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Adsorption of α-Chymotrypsin on Plant Biomass Charcoal

Hidetaka Noritomi[1*], Keito Hishinuma[1], Shunichi Kurihara[1], Jumpei Nishigami[1], Tetsuya Takemoto[2], Nobuyuki Endo[3], Satoru Kato[1]

[1]Department of Applied Chemistry, Tokyo Metropolitan University, Minami-Ohsawa, Hachioji-shi, Tokyo, Japan; [2]Osaka Gas Co., Ltd., Konohana-ku, Osaka, Japan; [3]EEN Co., Ltd., Bunkyo-ku, Tokyo, Japan.
Email: [*]noritomi@tmu.ac.jp

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ABSTRACT

The adsorption of α-chymotrypsin onto plant biomass charcoal (PBC), which was prepared from plant biomass wastes such as bagasse and dumped adzuki beans by pyrolysis, has been examined. The PBC was characterized by SEM, specific surface area, and pore size distribution. The adsorption isotherms were successfully correlated by the Freundlich equation. The amount of α-chymotrypsin adsorbed on PBC was dramatically dependent upon the solution pH and temperature. Maximum adsorptions of α-chymotrypsin on adzuki bean charcoal and bagasse charcoal were observed at weak acidic and near neutral pH, respectively. The amount of α-chymotrypsin adsorbed on PBC decreased with an increase in the concentration of salts. Plots of the amount of α-chymotrypsin adsorbed on PBC versus temperature exhibited an optimum temperature.

Keywords: Adsorption; Characterization; Plant Biomass Charcoal; α-Chymotrypsin; Protein

1. Introduction

The use and application of boimass for renewable resources and energies are one of the most important challenges to establish a recycling society. Recently, much attention has been given to the charcoal prepared from plant biomass wastes in order to use soil amendments, adsorbents, humidity control materials, materials for wastewater treatment, and catalysts [1-6]. However, plant biomass wastes have not sufficiently been recycled yet, compared to other wastes, although an enormous amount of plant biomass waste has been discharged in the world. Moreover, the development in the high value-added function of charcoal derived from plant biomass wastes has been desired.

The adsorption of proteins onto the surface of solids has been studied extensively in the biotechnological, medical, pharmaceutical, and food fields in order to apply it to the immobilization of biocatalyst in the bioreactor, the separation of proteins, and the removal of protein contamination from food and medicine [7,8]. In order to assess the property of plant biomass charcoal (PBC) as a biomaterial, we have so far investigated the interaction between a protein and PBC derived from dumped adzuki beans and so on, when hen egg white lysozyme (HEWL) was used as a model protein, and have found out that PBC effectively adsorbs HEWL, and HEWL adsorbed on PBC exhibits the enhanced storage stability at low temperatures and the excellent thermal stability at high temperatures, compared to those of native HEWL [9-11].

In the present work, to test the generality on the adsorption efficiency of PBC for proteins, we employed bovine pancreas α-chymotrypsin as a model protein, since it is well investigated regarding its structure, functions, and properties [12]. In addition to adzuki bean charcoal and bamboo charcoal, which were used in our previous work [9], bagasse charcoal and wood charcoal have newly been used as PBC. Moreover, we investigated structural characteristics of PBC.

2. Experimental

2.1. Materials

α-Chymotrypsin (EC 3.4.21.1 from bovine pancreas) (type II, 52 units/mg solid) was purchased from Sigma-Aldrich Co. Plant biomass charcoal derived from bagasses was supplied from Osaka Gas Co. Ltd. Plant biomass charcoals derived from adzuki beans, bamboos, and woods were from EEN Co. Ltd. Medicinal carbon was

[*]Corresponding author.

