Properties of Protein Adsorption and Desorption of Alumina Ceramic Membrane

Takahiro KAWAKATSU,1 Toshikuni YONEMOTO1 and Mitsutoshi NAKAJIMA2

1Department of Chemical Engineering, Tohoku University, Aoba, Sendai, Miyagi 980-8579, Japan
2National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

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The properties of protein adsorption and desorption of alumina ceramic membranes were investigated using several types of proteins. The effect of pH on the protein adsorption was studied by soaking the membrane in protein solution. For basic proteins, the amount of protein adsorbed increased at pHs lower than the isoelectric point due to an electrostatic interaction between the protein and the membrane. A single protein solution was filtered and the protein was adsorbed on the membrane. Most of the adsorbed protein was desorbed and concentrated by changing the ionic strength or pH of the filtered solvent, however, the protein remaining on the membrane was not washed out even by soaking in sodium lauryl sulfate (SDS) solution. The adsorption-desorption experiments were carried out using a binary protein solution of lysozyme and β-galactosidase of pH 3 for the adsorption step and phosphate buffer solution of pH 7 for the desorption step. During the adsorption step, β-galactosidase was adsorbed more than the lysozyme, however, the absolute adsorbed amount was smaller than that in the single protein solution of β-galactosidase since the adsorption site was partially occupied by lysozyme. β-Galactosidase was concentrated seven-fold in the permeate during the desorption step in the first 20 s, while the concentration of lysozyme was one-tenth of its original value.

Keywords: protein adsorption, protein desorption, ceramic membrane, microfiltration

Protein adsorption and desorption are important subjects during ultrafiltration and microfiltration processes. For ultrafiltration, the protein itself is usually a separation target and rejected protein is adsorbed on the membrane surface using lower-molecular-weight cut-off membranes (Matthiasson, 1983, Turker & Hubble, 1987) or partially permeable membranes (Fane et al., 1983). Most polymeric membranes are negatively charged in the range of pH 3 to 7, and the adsorbed amount of protein (BSA) increased as the pH decreased in the region below the isoelectric point because of an electrostatic interaction between the protein and membrane surface (Matthiasson, 1983, Turker & Hubble, 1987). In another paper, the maximum adsorption was observed at the isoelectric point due to a weakness of the repulsive interaction between BSA molecules (Fane et al., 1983). For the microfiltration membrane, Bowen and Gan (1990) studied the adsorption properties of BSA on a hydrophilic polyvinylidene fluoride (PVDF) membrane. In this case, both of the protein-membrane and protein-protein interactions were observed. The maximum adsorbed amount of protein (0.2-0.4 mg·m⁻²) was obtained in the region of the isoelectric point, pH 4.9. The minimum point was observed at around pH 4, and at lower pHs, the adsorbed amount of protein increased again. Persson et al. (1993) studied the adsorption properties of β-lactoglobulin on four kinds of organic membranes made of nylon-66, cellulose acetate, and hydrophilic and hydrophobic types of PVDF. The adsorption amount using a hydrophilic PVDF membrane was the least, almost one-tenth that with the other membranes.

Inorganic microfiltration membranes are generally hydrophilic, and the electrostatic interaction between a protein and membrane surface is considered to be a dominant factor for protein adsorption and desorption compared to a hydrophobic interaction. Using several types of solids (polystyrene, polyoxymethylene, silver iodide, hematite and silica), Norde et al. (1986) investigated the adsorption properties of human plasma albumin (HPA). They also examined the effect of several methods on the desorption of HPA, such as dilution and at lower pHs, the adsorbed amount of protein increased again. Persson et al. (1993) studied the adsorption properties of β-lactoglobulin on four kinds of organic membranes made of nylon-66, cellulose acetate, and hydrophilic and hydrophobic types of PVDF. The adsorption amount using a hydrophilic PVDF membrane was the least, almost one-tenth that with the other membranes.

Competitive adsorption occurs in binary protein systems. With a binary solution of BSA and bovine fibrinogen, BSA was adsorbed about 3-4 times more than fibrinogen on silicon rubber although BSA adsorption with a single solution was less than that of fibrinogen (Lok et al., 1983). It was suggested that such competitive adsorption was dominated by the adsorption rate and site of these proteins.

