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Author(s)	Seki, Hideshi; Suzuki, Akira; Maruyama, Hideo
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Adsorption of Egg Albumin to Methylated Yeast Biomass

HIDESHI SEKI, AKIRA SUZUKI AND HIDEO MARUYAMA

Division of Marine Biosciences, Graduate School of Fisheries Sciences,

Hokkaido University,

Minato-cho 3-1-1 Hakodate 041-8611, Japan

Abbreviated title:

EGG ALBUMIN ADSORPTION TO METHYLATED YEAST

Hideshi Seki

Division of Marine Biosciences, Graduate School of Fisheries Sciences

Hokkaido University,

Minato-cho 3-1-1 Hakodate 041-8611, Japan

E-mail: seki@elsie.fish.hokudai.ac.jp.

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 to MeYE was studied to evaluate the protein adsorption ability of MeYE. At near neutral pH, egg albumin was scarcely adsorbed to unmethylated yeast and the adsorption amount of egg albumin increased with increasing the methylation degree. The adsorption amount of egg albumin to 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 adsorption amount of egg albumin to MeYE. Both the apparent adsorption constant and the saturated adsorption amount increased with the degree of methylation. The saturated adsorption amount of egg albumin to MeYE having methylation degree 77% was 8.41×10^{-6} mol g^{-1} or 0.378 g g^{-1} at near neutral pH.

Key Words: methylation, yeast biomass, adsorption, protein, egg albumin

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 industry. The protein bioseparation is a time and cost consuming process and it 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 the studies on the 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 by 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 environment (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 the 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 been scarcely 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 at 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 it also has a negative charge above pH 4.5. Therefore, it is expected that egg albumin can hardly adsorb to 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 mineral acid (0.02 to 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 carboxylic group (11, 14, 17). Therefore, it is expected that the highly methylated yeast surface become to have 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 behavior of egg albumin to MeYE, adsorption experiments were performed at the pH range from pH4 to pH8. 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.

MATERIALS

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 a nitrogen atmosphere, was used in all experiments.

Methylated Yeast

Yeast (dried) of practical-grade was purchased from Wako Pure Chemical Industries (Japan) and it was washed in the following manner. A 100 g of yeast was suspended in a 1 dm^3 of 0.01M NaOH solution and mechanically stirred for 2 h. The yeast was separated in a centrifuge at 3,300 rpm for 30 min and washed repeatedly with distilled water. Then it was suspended in a 1 dm^3 of methyl alcohol and stirred at room temperature. After 24 h of stirring, yeast was separated in a centrifuge at 3,300 rpm for 30 min, and it was freeze-dried and ground to a fine powder. The ground yeast was sieved through a 120-mesh (0.125 mm) sieve and the undersize 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 10 g of YE was dispersed in a 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 3,300 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 have 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.

EXPERIMENTAL METHODS

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 a 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 atmosphere. 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 by 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.

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 the thermal equilibrium was reached at 30□, 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 3,300 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 to MeYE/YE was determined from the difference between the egg albumin concentrations in the initial and the equilibrium states. The molar concentration of egg albumin was calculated assuming the molecular weight as 45,000.

RESULTS AND DISCUSSION

Determination of Methylation Degree

Figure 1 shows a typical example of the proton adsorption isotherm of MeYE (methylated for 9h) obtained from potentiometric titration (open circles). As for the comparison, the result of YE is also presented in Fig. 1 (solid circles). The ordinate of the figure, X_H, represents the equilibrium number 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 is considerably lower than that of YE at below pH 5.

The proton adsorption isotherm 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 adsorption amount of proton to 1-dry g of microorganism, X_H, can be expressed by the following equation (11, 14).

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

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. Type 1 site, which has the acid-dissociation constant of pK_{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.

pH Dependence of Egg Albumin Adsorption to 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 porous structure, the adsorption

proceeded rapidly and 30 min or so was enough to attain the 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 adsorption amount of egg albumin to 1 dry-g of MeYE/YE at equilibrium. MeYE methylation degree of 23, 54, 68, and 77% were used in the experiment. As for the comparison, the result of YE was 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 iso-electric point of egg albumin). The adsorption amount of egg albumin at near neutral pH increased significantly with increasing the methylation degree. The result suggests that the yeast surface has been modified to have a positive charge by the methylation and thus the adsorption amount of negatively charged egg albumin has increased with increasing the methylation degree.

