF	0.1342	0.8413	-6.456	19.658	-21.86	8.445	1.000
o-DCB	0.9793	-0.05810	0.3253	-0.2351	0.3990	0.1982	1.000
Dioxane	10.02	0.5430	1.879	2.616	-0.2129	2.368	1.000
Decane	0.02031	0.1489	-0.7667	1.796	-1.046	0.6126	1.000
Methyl Sal.	-0.004314	0.1015	-0.3815	0.5747	-0.3976	0.2302	1.000
DCA	-0.01276	0.5712	-2.620	4.363	-3.103	1.032	1.000

Table 2 Vapor pressure estimated from the fitted equation (2) and the Antoine's equation (1)

Compound	EtOH	DMF	o-DCB	Dioxane	Decane	Methy Sal.	DCA
P at 25℃ calcd. from (2)	59.30	3.82	1.37	38.80	1.40	0.875	3.60
P at 25℃ calcd. from (1)	59.20	3.76	2.41	35.20	1.30	—	—
P/bp calcd. from (2)*	760/78.3	760/153	760/180.4	760/101.3	760/174.12	759/223.2	760/194.4
P/bp calcd. from (1)	759/78.3	753/153	771/180.4	759/101.3	760/174.12	—	—

$^*P$/bp means vapor pressure (P) at boiling point (bp).

where a_0, a_1, a_2, a_3, a_4 and a_5 are regression coefficients. The fitted equation is indicated by curves in Figs. 1-5. The coefficients and the square of correlation coefficient, R^2 (coefficient of determination) are given in Table 1. In each analysis, R^2 was 1.00. When no data were given in the temperature range where P seemed close to zero, the fitted equation intended to give minus value of P. In order to avoid this discrepancy, $P = 0$ at 0 ℃ and presumed P values at low temperatures were used for the curve fitting. The authors examined the sorption of these five compounds onto polymer substances mainly at 25 ℃. Table 2 gives the vapor pressure at 25 ℃ for each compound estimated from the fitted equation. Values of P at 25 ℃ estimated from the Antoine's equation were also given in Table 2. The constants in the Antoine's equation for the five compounds were obtained from the literature.[4] We can see considerable agreement between the values calculated from the fitted equation (2) and those from the Antoine's equation (1). The most reliable value of P should be 760 mmHg at boiling point (bp) for each compound. The vapor pressure at bp for each compound was estimated from the equations (1) and (2). The fitted equation gave the vapor pressure, 760 mmHg for EtOH, DMF, o-DCB, dioxane and decane, while the Antoine's equation gave 753 mmHg and 771 mmHg for DMF and o-DCB, respectively. This fact suggests the present curve fitting is very useful. When the constants in the Antoine's equation are not obtained from the literature, the present method is very convenient for the estimation of P at a given temperture. For instance, the constants in the Antoine's equation for methyl salicylate and DCA are not given but several data of P are given in the literature.[4] The values of P were plotted against temperatures and the curve fitting was made as shown in Figs. 6 and 7. The coefficients of the fitted equations are given in Table 1. These equations give the values of P at 25 ℃ and bp

as given in Table 2.

4. Conclusion

Reference and experimental data of vapor pressure (P/mmHg) of some organic compounds were given by the polynomials of the fifth degree in temperature (t/°C). The fitted equation is very convenient for the estimation of vapor pressure of organic compounds of which constants in the Antoine's equation are unknown.

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Adsorption of Organic Compounds on Proteins and Synthetic Polypeptides

Hitoshi Kanazawa

Faculty of Education, Fukushima University

Abstract

Adsorption of volatile organic compounds such as alcohols, N,N-dimethyl formamide, o-dichlorobenzene, anisole, acetonitrile, etc. on silk proteins wool keratin and synthetic polypeptides was studied. Each of proteins and polypeptides gave its own adsorption property, which seemed convenient for the identification of amino acid residues in the polymer. Methanol and ethanol gave high adsorption property in almost every polymer except poly(L-valine) and poly(L-leucine) because of the high vapor pressure, but the tendency of adsorptionn of seven kinds of alcohols suggests that the molecular interaction between polypeptides and organic compound vapor should play an important role in the adsorption property.

1. Introduction

Adsorption of organic compounds on materials is a very important phenomenon in science or even in our life. But, the mechanism seems to have a lot of unsolved problems. When it is known that proteins have an adsorbability of some odor organic compounds. When usual fibers were exposed to saturated vapor of organic compounds, each fiber gave the inherent adsorption behavior of the compounds. The authors proposed a novel method to identify the kind of fibers by using the adsorption property. [1,2] The present study has been carrying out to reveal the mechanism of the adsorption of usual volatile organic compound on natural proteins. Synthetic polypeptides with several amino acid residues and a silk fibroin model are prepared to discuss essentially the adsorption behavior of organic compounds on proteins. In addition, the present study will head for the design of the materials with the adsorption selectivity of organic compounds.