In this work, properties of protein adsorption onto and desorption from alumina ceramic membranes were investigated by considering the ionic strength and pH effect in a static method by soaking and a dynamic method using filtration. The washing effect with use of sodium lauryl sulfate (SDS) solution was also studied for strongly adsorbed protein. Based on the single protein solution experiment, one couple of basic and acidic proteins, which have different
adsorption and desorption properties for change in pH, were selected to elucidate the competitive adsorption by filtration of a binary protein solution and selective desorption using buffer solution.

Materials and Methods

Membranes and chemicals  Alumina ceramic membranes, Cefilt-MF (Nihon Gaishi Co., Tokyo) and MEBRALOX (Toshiba Ceramics Co., Tokyo), were used. The nominal pore size of each membrane was 0.2 μm. BSA, lysozyme and trypsinogen were purchased from the Sigma Chemical Company (St. Louis, Mo.). Cytochrome c was obtained from Wako Pure Chemical Industries, Ltd (Osaka). β-Galactosidase was donated by K.I. Chemical Industry Co., Ltd (Shizuoka). Characteristics of the proteins are summarized in Table 1. The molecular weight (MW), and isoelectric point (pI) were quoted from the paper on β-galactosidase (Takenishi et al., 1983) and from a data book (Anonymous, 1975) for other proteins. For all other chemicals, fine grade was used.

Measurement of protein concentration  In order to measure the protein concentration, an UV spectrophotometer (220, 280 nm) was used for single protein solutions. For dilute protein solutions, the concentration was measured using a Micro BCA Protein Assay Reagent Kit (Pierce Chemical Co., Rockford). An HPLC system (Japan Spectroscopic Co., Ltd., Tokyo) was used for the binary protein solutions, which consists of a high pressure pump; 880-PU, degasser; 880-51, column temperature controller; TU-100 and UV detector; 875-UV. A gel permeation column, Asahipak GF-510 HQ (GL Sciences Inc., Tokyo), was fitted to the HPLC system and the column temperature was adjusted to 50°C. The mobile phase was 0.2 mol•L⁻¹ phosphate buffer (pH 7.0) and the flow rate was 0.7 mL•min⁻¹.

Surface area and zeta-potential of ceramic membranes  The surface area of a ceramic membrane was measured by the nitrogen adsorption method. The zeta-potential of the membrane was obtained by a nondestructive method to measure the trans-membrane stream potential (E) between two electrodes set near the membrane surfaces on the feed and permeate sides. A one mmol•L⁻¹ NaCl solution was filtered at a pressure, AP. The ratio of E to AP is constant and proportional to the zeta-potential (ζ) according to the Helmholtz-Smoluchowski equation.

\[ E/AP = \varepsilon \zeta / (4\pi \mu \lambda) \]  

where ε is the dielectric constant of the solution, μ is the viscosity of the solution and λ is the specific conductance of the solution.

| Protein       | Source          | MW    | pI    |
|---------------|-----------------|-------|-------|
| Lysozyme      | Chicken egg     | 14600 | 10.5  |
| Cytochrome c  | Horse           | 11702 | 10.1  |
| Trypsinogen   | Bovine          | 23991 | 9.3   |
| β-Galactosidase⁴ | P. multicolor  | 126000| 5.9   |
| BSA           | Bovine          | 66296 | 4.9   |

⁴Takenishi et al., 1983.

Adsorption of protein by soaking  A 1 g piece of a ceramic membrane was soaked in 200-800 mg•L⁻¹ protein solution of various pHs at 25°C. The protein adsorption reached equilibrium in 4 days. The amount of protein adsorbed was calculated from the decrease in concentration of the protein solution.

Adsorption and desorption of protein using filtration and desorption by soaking in SDS solution—Single protein system

(S-1) Adsorption  Three liters of 60 mg•L⁻¹ protein solution (absorbance at 220 nm was 1) in 20 mmol•L⁻¹ phosphate buffer, pH 3 or 7, was cross-flow-filtered at 50 kPa with a ceramic membrane (5 cm length, 0.6 cm inner diameter) at 25°C for 60 min. The amount of adsorbed protein was calculated from the concentration difference between the feed and permeate.