The adsorption amount of egg albumin to 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 the net negative charge density of egg albumin, and thus the adsorption 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 the number of negatively charged phosphatic groups. Thus, the adsorption amount of egg albumin steeply decreased above pH 7.

Adsorption Isotherm of Egg Albumin to MeYE

Figure 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 to MeYE/YE has been described by the Langmuir isotherm:

$$K = X/(N - X)C \qquad [2]$$

or

$$C = NC/X - 1/K \qquad [3]$$

where K (dm^3 mol^{-1}) and N (mol g^{-1}) are the apparent adsorption constant and

the saturated adsorption amount of egg albumin, respectively. Figure 4 shows the Langmuir plot of the experimental data in Fig. 3. The adsorption of egg albumin to MeYE and YE agreed well with the Langmuir isotherm and the apparent adsorption constants and the saturated adsorption 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, were listed in Table 2. Both the apparent adsorption constant and the saturated adsorption amount increased markedly with increasing the 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 pH8. Thus, it is obvious that the negatively charged carboxylic groups on the yeast surface inhibit the adsorption of egg albumin.

The saturated adsorption amount of MeYE methylation degree of 77% was 8.41×10^{-6} mol g^{-1} or 0.378 g g^{-1}. Gun'ko et al. (1) studied the adsorption of bull serum albumin to fumed silica (Aerosil A-300, diameter ca. 2 μm, specific surface area ca. 300 m^2 g^{-1}) at about pH 6. The saturated adsorption amount of bull serum albumin to fumed silica was about 0.5 g g^{-1} and it was slightly larger than that of egg albumin to MeYE. However, the saturated adsorption amount of egg albumin to MeYE was much larger than those of catalase to a

hydroxyl apatite (specific surface area $80m^2\ g^{-1}$) at pH6.8, 0.08 g g^{-1} (2), bovine serum albumin and lysozyme to hydrophobic calcium hydroxyapatites (specific surface area $90m^2\ g^{-1}$) at pH6, 0.135 g g^{-1} (3), bovine serum albumin to a strong anion-exchange resin (Macroprep High Q, diameter 50μm) at pH6, 0.037 g cm^{-3} (4), and human serum albumin to a colloidal TiO_2 (primary particle size 30nm) at pH6, 0.125 g g^{-1} (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 to 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 to unmethylated yeast at near neutral pH and only a small amount of egg albumin was adsorbed to unmethylated yeast at an isoelectric point of egg albumin (pH 4.5). The adsorption amount of egg albumin to methylated yeast increased with increasing the methylation degree. The adsorption amount of egg albumin increased with increasing pH from 4 to 7 and steeply decreased above pH 7. The adsopriton of egg albumin was well described by the Langmuir isotherm. Both the apparent adsorption constant and the saturated adsorption amount increased with the degree of methylation. Methylated yeast having a methylation digree 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.

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 adsorption amount of egg albumin	[mol g^{-1}]
N_H	= Number of acidic/basic groups on MeYE/YE	[mol g^{-1}]
X	= Equilibrium adsorption amount of egg albumin	[mol g^{-1}]
X_H	= Equilibrium adsorption amount of proton	[mol g^{-1}]

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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

TABLE 2

Equilibrium Parameters for the Egg Albumin Adsorption to Methylated Yeast at pH 6.5