2. Experimental

2.1 Materials

Fibroin : A buffer solution (pH=9.0), 0.2 M boric acid/hydrochloric acid was prepared ; 50 ml of 0.025 mol/l $Na_2B_4O_7$ solution was mixed with 4.6 ml of 0.1 mol/l HCl solution and the whole volume was increased to 100 ml with water. Cocoon of Bombyx mori was mixed with the buffer solution of 50 times of the volume, and the mixture was boiled for one hour. The obtained solution was used as a sericin solution. The undissolved fiber was rinsed with hot water several times, and dissolved in an aqueous solution of calcium chloride (40 w/v %); the final concentration of fibroin in the solution was 10 w/v %. The undissolved impurities in the aqueous solution of fibroin in calcium chloride was removed by centrifugation. The solution was dialyzed against distilled water overnight on a cellulose membrane tube, 27/32 Lot. 907001 of Sanko Jyunyaku Co.Ltd. The dialyzed solution in the tube was concentrated to about 1/4 volume by an electric fun. The solution was gelated by mixing with HCl solution at pH = 3 - 4. The fibroin gel was freeze-dried. The fibroin powder was obtained by grinding the dried material.

Sericin : The remaining solution of sericin was concentrated to 1/10 volume by an rotary evaporator. Sericin was obtained by the recrystallization with ethanol and washed with water and dried.

Wool keratin : Wool fiber was reduced with 2-mercaptoethanol in an aqueous urea solution. The obtained solution was dialyzed against distilled water on a cellulose membrane tube. Wool keratin was precipitated by recombination between cystine residues in the solution. The precipitant was filtered off, washed and dried.

Synthetic polypeptides : Amino acid N-carboxy anhydrides (Amino acid NCAs) were prepared by the reaction of amino acids with triphosgen and purified by the recrystallization with ethyl acetate and hexane. γ-Benzyl-L-glutamate (BLG) and β-benzyl-L-aspartate (BLA) were prepared by the method reported by Yuki et al. with a slight modification.[3] The NCAs were polymerized in acetonitrile or hexane by butylamine as an initiator. The ratio of each NCA to the initiator was defined as 200. Poly(glycine), poly(L-alanine), poly(L-valine), poly(L-leucine), poly(L-phenylalanine), poly(BLA), poly(BLG) were prepared by the method. Copolymers of L-Ala and Gly were obtained by the reaction of the mixture of L-Ala NCA and Gly NCA by changing the mixing molar ratio of the two NCAs.

Reagents : Guaranteed grade reagents of methanol (MeOH), ethanol (EtOH), *1*-propanol (1-PrOH), 2-propanol (2-PrOH), 1-butanol (1-BuOH), N,N-dimethylformamide (DMF), decane, o-dichlorobenzene (DCB), dioxane, acetonitrile, p-xylene, anisole, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. and used as received.

2.2 Methods

Adsorption of organic compound vapor : Polymer materials were exposed in the vapor

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of volatile organic compounds(alcohols, N,N-dimethylformamide (DMF), o-dichloroben-zene (DCB), dioxane, decane, p-xylene, anisole and acetonitrile at given temperatures for 15 h and the adsorbed portion was extracted in ethyl acetate and analyzed by GC. The time 15 h seemed enough for getting results close to the adsorption equilibrium. The adsorbed or-ganic compounds on polymers were extracted in ethyl acetate at 40 °C three times and the extracts were analyzed by GC. A GC of injector 180°C, that of detector (FID) 200 °C, column initial temperature 70°C, initial time 1 min, raising rate 15 °C/min and final tempera-ture 145 °C. Capillary columns, a Shimadzu CBP1-M25-0.25 and a J & W Scientific DB624-M30-0.25-film thickness 0.14 μm were used.

3. Results and Discussion

The adsorption of organic compounds were different between the adsorption from the vapor mixture of organic compounds and that from a single vapor of each compound on polymers. Fig.1 gives the result for the adsorption of mixture of five organic compounds onto sericin, fibroin and keratin. Ethanol was adsorbed well on each of proteins. Keratin gives the adsorption maximum of ethanol though the other compounds did not adsorb so much.
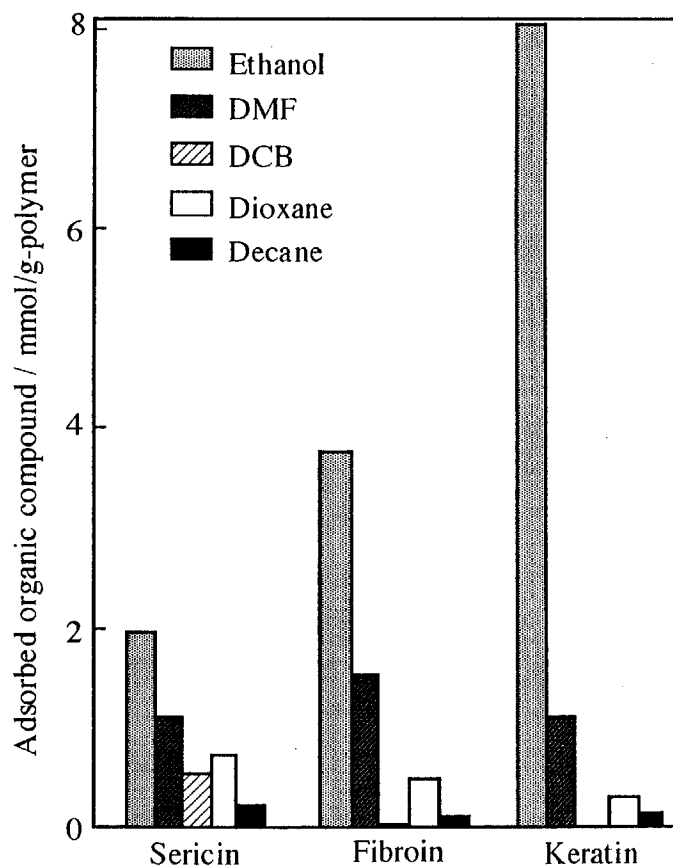

