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Adsorption of egg albumin onto methylated yeast biomass

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Abstract

A new biosorbent, methylated yeast (MeYE), was prepared for the adsorptive separation of proteins from aqueous solutions. Yeast was methylated in a 0.1 M HCl methyl alcohol solution at room temperature. About 80% of the carboxylic groups of yeast could be methylated within 9 h. The adsorption of egg albumin onto MeYE was studied to evaluate the protein adsorption ability of MeYE. At near neutral pH, egg albumin was scarcely adsorbed onto unmethylated yeast and the adsorbed amount of egg albumin increased with increasing methylation degree. The amount of egg albumin adsorbed onto MeYE increased with increasing pH from 4 to 7 and steeply decreased above pH 7. The Langmuir isotherm was applied to determine the apparent adsorption constant and the saturated adsorbed amount of egg albumin on MeYE. Both the apparent adsorption constant and the saturated adsorbed amount increased with the degree of methylation. The saturated adsorbed amount of egg albumin onto MeYE having methylation degree 77% was 8.41×10^{-6} mol g^{-1} or 0.378 g g^{-1} at near neutral pH.

Keywords: Methylation; Yeast biomass; Adsorption; Protein; Egg albumin

1. Introduction

The recovery and purification of protein products from various biological feed streams (protein bioseparation) is an important unit operation in the food, pharmaceutical, and biotechnological industries. Protein bioseparation is a time-consuming and costly process and often accounts for a significant percentage of the total processing cost. Thus, many protein bioseparation techniques such as precipitation, centrifugation, filtration, chromatography, and adsorption have been investigated to develop efficient and cost-effective technologies. The concentrations of target proteins in biological feed streams are generally very low and they need to be separated from a large number of impurities. Therefore, high-productivity (low-resolution) techniques are used first for the overall concentration of the feed stream. This is followed by high-resolution techniques for further purification of the target protein.

Adsorption is an effective and convenient technique in the primary bioseparation step since this technique can treat large liquid streams. Thus of protein adsorption onto various surfaces in aqueous systems has been studied over the past few decades by many researchers [1–5]. Recently, Peet et al. [4] have proposed a new technology, centrifugal adsorption technology (CAT), for efficient adsorption from large liquid streams using adsorbent particles in the micrometer range. CAT seems particularly suited for the recovery of macromolecules at low concentrations, because the small particle dimensions lead to fast mass transfer rates.

On the other hand, the use of microorganisms for the adsorptive separation of heavy metals from aqueous environments (biosorption) has attracted much attention in recent years. Many types of microorganisms have been investigated for use in this application [6–15]. Microorganisms would be suited for use in CAT, because they have a size in the micrometer range. However, the use of microorganisms for the adsorptive separation of proteins from aqueous solutions has scarcely been investigated.

In this study, a common and inexpensive microorganism, yeast, was applied to the adsorptive separation of egg albumin from aqueous solution. Most microorganisms have a negative surface charge near neutral pH [16,17]. Egg albumin, used as a model adsorbate in this study, has its isoelectric point at about pH 4.5 and a negative charge above pH 4.5. Therefore, it is expected that egg albumin can hardly adsorb onto yeast surface at near neutral pH.

Fraenkel-Conrat and Olcott [18] reported that the carboxylic groups of proteins were readily methylated at room temperature in methyl alcohol containing small amounts of

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mineral acid (0.02–0.1 mol dm^{-3}). The acid-catalyzed reaction of proteins with methanol was a specific one involving only the carboxylic groups; amino, phenolic, thiol, and indole groups and peptide and amide bonds were unaffected. The main negatively charged group on the surface of microorganisms is the carboxylic group [11,14,17]. Therefore, it is expected that the highly methylated yeast surface acquires a positive charge at near neutral pH and it can act as an adsorbent for negatively charged egg albumin.