Metylation 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

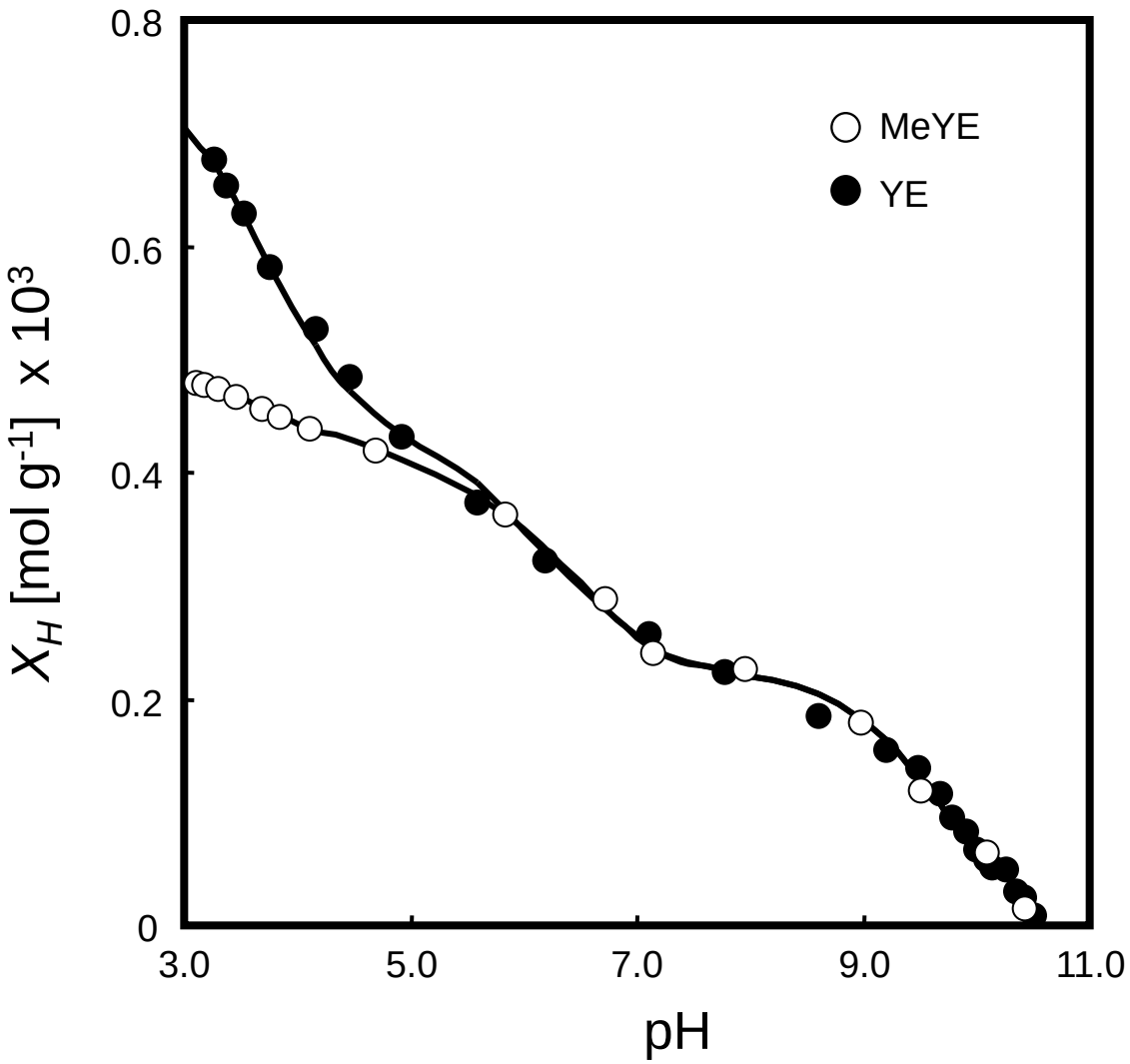
Figure Captions

FIG. 1. Number of protonated sites of the yeast (solid circles) and the yeast methylated for 9h (open circles). The number of protonated sites was determined by potentiometric titrations for 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 curve calculated from Eq. [1].