Fig.1 Adsorption of organic compounds from
their mixture onto protein powder at 25 °C for 25 h.

Fig.2 gives the result for the adsorption of eight compounds onto seven synthetic polypeptides with single amino acid residue (homopolymer) . It is remarkable that the adsorption tendency of the organic compounds on these polypeptides is different from each other. This suggests that the structure of polypeptides could be estimated by the adsorption tendency of organic compounds . In general, the identification of amino acid residues in polypeptides is very difficult by usual measurements.
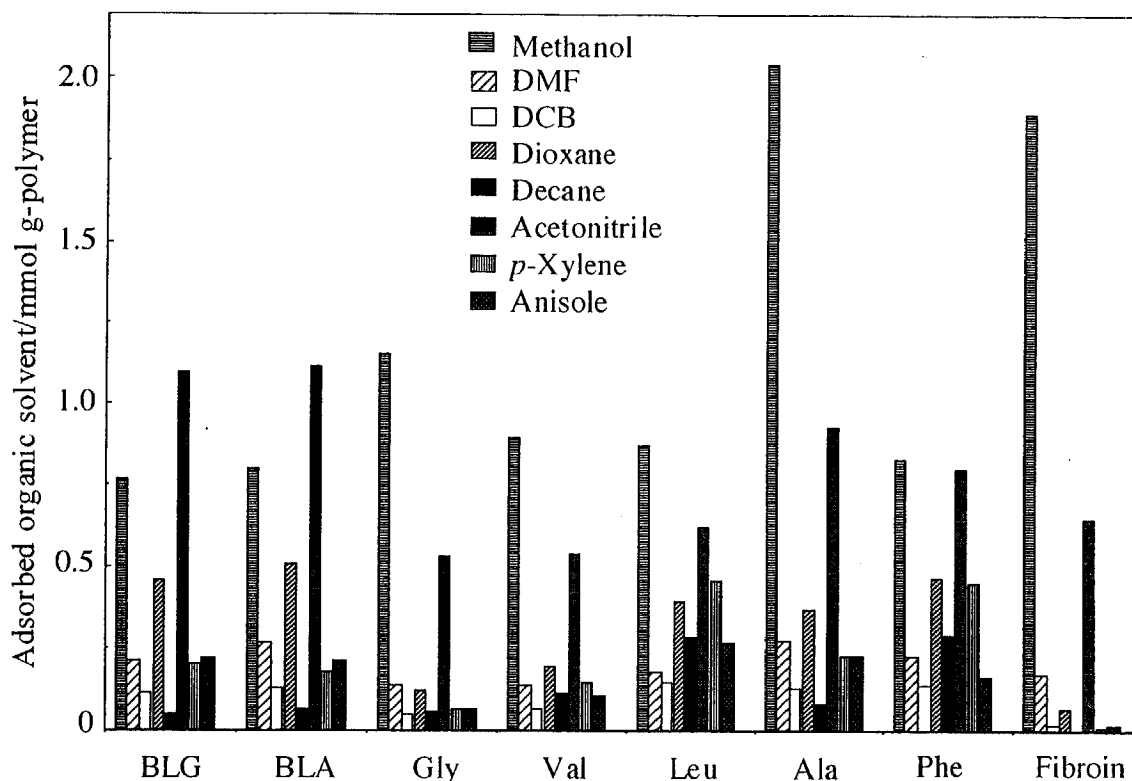

Fig.2 Adsorption of volatile organic compounds from their mixture onto polypeptides and fibroin-powder at 25°C at for 25 h.