In the present study, we prepared methylated yeast (MeYE) having a methylation degree of 23, 54, 68, and 77% according to the method reported by Fraenkel-Conrat and Olcott [18]. To confirm the effect of methylation on the adsorption of egg albumin onto MeYE, adsorption experiments were performed at pH from 4 to 8. Then we applied the Langmuir adsorption isotherm to the experimental data. Based on the Langmuir parameters, the effect of methylation on the adsorption ability of MeYE for egg albumin will be discussed.

2. Materials

2.1. Chemicals

Albumin (from egg), methyl alcohol, sodium chloride, sodium hydroxide, and hydrochloric acid were purchased from Kanto Chemical Co. Inc. (Japan). Albumin was of practical-grade quality and other chemicals were of reagent-grade quality. They were used with no further purification. Distilled water, boiled for 15 min and cooled under nitrogen, was used in all experiments.

2.2. Methylated yeast

Yeast (dried) of practical grade was purchased from Wako Pure Chemical Industries (Japan) and washed in the following manner. A sample of 100 g of yeast was suspended in a 1 dm^3 of 0.01 M NaOH solution and mechanically stirred for 2 h. The yeast was separated in a centrifuge at 3300 rpm for 30 min and washed repeatedly with distilled water. Then it was suspended in 1 dm^3 of methyl alcohol and stirred at room temperature. After 24 h of stirring, yeast was separated in a centrifuge at 3300 rpm for 30 min, freeze-dried, and ground to a fine powder. The ground yeast was sieved through a 120-mesh (0.125-mm) sieve and the undersized fraction was used. Hereafter, the yeast will be abbreviated as YE.

Methylated yeast was prepared according to the method reported by Fraenkel-Conrat and Olcott [18]. A sample of 10 g of YE was dispersed in 1 dm^3 of methyl alcohol containing HCl (0.1 mol dm^{-3}) as a catalyst and mechanically stirred at room temperature. The methylated yeast was collected in a centrifuge at 3300 rpm for 20 min and washed repeatedly with distilled water. Then it was freeze-dried and stored in a desiccator. Hereafter, the methylated yeast will be abbreviated as MeYE. In this study, we used four types of MeYE that had a methylation degree of 23, 54, 68, and 77%. They were prepared by changing the methylation time 1.5, 3, 6, and 9 h, respectively.

3. Experimental methods

3.1. Potentiometric titration of MeYE and YE

The degree of methylation was determined from the change in the number of carboxylic groups before and after methylation. The number of carboxylic groups was determined by potentiometric titration. A solution (0.3 dm^3) containing 1.0 g dm^{-3} of MeYE/YE was mechanically stirred at 30 °C. The ionic strength of the solution was adjusted to 0.1 mol dm^{-3} by the addition of NaCl. To eliminate CO_2, the titration was performed under nitrogen. After reaching thermal equilibrium, the solution was titrated with a volumetric standard solution of HCl or NaOH (0.1 mol dm^{-3}). The pH of the solution was measured using a pH meter (Orion Research 520-A). The number of protonated acidic groups was determined from the difference between the bulk proton concentrations in the presence of MeYE/YE and those in the absence of MeYE/YE.

3.2. Adsorption of egg albumin

A solution (90 cm^3) of NaCl (0.1 mol dm^{-3}) containing MeYE/YE (1.0 g dm^{-3}) was prepared. The pH of the solution was adjusted to the desired value with HCl or NaOH. After thermal equilibrium was reached at 30 °C, 10 cm^3 of a solution (10 g dm^{-3}) containing a certain amount of egg albumin was added to the suspension. The suspension was stirred for the time necessary to attain the adsorption equilibrium, and then MeYE/YE was separated from the liquid phase in a centrifuge (Kokusan H-1500F) at 3300 rpm for 20 min. The pH and egg albumin concentration of the supernatant were measured. The concentration of egg albumin was determined with a spectrophotometer (Hitachi U-1500) at 280 nm. The amount of egg albumin adsorbed onto MeYE/YE was determined from the difference between the egg albumin concentrations in the initial and equilibrium states. The molar concentration of egg albumin was calculated, assuming the molecular weight to be 45,000.