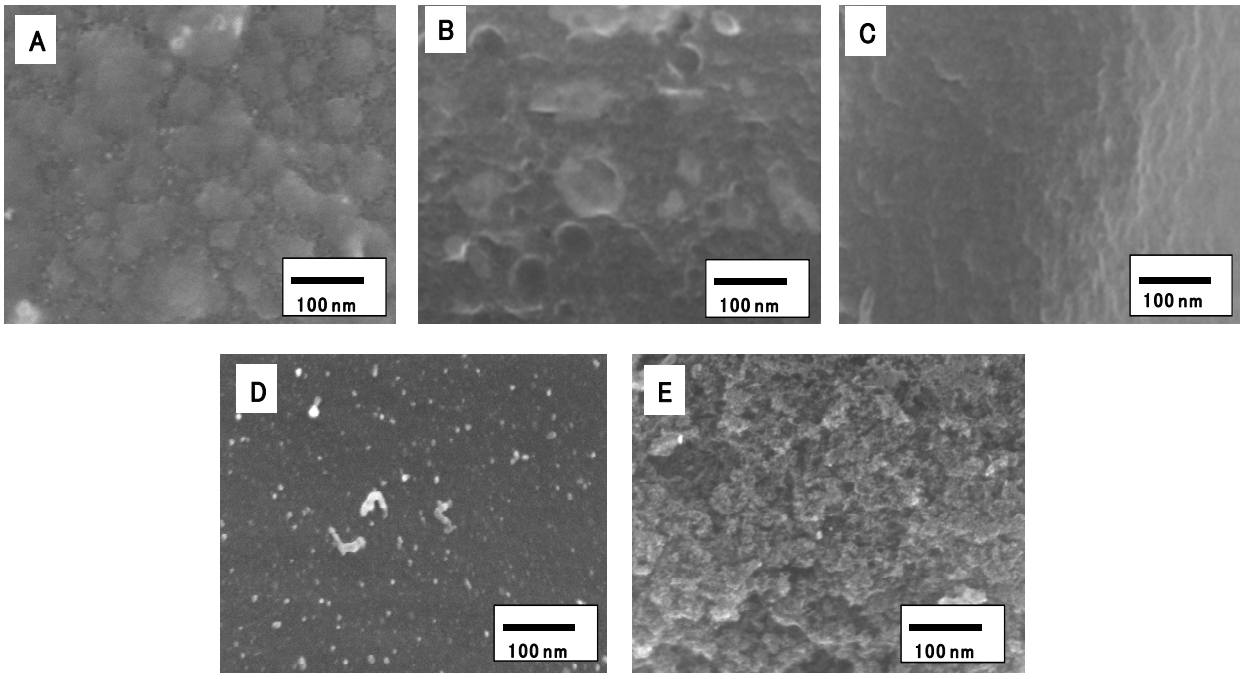

Figure 1. SEM images of (A) bagasse charcoal, (B) adzuki bean charcoal, (C) bamboo charcoal, (D) wood charcoal, and (E) medicinal carbon.

obtained from Nichi-Iko Pharmaceutical Co. Ltd.

2.2. Characterization of Biomass Charcoal Powder

The SEM micrograph was obtained using a scanning electron microscope (JSM-7500F FE-SEM, JEOL Ltd.) operating at 5 or 15 kV. The sample for SEM was prepared on a carbon tape without vapor deposition.

All samples were outgassed at 300˚C for 8 h prior to the nitrogen adsorption measurements. The specific surface area of PBC was calculated with the use of the Brunauer-Emmett-Teller (BET) method using a micropore system (BELSORP-mini II, BEL JAPAN, INC.).

2.3. Adsorption of α-Chymotrypsin onto Plant Biomass Charcoal

As a typical procedure, 5 mL of 0.01 M phosphate buffer solution at pH 7 containing 300 μM α-chymotrypsin and 3 g/L bagasse charcoal was placed in a 10-mL test tube with a screw cup, and was incubated at 25˚C and 120 rpm for 24 h. After adsorption, the mixture was filtrated with a membrane filter (pore size: 0.1 μm, Millipore Co. Ltd.). The amount of α-chymotrypsin adsorbed on PBC was calculated by subtracting the amount of α-chymotrypsin included in the supernatant liquid after adsorption from the amount of α-chymotrypsin in the aqueous solution before adsorption. The amount of α-chymotrypsin was measured at 280 nm by UV/vis spectrophotometer (UV-1800, Shimadzu Co. Ltd.).

The aqueous solutions used in this study were acetate buffer solutions at pH 4 and 5, phosphate buffer solutions at pH 6 and 7, borate buffer solutions at pH 8, 9, and 10, and disodium hydrogen phosphate buffer solutions at pH 11 and 12. The concentration of buffer solution was prepared at 0.01 M. Each data point for the amount of α-chymotrypsin adsorbed represents an average of three measurements with a standard error less than 10%.