As the above results gave the high adsorption of methanol and ethanol, the adsorption of five kinds of alcohols onto proteins and synthetic polypeptides. Methanol and ethanol adsorbed well to fibroin powder and sericin powder. The powder gave higher adsorbability of them than fibers. This is cosidered from the difference in surface area between powder and fiber. Fig.3 gives that fibroin fiber adsorbed organic compounds more than cotton fiber. Fig.4 gives the result for seven polypeptides in the adsorption from single alcohol. Methanol adsorbed well on almost of the polymer, but, only poly(L-leucine) and poly(L-valine) adsorbed 2-propanol more than methanol. This suggests that the interaction between isopropyl groups of the polymer and the alcohol is important. The effect of high vapor pressure of methanol should affect the adsorption results, but the obtained result suggests that the adsorption is affected by not only vapor pressure of adsorbates (volatile organic compounds in the presen case) but by the interation between the polymer and the organic compounds. The effect of vapor pressure of the compounds will be investigated soon.
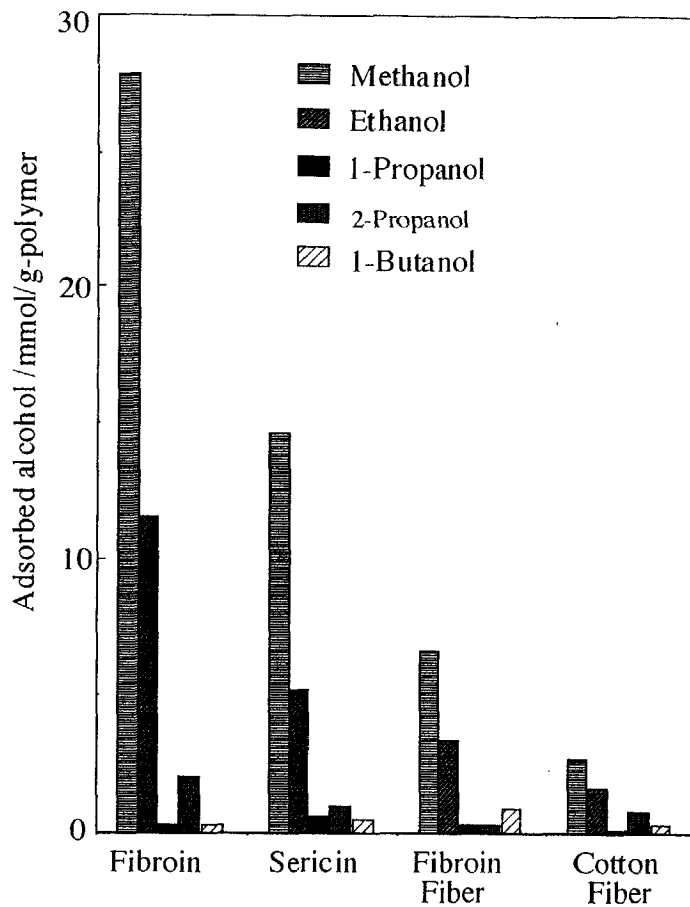
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Fig.3 Adsorption of alcohol vapor from each alcohol alone onto silk fibroin powder, silk sericine powder, silk fibroin fiber and cotton fiber at 25°C for 25 h.
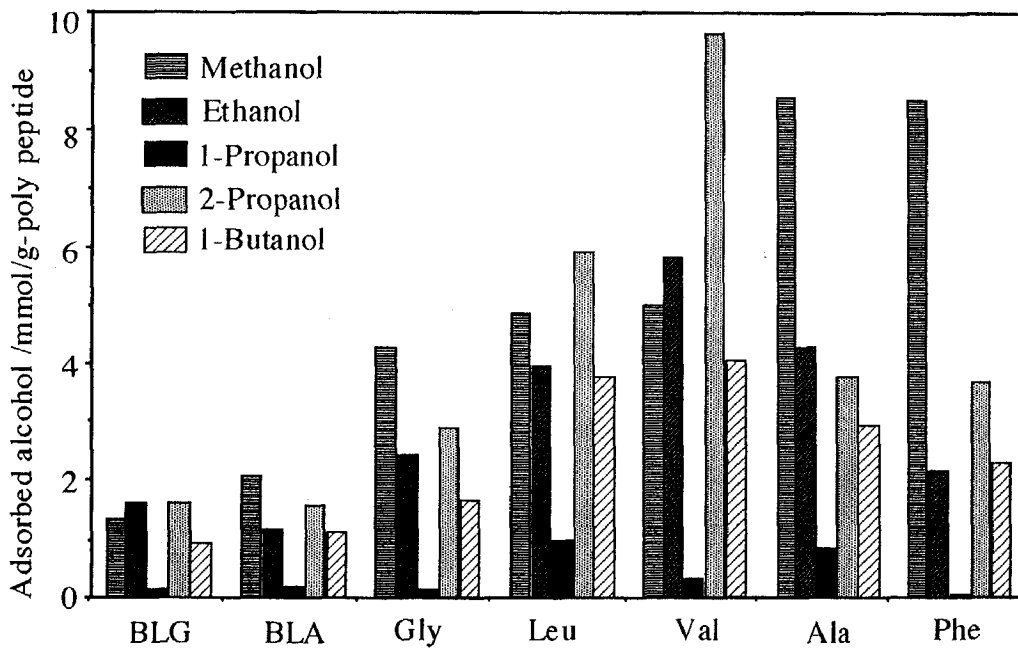

Fig.4 Adsorption of alcohols on synthetic polypeptides at 25°C for 25 h. Each alcohol was used without mixing with the other compounds.

Each of proteins and synthetic polypeptides gave its own adsorption tendency of organic compounds, respectively. Even small differences in side chains of amino acid residues in polypeptides seemed to be reflected in the results. This characteristic absorption pattern can be available for the estimation of the structure of polypeptides. More essential investigation of the present adsorption system will be continued; the the techniques in the physical chemistry will be examined. The results with proteins, cellulose and synthetic polypeptides, chemically-modified proteins will be compared with each other.