4. Results and discussion

4.1. Determination of methylation degree

Fig. 1 shows a typical example of the proton adsorption isotherm of MeYE (methylated for 9 h) obtained from potentiometric titration (open circles). For comparison, the result with YE is also presented in Fig. 1 (solid circles). The ordinate of the figure, X_H, represents the equilibrium number

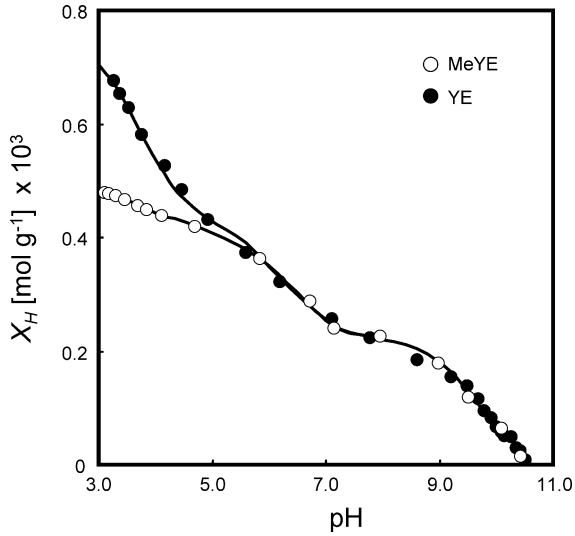

Fig. 1. Number of protonated sites of yeast (solid circles) and yeast methylated for 9 h (open circles). The number of protonated sites was determined by potentiometric titration of 0.3 dm^3 of a suspension containing 1.0 g dm^{-3} of yeast or methylated yeast. Ionic strength was adjusted to 0.1 mol dm^{-3} by NaCl. The solid lines represent the theoretical curves calculated from Eq. (1).

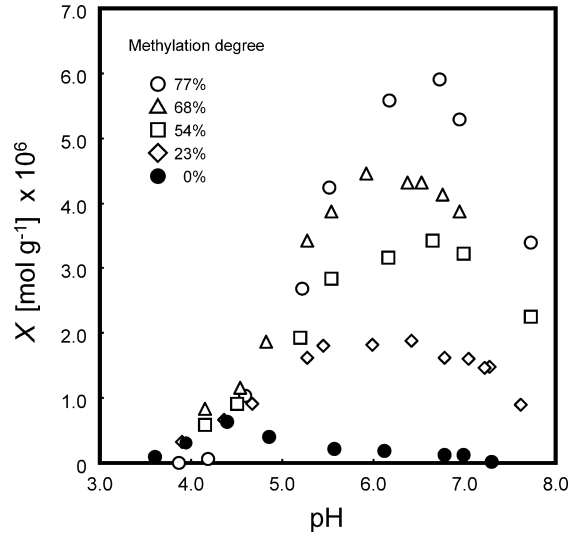

Fig. 2. pH dependence of egg albumin adsorption onto methylated yeast at 30 °C. The concentration of methylated yeast and the initial concentration of egg albumin were 1.0 g dm^{-3} and 1.0×10^{-5} mol dm^{-3}, respectively. Ionic strength was adjusted to 0.1 mol dm^{-3} by NaCl.

Table 1
Equilibrium parameters for the acid dissociation of yeast

	Type 1	Type 2	Type 3
pK_{H}	3.73	6.24	9.64
N_{H} (mmol g^{-1})	0.336	0.199	0.244

of protons bound to 1 dry g of MeYE/YE. The X_{H} values of MeYE and YE are almost the same at above pH 5, while the X_{H} values of MeYE are considerably lower than those of YE below pH 5.