3. Results and Discussion

3.1. Characterization of Plant Biomass Charcoal

In order to confirm the morphology of PBC, we have obtained SEM images presented in **Figure 1** for bagasse charcoal, adzuki bean charcoal, bamboo charcoal, and wood charcoal. Additionally, SEM image of medicinal carbon is shown as typical activated carbon in **Figure 1**. As seen in the figure, the morphology of PBC surface is strongly dependent upon the kind of materials. Bagasse charcoal was produced under 600˚C by a charcoal kiln. Adzuki bean charcoal, bamboo charcoal, and wood char coal were prepared under 450˚C by pyrolysis without combustion under a nitrogen atmosphere [9]. Consequently, the preparation of PBC used in the present work was not carried out by activation treatment. On the other hand, as seen in **Figure 1(E)**, the surface of medicinal carbon was obviously much rougher than that of PBC, and many pores were observed on the surface.

Table 1 shows the textural parameters of PBC obtained from low-temperature (−196˚C) nitrogen adsorption

Table 1. Structural characteristics of PBC.

Charcoals	Specific surface area [m^2/g]	Pore volume [cm^3/g]	Pore diameter peak [nm]
Bagasse	459	0.047	less than 2.6
Adzuki bean	204[a]	-	-
Bamboo	294	0.041	less than 2.6
Wood	117	0.025	less than 2.6
Medicinal carbon	1158	0.32	less than 2.6

[a]Specific area of adzuki bean charcoal was obtained from the CO_2 isotherm.

isotherms, which allow the calculation of specific surface area, specific pore volume, and pore diameter peak. In the table, the specific area of adzuki bean charcoal depicted the value obtained from the CO_2 isotherm in our previous work [9], since it was too long to reach the adsorption equilibrium, and the exact value could not be obtained. The specific surface area of PBC was much smaller than that of medicinal carbon, and the specific pore volume of PBC was one order of magnitude lower than that of medicinal carbon. The characteristics of pores and surface chemistry of charcoal are influenced by carbonizing temperature [13]. The specific pore volume tends to increase as the carbonizing temperature of charcoal increase. It was presumed that the formation of pores of PBC was not enhanced, since PBC was prepared at low temperatures.

Figure 2 shows the pore size distribution of PBC obtained by the Barrett-Joyner-Halenda (BJH) method [14], which is based on a model of the adsorbent as a collection of cylindrical pores. The theory accounts for capillary condensation in the pores using the classical Kelvin equation, which in turn assumes a hemispherical liquid-vapor meniscus and a well-defined surface tension. The pore size distribution of PBC was less than 10 nm, while that of medicinal carbon was less than 100 nm. Thus, the pore size of PBC was mainly in the micro-pore range, whereas that of medicinal carbon was in the meso-pore and macro-pore ranges.

3.2. Adsorption Isotherms

We used α-chymotrypsin as a model protein. The amount of α-chymotrypsin adsorbed on BCP increased with an increase in the time of adsorption, and reached a plateau around 24 h. **Figure 3** shows the amount of α-chymotrypsin adsorbed on PBC. The amount of α-chymotrypsin adsorbed varied with the kind of materials. As seen in **Figure 3**, the sequence of the amount adsorbed went as follows: medicinal carbon > bagasse charcoal > wood charcoal > adzuki bean charcoal > bamboo charcoal, while that of the specific surface area went as follows: medicinal carbon > bagasse charcoal > bamboo charcoal > adzuki bean charcoal > wood charcoal, as shown

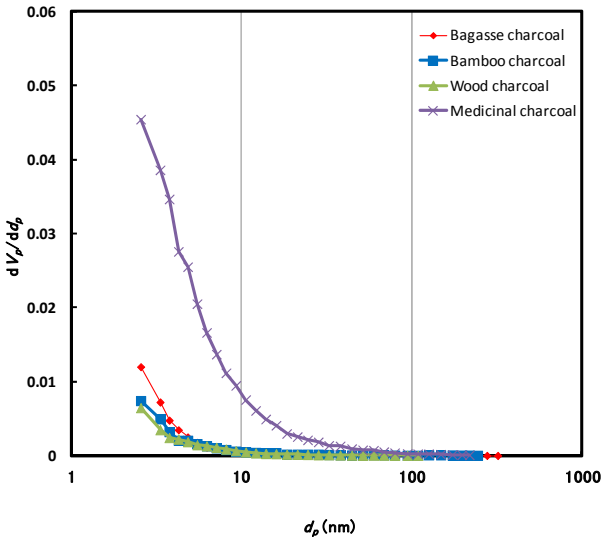

Figure 2. Pore size distribution of plant biomass charcoals.