The role of hydroxyl groups in L-serine and L-tyrosine in fibroin will be investigated in detail using several kinds of synthetic polypeptides. The application of the adsorption pattern for the identification of the structure polypeptides will be studied still more.

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Adsorption of Amino Acids to Proteins and Protein Models

Hitoshi KANAZAWA, Mina AKAMATSU and Tetsuo ONAMI*

Faculty of Education, Fukushima University
Matsukawa-machi, Fukushima 960-1296, Japan
*Department of Chemistry, Fukushima Medical College,
Hikarigaoka, Fukushima 960-1295, Japan
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ABSTRACT

Adsorption of amino acids to silk fibroin and synthetic polypeptides was studied. Fine-cut silk fibroin fiber adsorbed preferably L-lysine but fibroin-coated silica gel gave a selective adsorption of L-aspartic acid and L-glutamic acid. Poly(Gly) and poly(L-Ala) gave the similar result to fibroin-coated silica gel. The molecular conformation or surface property of fibroin in these materials were considered to be different from each other. Copolypeptide containing L-Ala, L-Ser, and O-benzyl-L-Ser residues adsorbed all of the examined amino acids.

1. INTRODUCTION

The adsorption of organic compounds to materials is a very important phenomenon in science or even in our life. But, the mechanism seems very complicated and have a lot of unsolved problems. Protein fibers are empirically known to adsorb some odorous substances. The authors studied the adsorption of various organic compounds to proteins such as silk fibroin, silk sericin and wool keratin,[1-6] and found that proteins gave the characteristic adsorbability of amino acids. In the present article, the adsorption property of amino acids to silk fibroin materials such as fine-cut fibroin fiber, fibroin powder and fibroin coated silica gel was investigated. As 99.6% of amino acid residues in silk fibroin is composed of only four amino acids of glycine (content 42.8%), L-alanine (content 28.3%), L-serine (content 14.7%) and L-tyrosine (content 13.8%),[7] the polypeptides containing these amino acid residues should be useful for the fibroin model. Thus, poly (Gly), poly (L-Ala) and copolypeptide of L-Ala and L-Ser were prepared and their adsorption property of amino acids was compared with that of fibroin materials.

2. EXPERIMENTAL

2. 1 Materials

Commercial reagent grade organic solvents were refluxed over general drying agents and

distilled fractionally before use. The other reagents were used without any purifications.

Fibroin powder: A buffer solution, M/5 boric acid/hydrochloric acid was prepared; 50 ml of 0.025 mol/l Na₂B₄O₇ solution was mixed with 4.6 ml of 0.1 mol/l HCl solution and the whole volume was increased to 100 ml with water. Cocoon of Bombyx mori was mixed with the buffer solution of 50 times of the volume, and the mixture was boiled for one hour. The resulting fibroin fiber was washed sufficiently with water. The fibroin was dissolved in a solution of calcium chloride in water and ethanol (the ratio, 8:2:1). After removing contaminations by the centrifugation, the fibroin solution was dialyzed overnight in water and it was concentrated until the volume became 30% of the original one by a fun. When an aqueous solution of hydrochloric acid was added into the concentrated fibroin solution until pH was 3.5, the gelation occurred. The gel was freeze-dried and fiboroin powder was obtained.

Fine-cut fibroin fiber: Fibroin fiber was washed in the same manner as the above. The fiber was dried and mixed with an aqueous sodium carboxymethyl cellulose (CMC) solution (fiber 0.5g, water 20ml and CMC 6 g). The mixture was washed with methanol and dried in vacuum. The CMC solid containing fiber was obtained. The solid was cut finely with a powder mixer (Yamamoto Denki Co. Ltd., Y308B). The fine-cut solid was washed in boiling water and dried in vacuum. The length of the fine-cut fibroin fiber was 50 μm-150 μm.

Fibroin coated silica gel: Silica gel (diameter 5 μm;Silica-150, Toso Corpo.) was dispersed in a fibroin solution (concn.:10 wt%) at 1/100 volume ratio and the mixture was stirred. The fibroin coated silica gel was put into ethanol and stirred. The filtrated solid portion was put into a mixture of chloroform and hexane and the fibroin coated silica gel was obtained as a precipitated portion.

Polypeptides: N-Carboxy amino acid anhydrides (amino acid NCAs) were synthesized by the reaction between amino acids and triphosgen, and recrystallized from a mixture of ethyl acetate and hexane. Polypeptides were prepared by the polymerization of amino acid NCAs in acetonitile with butylamine as an initiator at 40 ℃ for 14 days. Poly (L-Ala) and poly (Gly) were prepared using the corresponding NCA, respectively. The polymer was hydrolyzed in the manner described later.