The proton adsorption isotherms of microorganisms are usually broad and ill-defined, thus reflecting the diversity in the proton binding sites. The proton binding sites of microorganisms can be divided into three main types [9,11,14,17]. The total amount of protons adsorbed onto 1 dry g of microorganism, X_{H}, can be expressed by the equation [11,14]

$$X_{\mathrm{H}} = N_{\mathrm{H1}}[\mathrm{H}^+]/\big(K_{\mathrm{H1}} + [\mathrm{H}^+]\big) + N_{\mathrm{H2}}[\mathrm{H}^+]/\big(K_{\mathrm{H2}} + [\mathrm{H}^+]\big) + N_{\mathrm{H3}}[\mathrm{H}^+]/\big(K_{\mathrm{H3}} + [\mathrm{H}^+]\big), \quad (1)$$

where K_{H} and N_{H} represent the dissociation constants of proton binding sites and the number of binding sites on 1 dry g of microorganism, respectively. A nonlinear least-squares method was applied to find the constants, K_{H} and N_{H}, of three type sites. The constants for YE which gave the best fit with the experimental data are listed in Table 1. The type 1 site, which has the acid-dissociation constant of p$K_{\mathrm{H1}} = 3.73$, can be considered as the carboxylic groups. As mentioned above, the acid-catalyzed reaction of proteins with methanol was a specific one involving only the carboxylic groups. Therefore, the number of carboxylic groups of MeYE was determined using the same constants as YE except N_{H1}. The number of carboxylic groups of MeYE methylated for 9 h decreased to 0.077 mmol g^{-1} and the methylation degree of the present MeYE was obtained to be 77%. The solid lines in Fig. 1 represent the theoretical curve calculated from Eq. (1). The experimental results agreed well with the theoretical values calculated using Eq. (1) and at pH above 5 the X_{H} values of MeYE coincided well with that of YE. The result suggests that the present methylation reaction did not affect any functional group other than the carboxylic groups.

4.2. pH dependence of egg albumin adsorption onto MeYE

The pH dependence of egg albumin adsorption to MeYE is shown in Fig. 2. Preliminary kinetic experiments for the egg albumin–MeYE system were conducted. Since MeYE does not have a porous structure, the adsorption proceeded rapidly and 30 min or so was enough to attain equilibrium. From the results, we determined the contact time for the equilibrium experiments as 2 h. The ordinate, X (mol g^{-1}), of Fig. 2 represents the amount of egg albumin adsorbed onto 1 dry g of MeYE/YE at equilibrium. MeYE methylation degrees of 23, 54, 68, and 77% were used in the experiment. For comparison, the results of YE are also presented. The initial concentration of egg albumin was 1.0×10^{-5} mol dm^{-3}. In the case of YE, only a small amount of egg albumin was adsorbed at about pH 4.5 (which is the isoelectric point of egg albumin). The adsorbed amount of egg albumin near neutral pH increased significantly with increasing methylation degree. The result suggests that the yeast surface has been modified to have a positive charge by the methylation and thus the adsorbed amount of negatively charged egg albumin has increased with increasing methylation degree.

The amount of egg albumin adsorbed onto MeYE increased with increasing pH from 4 to 6 and steeply decreased above pH 7. The number of positively charged amino groups on MeYE is constant in the pH range, while the net negative charge density of egg albumin steeply increases above pH 4.5. The attractive force between the positively charged MeYE surface and the negatively charged egg albumin may become stronger with increasing net negative charge density of egg albumin, and thus the adsorbed amount increase with increasing pH from 4 to 6. On the other hand, the number of negatively charged phosphatic groups on MeYE steeply increases above pH 6. Although the number of positively charged amino groups on MeYE is constant below pH 8, the net positive charge density of MeYE should decrease with increasing number of negatively charged phosphatic groups. Thus, the adsorbed amount of egg albumin steeply decreased above pH 7.