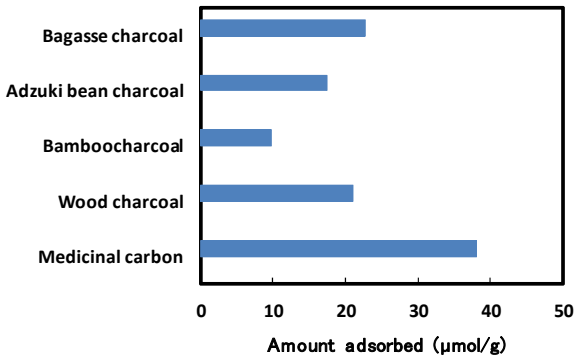

Figure. 3 Effect of the kind of materials on the amount of α-chymotrypsin adsorbed; adsorption was carried out by incubating buffer solution (pH 7) containing 300 μM α-chymotrypsin and 3 g/L PBC or medicinal carbon at 120 rpm and 25°C for 24 h.

in **Table 1**. Concerning PBC, the amount adsorbed did not correspond to the specific surface area. As seen in **Figures 1** and **2**, the morphology of PBC surface and the pore size distribution of PBC markedly depended upon the kind of raw materials. Consequently, it is assumed that they affect the adsorption efficiency of PBC. The

amount of α-chymotrypsin on bagasse charcoal was 0.6-fold that on medicinal carbon, although the surface area of medicinal carbon was about 2.5 times larger than that of bagasse charcoal. The scale of α-chymotrypsin is 5.1 × 4.0 × 4.0 nm [12]. Thus, it is considered that pores having less than the size of proteins do not work effectively against the adsorption of proteins. This indicates that bagasse charcoal has reasonable adsorption efficiency for α-chymotrypsin.

Figure 4 shows the adsorption isotherms of α-chymotrypsin on adzuki bean charcoal and bagasse charcoal. These isotherms exhibit a gradual increase. The amount of α-chymotrypsin adsorbed on bagasse charcoal is superior to that of α-chymotrypsin adsorbed on adzuki bean charcoal. The solid lines presented in the figure are the best fit Freundlich isotherm characterization of the experimental data using Equation (1).

$$W = K_F C^{1/n} \quad (1)$$

Here, W is the amount of α-chymotrypsin adsorbed on PBC, C is the α-chymotrypsin concentration, and K_F and n are experimental constants [15]. The correlation constants (r^2) of adzuki bean charcoal and bagasse charcoal were both 0.990. With regard to other adsorption isotherm models, for example, when the data were fitted for the adsorption isotherm model of Langmuir, the correlation constants (r^2) of adzuki bean charcoal and bagasse charcoal were 0.853 and 0.815, respectively. The present isotherm type was similar to that of the adsorption of HEWL onto PBC, and α-chymotrypsin was more effectively adsorbed on PBC, compared with HEWL [9]. The isotherm of bagasse charcoal displayed upward curvature, compared to that of adzuki bean charcoal. This indicates that the adsorption of α-chymotrypsin on bagasse charcoal is more effective than that on adzuki bean charcoal.

3.3. Effect of pH Value on α-Chymotrypsin Adsorption

Figure 5 shows the relationship between the pH value of aqueous solutions and the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal at 25˚C. The amount of α-chymotrypsin adsorbed on bagasse charcoal sharply increased with an increase in the pH value, reaching the optimum value around neutral pH, and tended to decrease in the alkaline region. The pH profile in the case of adzuki bean charcoal was similar to that in the case of bagasse charcoal, although the optimal value of adzuki bean charcoal is slightly shifted to acidic pH, compared to that of bagasse charcoal. The pH profile in the adsorption of α-chymotrypsin on PBC was similar to that in the adsorption of HEWL on PBC [9]. The net charge on the protein molecules is varied by adjusting the pH of the solution, since the protein molecule is constructed by amino acid residues containing positive- and negative-charged side chains. α-Chymotrypsin belongs to basic proteins, and the isoelectric point (pI) of α-chymotrypsin is 9.1 [12]. The lower the pH of solution containing α-chymotrypsin becomes below the pI of α-chymotrypsin, the more positive the net charge of α-chymotrypsin becomes. The ζ potential of PBC drastically decreases with increasing the pH value, exhibiting a negative value above pH 4, drops till pH 7, and is almost constant in the alkaline region [9]. When the pH value was around the pI of α-chymotrypsin or the pH where the ζ