2. 2 Adsorption of amino acids

Proteins or synthetic polypeptides were packed in stainless columns (i.d. 4 mm, length 150 mm) for the LC use, and the aqueous solution of eight kinds of amino acids, Gly, L-Ala, L-Val, L-Leu, L-Phe, L-Asp, L-Glu and L-Ser was passed through the column. The fractions were collected at given times and were analyzed by the Shimadzu amino acid LC system (which was composed of Shimadzu Liquid Chromatograph LC10-AS, System Controller SCL-10A, Column Oven CTO-10A, Auto Injector SIL10A and Fluorescence Detector RF-10AL).

2. 3 NMR

Nuclear magnetic resonance (¹H NMR) spectra were recorded on JEOL JNM-EX270

distilled fractionally before use. The other reagents were used without any purifications.

Fibroin powder: A buffer solution, M／5 boric acid／hydrochloric acid was prepared; 50 ml of 0.025 mol／l Na$_2$B$_4$O$_7$ solution was mixed with 4.6 ml of 0.1 mol／l HCl solution and the whole volume was increased to 100 ml with water. Cocoon of Bombyx mori was mixed with the buffer solution of 50 times of the volume, and the mixture was boiled for one hour. The resulting fibroin fiber was washed sufficiently with water. The fibroin was dissolved in a solution of calcium chloride in water and ethanol (the ratio, 8:2:1). After removing contaminations by the centrifugation, the fibroin solution was dialyzed overnight in water and it was concentrated until the volume became 30% of the original one by a fun. When an aqueous solution of hydrochloric acid was added into the concentrated fibroin solution until pH was 3.5, the gelation occurred. The gel was freeze-dried and fiboroin powder was obtained.

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2. 3 NMR

Nuclear magnetic resonance (¹H NMR) spectra were recorded on JEOL JNM-EX270

spectrometer, and were recorded in trifluoroacetic acid-d_1. Tetramethyl silane (TMS) was used as internal standard;chemical shifts are denoted in ppm downfield to TMS.

3. RESULTS AND DISCUSSION

Aqueous solutions of the mixture of amino acids were passed through the column packed with fine-cut fibroin fiber, fibroin powder or fibroin-coated silica gel. The pH of the amino acid mixture was 5.5. Tables 1 and 2 give the amount of adsorbed amino acids calculated from the concentration of amino acids contained in the fraction at flow volnme of 5.5 ml or 20.5 ml. Fine-cut fibroin fiber adsorbed L-lysine more than the other amino acids. Fibroin powder gave the similar result to the fine-cut fibroin fiber. On the other hand, fibroin-coated silica gel adsorbed completely L-aspartic acid and L-glutamic acid. The molecular conformation or surface structure of fibroin in both adsorbents are considered to be different from each other.

Table 1   Adsorption of amino acids to fibroin fiber

Amino acid L- or gly	Concn. of original solution $\times 10^5$ mol/l	Concn. of amino acid in fraction at 5.5ml flow volume $\times 10^6$ mol/l	Adsorbed amino acid mol%
Asp	1.6	4.27	27
Ser	1.6	8.00	0
Glu	1.6	5.69	15
Gly	1.6	7.65	4.2
Ala	1.6	7.74	0.4
Val	1.6	7.95	2
Leu	1.6	7.43	4.5
Phe	1.6	8.03	1
Lys	1.6	0.34	95

Used fibroin fiber: 0.123g
Adsorbed amount of L-Lys was estimated as 8.9×10^{-7} mol/g

Table 2   Adsorption of amino acids to fibroin-coated silica gel

Amino acid L- or gly	Concn. of original solution $\times 10^6$ mol/l	Concn. of amino acid in fraction at 20.5ml flow volume $\times 10^5$ mol/l	Adsorbed amino acid mol%
Asp	8.00	0.00	100
Ser	8.00	7.14	0
Glu	8.00	0.00	100
Gly	8.00	8.00	0
Ala	8.00	8.00	0
Val	8.00	8.00	0
Leu	8.00	8.00	0
Phe	8.00	0.70	2.5
Lys	8.00	0.73	24

Used fibroin-coated silica gel: 1.84g
Adsorbed amount : L-Glu over 6.5×10^{-8} mol/g, L-Asp over 6.50×10^{-8} mol/g

passing through sulfuric acid. The HBr was introduced in the poly (L-Ala-O-benzyl-L-Ser) solution in TFA at about 0 ℃ for six hours. Subsequently, the reaction mixture was stirred for six hours at 20℃. The reaction mixture was concentrat..d by the distillation at reduced pressure until the volume became one third of the original one. The concentrated reaction mixture was poured into large amount of ethanol and the precipitate was collected and dried under vacuum. The NMR spectra of poly (L-Ala-O-benzyl-L-Ser) and the hydrolyzed one were compared to each other. Figures 1 and 2 give the spectra. Signals at 7.4 ppm and at 1.58 ppm are due to the hydrogens in benzene rings and methyl groups, respectively. The ratio of intensity of signal at 7.4 ppm to that at 1.58 ppm gives the molar ratio of benzyl group to methyl group in the polymer. Fig. 1 gave the ratio 1.194 and Fig. 2 0.684. The ratio 0.684/1.194 should give the molar ratio of benzyl groups in the copolymer after and before the hydrolysis. Thus, 43% of O-benzyl groups was estimated to be converted to hydrogen in the copolymer by the hydrolysis. In the present article, the partially converted polymer is given by poly (L-Ala-L-Ser-O-benzyl-L-Ser) for convenience.