4.3. Adsorption isotherm of egg albumin onto MeYE

Fig. 3 shows the adsorption isotherm of egg albumin to MeYE at pH 6.5 ± 0.2. The abscissa, C (mol dm^{-3}), of Fig. 3 represents the equilibrium concentration of egg albumin. According to the literature [19–21], adsorption of egg albumin onto MeYE/YE is described by the Langmuir isotherm,

$$K = X/(N - X)C \tag{2}$$

or

$$C = NC/X - 1/K, \tag{3}$$

where K (dm^3 mol^{-1}) and N (mol g^{-1}) are the apparent adsorption constant and the saturated adsorbed amount of egg albumin, respectively. Fig. 4 shows the Langmuir plot of the experimental data in Fig. 3. The adsorption of egg albumin onto MeYE and YE agreed well with the Langmuir isotherm and the apparent adsorption constants and the saturated adsorbed amounts were determined from the slopes and intercepts of the lines in Fig. 4. The constants, K and N, and the correlation coefficients between the experimental and the predicted values, R^2, are listed in Table 2. Both the apparent adsorption constant and the saturated adsorbed amount increased markedly with increasing methylation degree. As mentioned above, the methylation reaction has no effect on any functional groups other than the carboxylic groups and the number of positively charged amino groups on the yeast surface is constant below pH 8. Thus, it is obvious that the negatively charged carboxylic groups on the yeast surface inhibit the adsorption of egg albumin.

The saturated adsorbed amount of MeYE with a methylation degree of 77% was 8.41×10^{-6} mol g^{-1} or 0.378 g g^{-1}.

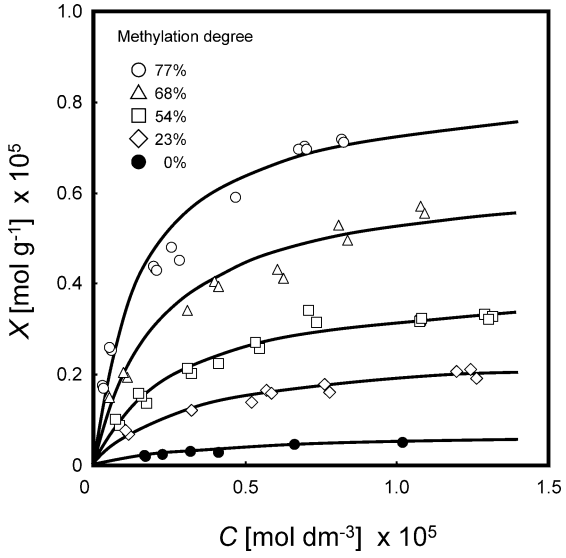

Fig. 3. Adsorption isotherm of egg albumin onto methylated yeast at 30 °C and at pH 6.5 ± 0.2. Ionic strength was adjusted to 0.1 mol dm^{-3} by NaCl. The concentration of methylated yeast was 1.0 g dm^{-3}. The solid lines represent the theoretical curves calculated using the parameters shown in Table 2.

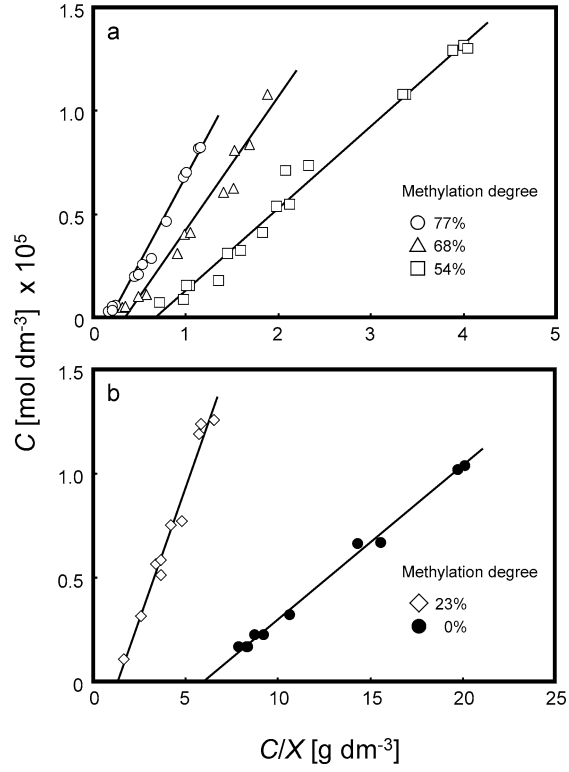

Fig. 4. Langmuir plots of the data in Fig. 3.