(S-2) Washing  The filtration apparatus was washed with water and phosphate buffer of the same pH as the protein solution. The membrane was detached from the module during the washing to retain the adsorbed protein.

(S-3) Desorption For protein desorption, the membrane was flushed with 20 mmol•L⁻¹ phosphate buffer, pH 3, 7 or 11, under the same conditions as for protein adsorption. After 60 min, it was flushed with 1 mol•L⁻¹ NaCl in the same buffer. The permeate was collected to measure the protein concentration.

(S-4) Desorption by SDS solution  After flushing with the NaCl solution, the membrane was detached from the module and soaked in 10 g•L⁻¹ SDS solution of 10-20 mL for 18 h. Protein concentration in the SDS solution was measured using a Micro BCA Protein Assay Reagent Kit and the amount of protein desorbed from the membrane was calculated.

Competitive adsorption and selective desorption of proteins using filtration—Binary protein system

(M-1) Adsorption  Three liters of 120 mg•L⁻¹ protein mixture in 20 mg•L⁻¹ phosphate buffer, pH 3, was cross-flow-filtered for 10 min under the same conditions as mentioned in (S-1). The solution contained β-galactosidase (60 mg•L⁻¹) and lysozyme (60 mg•L⁻¹). The amount of adsorbed protein was calculated from the concentration difference in the feed and permeate.

(M-2) Washing  The filtration apparatus was washed in the same way as described in (S-2).

(M-3) Desorption  For protein desorption, 20 mmol•L⁻¹ phosphate buffer of pH 7 was filtered at the same condition as for protein adsorption and the permeate was fractionated into sampling tubes to measure the protein concentration.

Results and Discussion

Surface area and zeta-potential of ceramic membranes  The specific surface areas of the Cefilt-MF (NGC) and MEBRALOX (TC) membranes were 0.29 and 0.08 m²•g⁻¹, respectively. Both ceramic membranes were composed of three layers of sintered alumina particles of different sizes. It is considered that the surface area is mainly affected by the thickness of the skin layer made of the finest particles at the final sintering step. Using scanning electron microscopy (SEM), the thickness of the skin layer of the NGC membrane...
was approximately 3.5 times larger than that of a TC membrane.

The zeta-potential values of the NGC and TC membranes are shown in Fig. 1. Using the nondestructive method, the isolectric points of the NGC and TC membranes were estimated as 5.5 and 3.5, respectively. In different studies, the isolectric point of aluminum oxide ranged from 2.3 to 10 (Parks, 1965). It is considered that the difference in the isolectric points is due to the calcination temperature since aluminum oxide becomes acidic after calcination (Robinson et al., 1964). A ceramic membrane made of sintered aluminum oxide particles is negatively charged with hydroxyl groups on its surface (Shimizu et al., 1989). The alumina ceramic membrane exhibited a tendency to shift its isolectric point to more basic when in contact with distilled water. However, the isolectric point slowly changed from 5.5 to 6.0 by soaking in water at 37°C for 1000 h, and from 5.5 to 6.5 by refluxing with water at 37°C for 100 h (Shimizu et al., 1989). This suggests that the variation in the zeta potential of the membrane is negligible in the experiments (soaking for 96 h, filtration for 20 min - 3 h) at 25°C done in this study.

Adsorption of protein by soaking Figure 2 shows the adsorbed amount of β-galactosidase on NGC and TC membranes at pH 6 as a function of the protein concentration. Based on membrane mass, the amount of protein using NGC membranes was 3.5 times larger than that with TC membranes. However, on the basis of the unit surface area, the amount of protein was approximately the same with each membrane although their zeta-potential profiles were different. Since the NGC membrane has a larger capacity to adsorb protein on the basis of unit mass, which is advantageous in measuring the amount of protein adsorbed, it was used in subsequent experiments.