The adsorption of amino acids was examined with poly (L-Ala-O-benzyl-L-Ser) and poly (L-Ala-L-Ser-O-benzyl-L-Ser). But, the difference in the adsorption property was not seen between both polymers. They adsorbed each amino acid similarly. Thus, L-Ser residues do not seem to play an important role in the adsorption property of silk fibroin. Further investigation will be necessary to discuss the adsorption of amino acids in more detail.
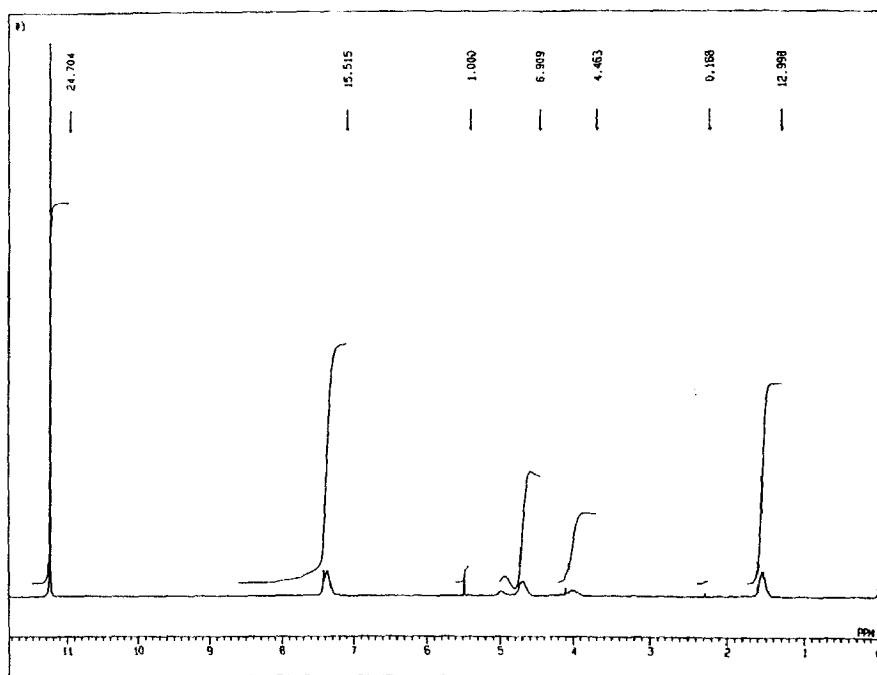

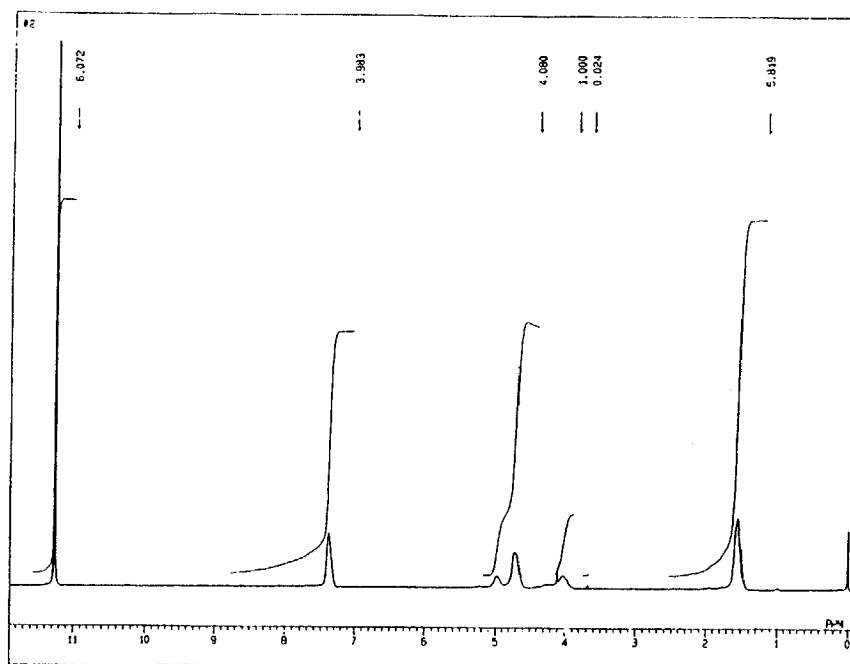**Fig.1** NMR spectrum of poly (L-Ala-O-benzyl-L-Ser).

Fig.2 NMR spectrum of poly (L-Ala-L-Ser-O-benzyl-L-Ser).