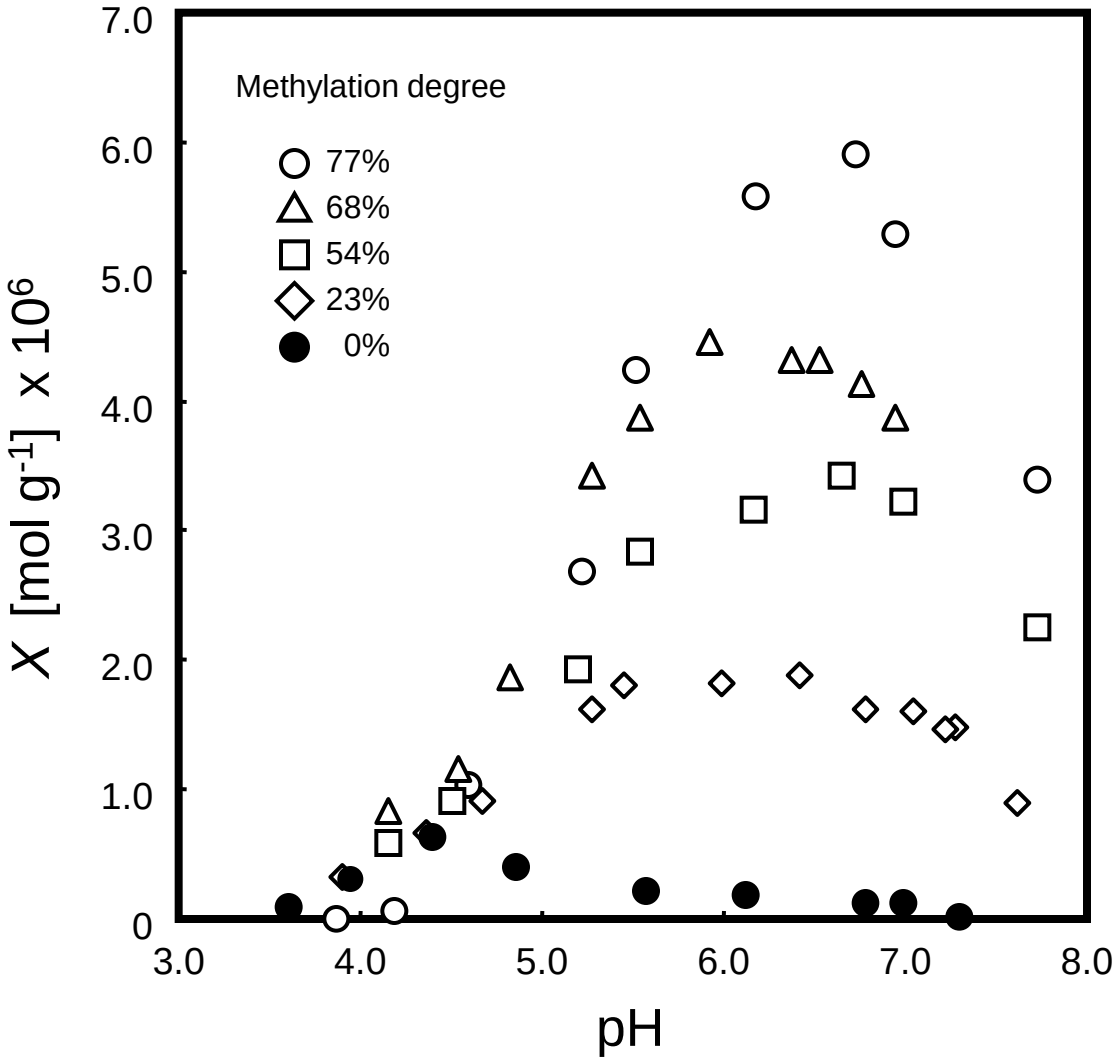
FIG. 2. pH dependence of egg albumin adsorption to methylated yeast at 30°C. The concentration of methylated yeast and the initial concentration of egg albumin were 1.0 g dm^{-3} and 1.0×10^{-5} mol dm^{-3}, respectively. Ionic strength was adjusted to 0.1 mol dm^{-3} by NaCl.

FIG. 3. Adsorption isotherm of egg albumin to 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.