The effect of pH on protein adsorption was investigated by soaking the NGC membranes in 800 mg·l⁻¹ protein solution and the results are summarized in Fig. 3. It is considered that the adsorbed protein formed a monolayer by referring to the reported specific amount of protein adsorbed in previous papers: 0.2 - 0.4 mg·m⁻² (BSA) (Bowen & Gan, 1990), 1.4 mg·m⁻² (BSA) (Lok et al., 1983), 2.7 mg·m⁻² (β-lactoglobulin) (Persson et al., 1993) and 3.5 mg·m⁻² (human albumin) (Brash & Samák, 1978). The proteins were positively charged at pHs lower than their pI, and the NGC membrane was negatively charged at pHs higher than its pI of 5.5. For basic proteins, such as lysozyme, cytochrome c, and trypsinogen, the amount of adsorbed protein was relatively larger in the range of pH 5.5 than that in each pI due to the electrostatic interaction. In the case of BSA, there was a peak of protein adsorption around pI 4.9, which is close to the pI of the NGC membrane. Both BSA and the membrane were charged to the same sign, positively on the acidic side to pI, and negatively on the basic side. This adsorption profile for β-galactosidase was different from the other proteins used in this study. Although β-galactosidase and the membrane were positively charged below pH 5.5, the protein adsorption value was still high at pH 3. Similar phenomena were reported by Pouliot et al. (1994) in whey filtration using an alumina ceramic membrane. The hydraulic resistance caused by protein adsorption increased at pH 4.8, though the alumina ceramic
membrane was positively charged and the main whey proteins, α-lactalbumin (pI: 4.8), and β-lactoglobulin (pI: 5.18), were neutral and positively charged, respectively. This suggested that the electrostatic interaction is not always the dominant factor for protein adsorption.

**Adsorption and desorption of protein by filtration and soaking in SDS solution—Single protein system**

Figure 4 shows the time courses of the changes in the permeate flux, concentration of lysozyme in the permeate, and the amount of lysozyme remaining on the NGC membrane during the adsorption (pH 7)-desorption (pH 7 or 11) process. Lysozyme solution in pH 7 buffer was filtered at the adsorption step, the membrane was flushed with pH 7 or 11 buffer at the first desorption step, and it was flushed with 1 mol·L⁻¹ NaCl solution in pH 7 or 11 buffer at the second desorption step. The permeate flux was slightly affected by the protein adsorption on the membrane surface but stable in the range of 3-4×10⁻⁴ m²·m⁻²·s⁻¹. Lysozyme was adsorbed up to 3 mg·m⁻² in the adsorption step, which nearly coincided with the result from the adsorption experiment by soaking. The slight change in flux and the value of the adsorbed lysozyme suggested that the monolayer of the lysozyme was formed on the membrane surface during filtration. By changing the buffer pH from 7 to 11, the protein was rapidly released and a six-fold concentrated solution over the filtered one was obtained in the permeate side within one minute. The operating pressure was constant at 50 kPa. It is considered that if a lower pressure was applied, the flux would be lower and a more concentrated protein might be obtained. The amount of protein remaining on the membrane at pH 11 was less than that obtained by the soaking method at pH 11. It is suggested that the adsorption strength was affected by the pH condition of the protein solution. The mechanism is considered as follows: at pH 7 an electrostatic interaction was the dominant factor for the adsorption since the charges of lysozyme and the membrane surface had opposite signs, and therefore, the protein was easily desorbed by changing pH; on the other hand, lysozyme was almost neutral at pH 11 and the protein was adsorbed by different mechanisms, such as hydrophobic interaction, deformation and aggregation which are considered to be irreversible. Changing the ionic strength using NaCl, a concentrated solution was also obtained. However, 20% of the protein remained on the membrane (0.6 mg·m⁻²) by increasing the ionic strength while more than 96% of the protein was eluted by changing the pH. The results of the adsorption (pH 3)-desorption (pH 3 or 7) process using the lysozyme are shown in Fig. 5. Lysozyme was not desorbed by changing the pH from 3 to 7 since the amount of lysozyme adsorbed at pH 3 was smaller than that at pH 7. These results correspond with those shown in Fig. 3. By changing the ionic strength, 70% of the protein was released, although the effect was not very strong.