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６．まとめ

　　本課題研究を始める以前に、各種繊維に対する揮発性有機化合物の吸着を検討した結果、タンパク質繊維の吸着能が高いことが注目された。そこで、絹フィブロイン、絹セリシンおよび羊毛ケラチンの各タンパク質繊維の吸着特性を、合成ポリペプチドと比較しながら、吸着を決定する要因について、系統的な検討を行い、選択的な吸着剤の可能性を追究した。なお、タンパク質繊維は、一度、溶解してから再生して粉末状とすることにより、表面積が大きくなり、吸着量が大きくなることがわかった。繊維の場合でも、それらを適当にカットすることによって、吸着剤として扱いやすくなる。従って、本研究におけるタンパク質繊維は、繊維廃材の再利用でも充分に利用できることになる。吸着の系統的な研究例は全く見られないために、引用すべき文献がない。その意味では、独創性は高いと思われる。吸着を決定する因子を挙げるためには、種々の吸着現象を観察して、多くの実験データを集めて、整理・解析する必要があった。そのために、多くの時間を要した。

　　吸着については、（１）水中での色素、（２）気相での有機化合物蒸気、（３）水中のアミノ酸、（４）水中の有機化合物について検討した。以下に、個々について総括する。

１）「水中での色素の吸着」

　　羊毛と絹について、繊維、再生粉末の両者について構造の異なる５種の酸性染料の吸着を検討した。羊毛繊維では、高温（７０℃）方が低温（30℃）よりも、吸着速度が大きいが、一方、絹繊維では、高温と低温における吸着速度の差異は明瞭に見られなかった。これは、羊毛繊維の表面のキューティクル層への色素の拡散が律速であるためと考えた。事実、これらの繊維を溶解して得られた再生粉末タンパク質を用いた結果、３０℃と７０℃での吸着速度の差は少ないが、３０℃での方が、少し吸着量が多いと見られた。これは、酸性染料とタンパク質との結合力は、主にイオン結合であり、温度の効果が認めにくいことによると解釈した。従って、羊毛をタンパク質源として用いる場合、キューティクル層を除去しないと、本質的な、アミノ酸残基の効果は見られないと考えた。なお、染料の構造による吸着特性の差は明瞭に見られ、吸着にはタンパク質分子の構造と染料の分子構造の間の相互作用が反映することがわかった。色素について、スルホンサン基が同じ側に２個あり、立体障害の少ない構造が吸着しやすいと見られた。詳細なアミノ酸残基の組成による違いは本質的な因子であると予想される、この段階では、論議できなかった。

２）「気相での有機化合物の吸着」

　　蒸気汎用の揮発性有機化合物の飽和蒸気に、タンパク質および合成ポリペプチドを曝して、吸着特性の差異を比較した。セリシン、フィブロイン、ケラチンの各タンパク質の吸着量には大きな違いが認められたが、吸着しやすい有機化合物を順に並べた結果（これを、「吸着傾向」とする）には、大きな差異は見られなかった。但し、セリシンのみが、ジクロルベンゼンを吸着することが見られた。７種の合成ポリペプチ

ドに対する8種の有機化合物の吸着では、ポリペプチドがそれぞれ、同様の粉末であるため、一律的な比較は可能である。ポリペプチドの多くは、アモルファスであり、β-構造は、ポリ（グリシン）、ポリ（L-アラニン）、ポリ（L-バリン）、ポリ（BLA）に見られ、その他は、αらせん構造が主である。これらの、分子形態の違いによる結果の両極化は認められない。例えば、ポリ（L-バリン）とポリ（L-ロイシン）の分子形態は、β構造とαらせんであるが、吸着傾向および吸着量は互いに類似した。Fig.2の結果は、ポリアミノ酸の吸着傾向には、組成アミノ酸残基の分子構造が極めて支配的因子となることを示唆している。このような吸着傾向のデータを保有することによって、吸着データから、ポリペプチドのアミノ酸組成について、推定可能となることがわかった。

なお、揮発性有機化合物の所定温度における蒸気圧を知る必要がある場合、データを容易に捜せないことがある。所定のデータを基に、温度と蒸気圧の関係について数値処理を行った結果、数式に当てはめて求められることができた。この報告書に掲載した（p. 10-16）。

3）「水中のアミノ酸の吸着」

アミノ酸混合物の水溶液から、特定のアミノ酸を吸着・分離できる可能性を求めた。その結果、絹フィブロイン粉末および細断繊維のみが、アミノ酸混合物からリジンを選択的に吸着することがわかった。一方。ポリグリシン、ポリ（L-アラニン）などは、グルタミン酸やアスパラギン酸を選択的に吸着することがわかった。この結果は、本課題研究に深く関わるが、別途の研究計画でも行い、その計画の中から特許出願を行った。研究そのものは本質的に、本課題研究の一部となるので、本報告書に掲載した。

4）「水中の有機化合物の吸着」

水中に存在する、微量有機化合物（クロロホルム、アルコール、アセトニトリルなど）のタンパク質による吸着除去の可能性を検討した。本報告書には、データを掲載しないが、再生タンパク質を吸着剤として用いることによって、効果的に吸着除去できることがわかった。この結果は、さらに、系統的な実験を続けてから、提出したい。

以上のように、「吸着」という、身近ではあるが、系統的検討および報告のなされていない現象について、系統的なデータ化を行った。その結果、多くのことがわかり、主題である選択的吸着剤の開発も、幾つか見通しがたった段階にある。引き続いて、蒸気圧、温度などの効果を含めた物理化学的検討を行っており、さらにこの研究課題を発展させている。