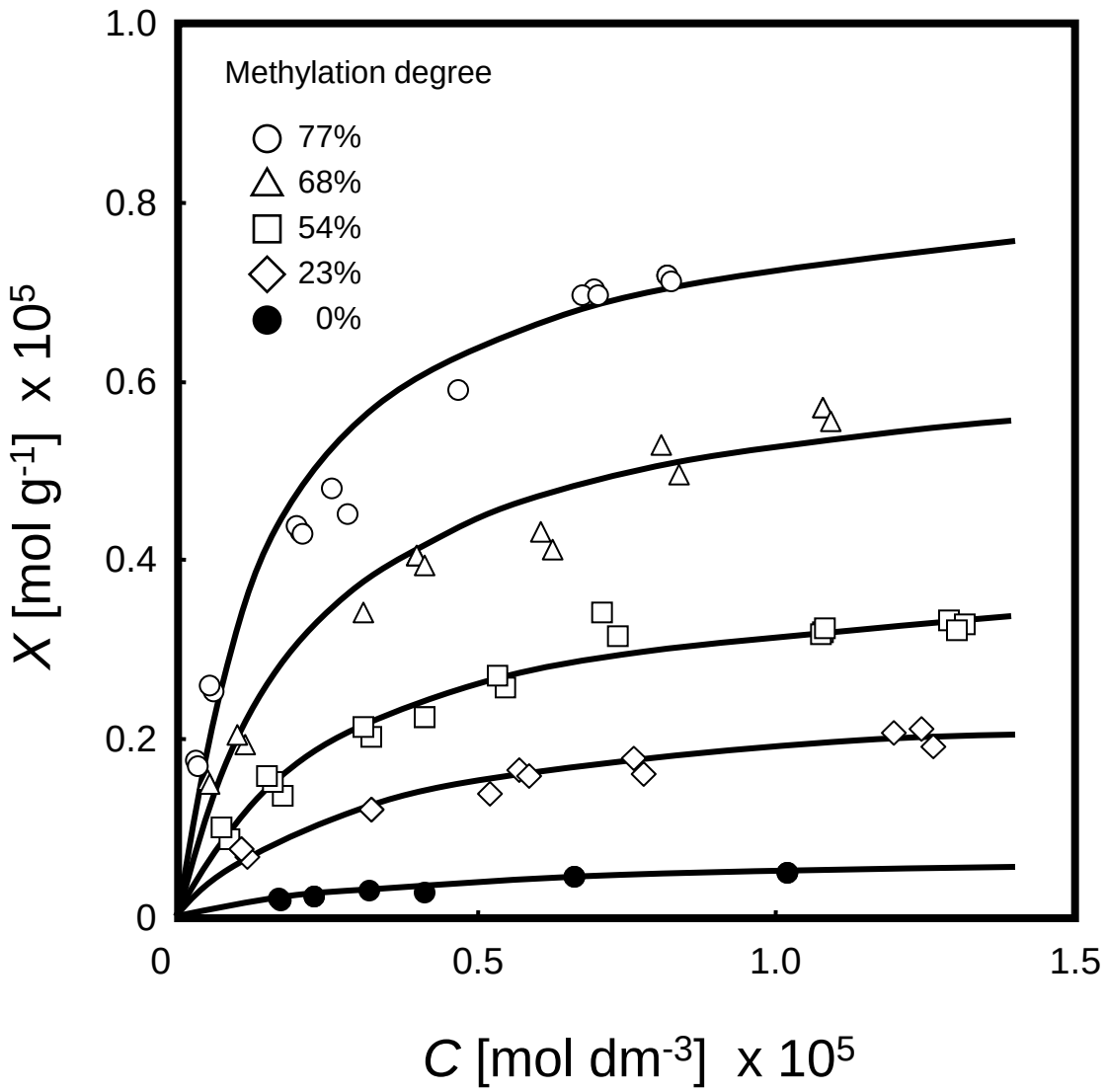
FIG. 4. Langmuir plots of the data in Fig. 3.