Figure 6 shows the time courses of the changes in the permeate flux, concentration of β-galactosidase in the permeate, and the amount of lysozyme remaining on the NGC membrane during the adsorption (pH 3)-desorption (pH 3 or 7) process. β-Galactosidase solution in pH 7 buffer was filtered at the adsorption step, the membrane was flushed with pH 3 or 7 buffer at the first desorption step, and it was flushed with 1 mol·L⁻¹ NaCl solution in pH 3 or 7 buffer at the first desorption step, and it was flushed with 1 mol·L⁻¹ NaCl solution in pH 3 or 7 buffer at the second desorption step. The permeate flux was slightly affected by the protein adsorption on the membrane surface but stable in the range of 3-4×10⁻⁴ m²·m⁻²·s⁻¹. Lysozyme was adsorbed up to 3 mg·m⁻² in the adsorption step, which nearly coincided with the result from the adsorption experiment by soaking. The slight change in flux and the value of the adsorbed lysozyme suggested that the monolayer of the lysozyme was formed on the membrane surface during filtration. By changing the buffer pH from 3 to 7, the protein was rapidly released and a six-fold concentrated solution over the filtered one was obtained in the permeate side within one minute. The operating pressure was constant at 50 kPa. It is considered that if a lower pressure was applied, the flux would be lower and a more concentrated protein might be obtained. The amount of protein remaining on the membrane at pH 11 was less than that obtained by the soaking method at pH 11. It is suggested that the adsorption strength was affected by the pH condition of the protein solution. The mechanism is considered as follows: at pH 7 an electrostatic interaction was the dominant factor for the adsorption since the charges of lysozyme and the membrane surface had opposite signs, and therefore, the protein was easily desorbed by changing pH; on the other hand, lysozyme was almost neutral at pH 11 and the protein was adsorbed by different mechanisms, such as hydrophobic interaction, deformation and aggregation which are considered to be irreversible. Changing the ionic strength using NaCl, a concentrated solution was also obtained. However, 20% of the protein remained on the membrane (0.6 mg·m⁻²) by increasing the ionic strength while more than 96% of the protein was eluted by changing the pH. The results of the adsorption (pH 3)-desorption (pH 3 or 7) process using the lysozyme are shown in Fig. 5. Lysozyme was not desorbed by changing the pH from 3 to 7 since the amount of lysozyme adsorbed at pH 3 was smaller than that at pH 7. These results correspond with those shown in Fig. 3. By changing the ionic strength, 70% of the protein was released, although the effect was not very strong.

**Fig. 4.** Time courses of the changes in permeate flux, concentration of lysozyme in permeate, and amount of lysozyme remaining on NGC membrane during adsorption (pH 7)-desorption (pH 7 or 11) process.

**Fig. 5.** Time courses of the changes in permeate flux, concentration of lysozyme in permeate, and amount of lysozyme remaining on NGC membrane during adsorption (pH 3)-desorption (pH 3 or 7) process.
second desorption step. β-Galactosidase was desorbed by changing the pH or ionic strength of the buffer for flushing. The flux did not significantly change during the process. Most of the adsorbed protein was eluted in the first 20 s of the desorption process by changing the pH from 3 to 7. The protein in the permeate was then concentrated more than seven-fold within 20 s. It is an important subject to examine the activity of the protein desorbed from the membrane. Miwa et al. (1991a, b) focused on the activity of β-galactosidase desorbed from an alumina ceramic membrane. They confirmed that β-galactosidase desorbed from a ceramic membrane retained its enzymatic activity and presented a novel membrane bioreactor system for continuous production and purification of β-galactosidase. In their system, the ceramic membrane were effectively utilized to retain microorganisms, to remove waste medium and to obtain purified and concentrated β-galactosidase as a product by filtering fermentation broth and repeatedly flushing with elution buffer. This application study to obtain an active enzyme using a ceramic membrane supports the experimental results by Norde et al. (1986) described in the introduction.