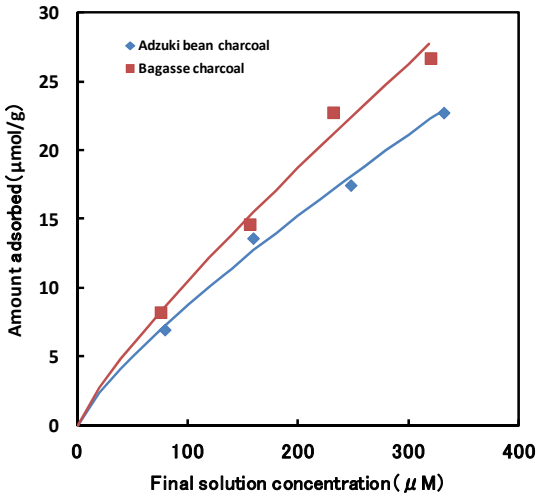

Figure 4. Adsorption isotherms of α-chymotrypsin on adzuki bean charcoal and bagasse charcoal; adsorption was carried out by incubating buffer solution (pH 7) containing a certain amount of α-chymotrypsin and 3 g/L PBC at 120 rpm and 25˚C for 24 h.

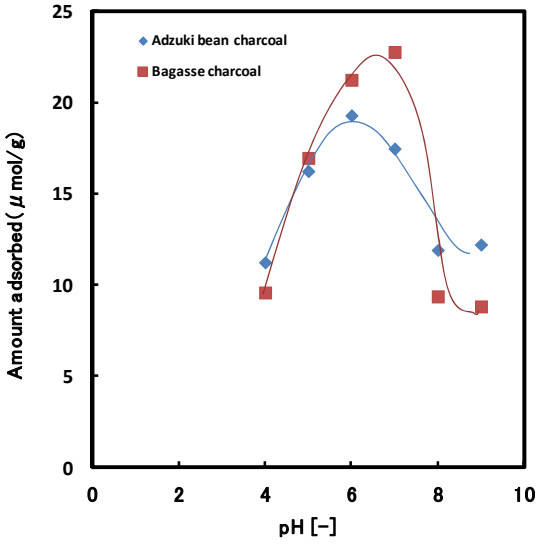

Figure 5. Effect of pH on the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal; adsorption was carried out by incubating buffer solution (appropriate pH) containing 300 μM α-chymotrypsin and 3 g/L PBC at 120 rpm and 25˚C for 24 h.

potential of PBC approached 0 volts, a dramatic decrease in the amount adsorbed was observed. The electrostatic interaction between the positive charge of α-chymotrypsin and the negative charge on PBC tends to decrease in the region of acidic or alkaline pH. Therefore, in the vicinity of neutral pH where the Coulomb force between PBC and α-chymotrypsin is high, a high amount of adsorption tends to be obtained. Consequently, the adsorption profiles seem to be related mainly with the electrostatic interaction.

3.4. Effect of Ionic Strength on α-Chymotrypsin Adsorption

Figure 6 shows the relationship between the KCl concentration of the solution and the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal. The amount of α-chymotrypsin adsorbed on PBC decreased with an increase in KCl concentration. The solutions of α-chymotrypsin at the KCl concentration employed at the present work were transparent, and no precipitate was observed. At first, it is considered that an increase in the ionic strength results in a decrease in the electrostatic interaction due to the electrostatic screening effect. Many radical species due to functional groups containing oxygen atoms, which are formed by thermal decomposition of cellulose and hemicelluloses, are detected in charcoals carbonized at 500˚C by the measurement of electron spin resonance, and functional groups decrease with increasing carbonization temperature [13, 16]. We have reported that the elemental ratio of oxygen on the surface of PBC was more than 15%, and C-O, O-C-O, C=O, and COOH were detected by X-ray photoelectron spectroscopy [9]. Consequently, it is suggested that electrostatic interactions and hydrogen bondings via functional groups on the surface of PBC largely contribute to the adsorption of α-chymotrypsin, since α-chymotrypsin has many ionic and hydroxyl amino acid residues, as seen in **Figure 7**. Thus, the addition of inorganic salts appears to weaken those interactions between PBC and α-chymotrypsin.