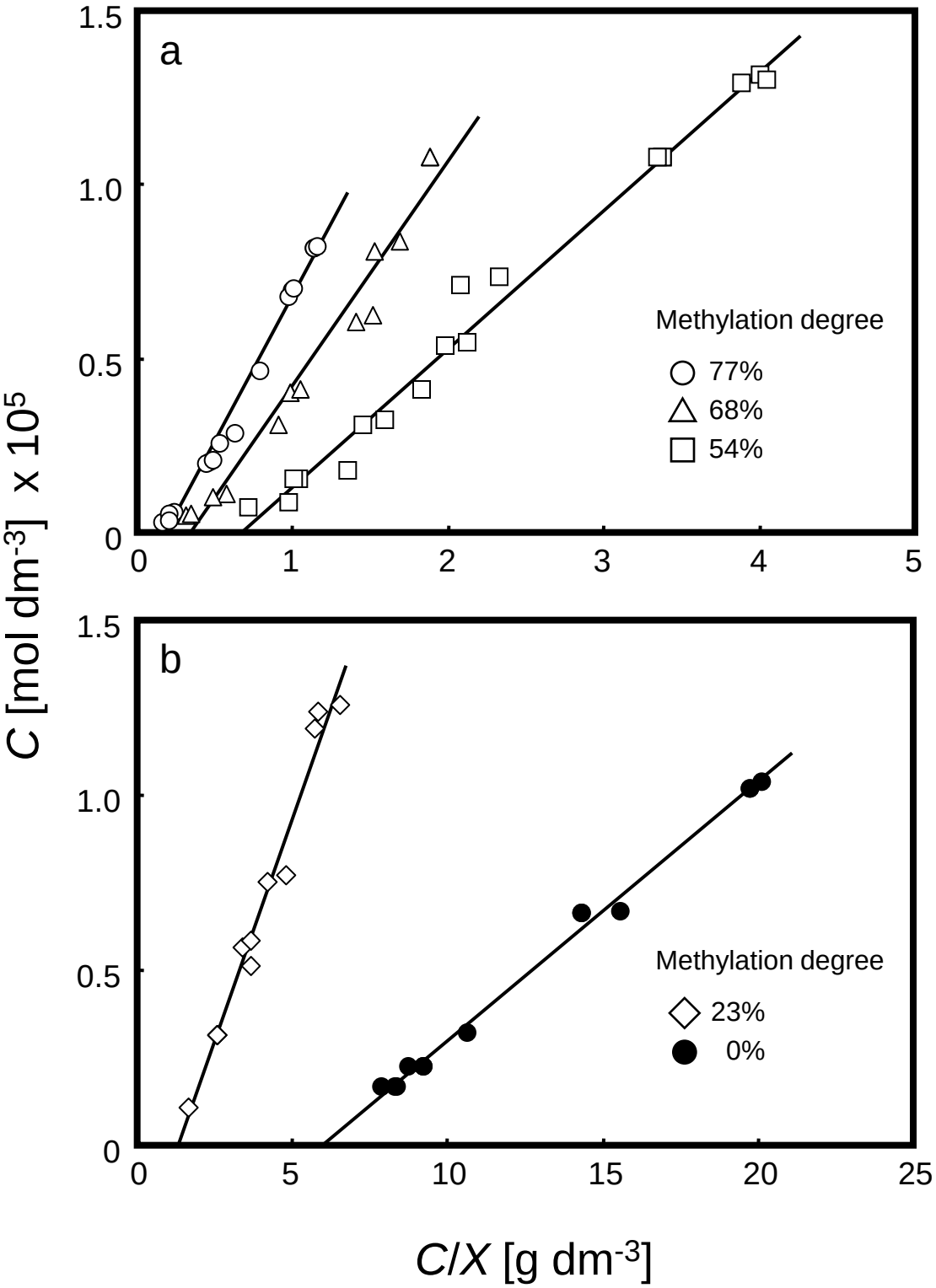
Seki et al. Fig.1



Seki et al. Fig.2



Seki et al. Fig.3



Seki et al. Fig.4