Table 2
Equilibrium parameters for the egg albumin adsorption onto methylated yeast at pH 6.5

Methylation degree (%)	K (dm^3 mol^{-1})	N (mol g^{-1})	R^2
0	2.29×10^5	0.74×10^{-6}	0.995
23	3.01×10^5	2.54×10^{-6}	0.976
54	3.76×10^5	3.98×10^{-6}	0.985
68	4.46×10^5	6.43×10^{-6}	0.969
77	6.23×10^5	8.41×10^{-6}	0.986

Gun'ko et al. [1] studied the adsorption of bull serum albumin onto fumed silica (Aerosil A-300, diameter ca. 2 μm, specific surface area ca. 300 $m^2\,g^{-1}$) at about pH 6. The saturated amount of bull serum albumin adsorbed onto fumed silica was about 0.5 $g\,g^{-1}$ and it was slightly larger than that of egg albumin adsorbed onto MeYE. However, the saturated amount of egg albumin adsorbed onto MeYE was much larger than that of catalase on a hydroxyl apatite (specific surface area 80 $m^2\,g^{-1}$) at pH 6.8, 0.08 $g\,g^{-1}$ [2], bovine serum albumin and lysozyme on hydrophobic calcium hydroxyapatites (specific surface area 90 $m^2\,g^{-1}$) at pH 6, 0.135 $g\,g^{-1}$ [3], bovine serum albumin on a strong anion-exchange resin (Macroprep High Q, diameter 50 μm) at pH 6, 0.037 $g\,cm^{-3}$ [4], and human serum albumin on colloidal TiO_2 (primary particle size 30 nm) at pH 6, 0.125 $g\,g^{-1}$ [5].

5. Conclusion

A common and inexpensive biomass, yeast, was applied to the adsorptive separation of proteins from aqueous solutions. Yeast was methylated according to the method reported by Fraenkel-Conrat and Olcott. The adsorption behavior of egg albumin on methylated yeast having a methylation degree of 23, 54, 68, and 77% was investigated at pH from 4 to 8. Egg albumin was scarcely adsorbed onto unmethylated yeast near neutral pH and only a small amount of egg albumin was adsorbed onto unmethylated yeast at an isoelectric point of egg albumin (pH 4.5). The amount of egg albumin adsorbed onto methylated yeast increased with increasing methylation degree. The adsorbed amount of egg albumin increased with increasing pH from 4 to 7 and steeply decreased above pH 7. The adsorption of egg albumin was well described by the Langmuir isotherm. Both the apparent adsorption constant and the saturated adsorbed amount increased with the degree of methylation. Methylated yeast having a methylation degree of 77% had a rather large adsorption capacity of 8.41×10^{-6} $mol\,g^{-1}$ or 0.378 $g\,g^{-1}$ for egg albumin at pH 6.5.

Appendix A. Nomenclature

C	Equilibrium concentration of egg albumin ($mol\,dm^{-3}$)
K	Adsorption constant of egg albumin ($dm^3\,mol^{-1}$)
K_H	Dissociation constant of acidic/basic groups on MeYE/YE ($dm^3\,mol^{-1}$)
N	Saturated adsorbed amount of egg albumin ($mol\,g^{-1}$)
N_H	Number of acidic/basic groups on MeYE/YE ($mol\,g^{-1}$)
X	Equilibrium adsorbed amount of egg albumin ($mol\,g^{-1}$)
X_H	Equilibrium adsorbed amount of proton ($mol\,g^{-1}$)

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