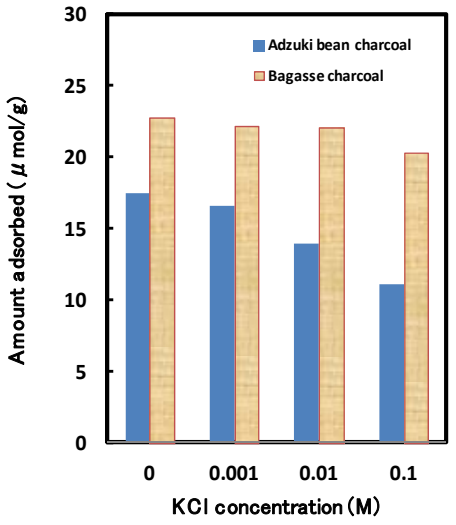

Figure 6. Effect of KCl concentration on the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal; adsorption was carried out by incubating buffer solution (pH 7) containing 300 μM α-chymotrypsin, 3 g/L PBC, and a certain amount of KCl at 120 rpm and 25˚C for 24 h.

3.5. Effect of Temperature on α-Chymotrypsin Adsorption

Figure 8 shows the plots of the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal against the adsorption temperature. The amount of α-chymotrypsin adsorbed on PBC was dramatically influenced by the temperature. The maximum amounts of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal were both observed around 25˚C. This tendency was similar to the case of the adsorption of HEWL onto PBC [9]. The temperature profile on the amount of proteins adsorbed on the water-insoluble matrix tends to exhibit an optimum temperature, since the conformation of proteins is generally sensitive to temperature [17]. The state on the surface of protein molecules

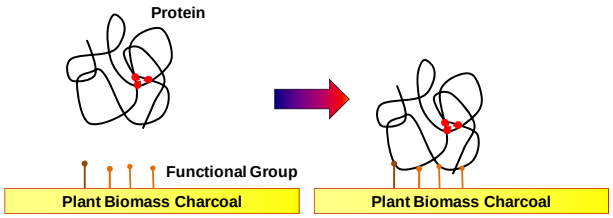

Figure 7. Schematic representation of adsorption of proteins onto the surface of PBC.

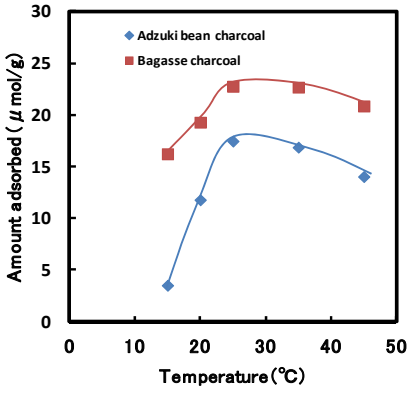

Figure 8. Effect of temperature on the amount of α-chymotrypsin adsorbed on adzuki bean charcoal and bagasse charcoal; adsorption was carried out by incubating buffer solution (pH 7) containing 300 μM α-chymotrypsin and 3 g/L PBC at 120 rpm and an appropriate temperature for 24 h.

such as the charge, hydrophilicity, and hydrophobicity due to their conformation affects the interaction of proteins with matrices. Moreover, the sufficient potential to weaken the hydration layer around a protein molecule is necessary to enhance the interactions between the amino acid residues of proteins and functional groups on the surface of PBC. Therefore, since the entropy effect of protein and the enthalpy effect of adsorption phenomenon are involved in the adsorption, the adsorption profile tends to exhibit the maximum at an appropriate temperature.

4. Conclusion

PBC had the adsorption efficiency for proteins, similar to medicinal carbon. The adsorption isotherms followed the Freundlish equation. PBC exhibited the optimum pH on the amount adsorbed due to the interaction between α-chymotrypsin and PBC, such as the electrostatic force, the hydrogen bonding. The adsorption temperature markedly affected the amount adsorbed.

5. Acknowledgements

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