Figure 7 demonstrates the amount of protein remaining on the ceramic membrane after filtration of the protein solution, buffer solution and 1 mol·l⁻¹ NaCl in buffer, and after soaking in 10 g·l⁻¹ SDS solution. With the other basic proteins, cytochrome c and trypsinogen, the results were similar to that obtained with lysozyme. The amount of trypsinogen remaining on the membrane was more than 40% even by changing the buffer’s pH, which was relatively large in comparison with those observed with lysozyme and cytochrome c. On the other hand, BSA was hardly released at pH 3 by changing the ionic strength. At pHs of both 3 and 7, BSA and the NGC membrane were charged with the same sign, therefore, the adsorption of BSA on the membrane was not due to an electrostatic interaction. By changing the pH from 3 to 7, 30% of the BSA was released approximately due to a charge inversion of the proteins. Kondo et al. (1991) studied the structural changes in protein molecules adsorbed on silica particles by measuring their CD spectra. The CD spectra of BSA was largely changed after adsorption on the silica particles at pH 5 while that of cytochrome c at pH 8.5 only slightly changed. The change in the CD spectra corresponded with that in an α-helix fraction. They considered that the BSA molecule was flexible and deformed on the silica surface since its helical content is 70%. On the contrary, cytochrome c, with a helical content of 27%, was considered to be rigid. Actually adiabatic compressibility of BSA is 10.5 cm²·dyne⁻¹, while that of cytochrome c is 0.066 cm²·dyne⁻¹ (Gekko & Hasegawa, 1986).

In Fig. 7, the black colored area denotes the amount of protein which was not desorbed even with SDS solution. The proteins are classified into several types based on the ratio of the amount unwashable with SDS solution to the total amount of adsorption. For lysozyme and cytochrome c, most of protein adsorbed was washed without SDS solution. For β-galactosidase, the adsorption amount itself was very large.
and the ratio of the amount unwashable to the adsorption amount was from 1/4 to 1/3. The ratios of BSA and trypsinogen were large and around 1/3 to 1/2. Especially, for BSA at pH 3, only a slight amount of protein was washed with pH 3 buffer and the pH 3 buffer with NaCl. Bohnert and Horbett (1986) reported that proteins, which adsorbed on a membrane, became unwashable by SDS solution with the passage of time, which suggests that the contact area of the protein expands on the membrane surface. It is concluded that BSA is the most flexible of all the examined proteins and has a tendency to expand on a membrane surface and to be tightly fixed.

**Competitive adsorption and selective desorption of proteins by filtration—Binary protein system** Based on the above experimental results, lysozyme and β-galactosidase were selected to examine the competitive adsorption and selective desorption of the proteins during filtration of the binary protein solution of pH 3 and flushing with buffer solution of pH 7. Figure 8 shows the time courses of the changes in concentrations of lysozyme and β-galactosidase in the permeate and the amount of lysozyme remaining on the NGC membrane during the adsorption (pH 3)-desorption (pH 7) process. The mixture of lysozyme and β-galactosidase in pH 7 buffer was filtered at the adsorption step and the membrane was flushed with pH 7 buffer at the desorption step. At the adsorption step, the concentration of β-galactosidase in the permeate was lower than that of the single protein system since the adsorption area was partially occupied by the lysozyme. The amount of adsorbed lysozyme was relatively lower than that of β-galactosidase. At the desorption step, the concentrations of β-galactosidase and lysozyme in the permeate in the first 20 s were 470 mg·L⁻¹ (about seven times larger than the applied concentration of 66 mg·L⁻¹) and less than 4 mg·L⁻¹ (applied concentration of 55 mg·L⁻¹), respectively. The amounts of protein desorbed by changing the buffer pH from 3 to 7 for both β-galactosidase and lysozyme are in good agreement with those shown in Fig. 7. The amount of β-galactosidase remaining on the membrane decreased to one sixth in this step while that of the lysozyme did not vary.

**Conclusion**

The zeta-potential of alumina ceramic membranes was evaluated and it was found that they were negatively charged at pH 7. Properties of the protein adsorption onto the alumina ceramic membrane were investigated by the soaking method. For basic proteins, the amount of protein adsorbed reached a maximum at a pH lower than the isoelectric point due to an electrostatic interaction between the protein and membrane. It is considered that BSA is the most flexible of all the proteins examined and has a tendency to expand on a membrane surface and to be tightly fixed. In filtration processes using the alumina ceramic membrane, the adsorbed protein, which was desorbed and concentrated by changing the ionic strength or pH of the solution, adequately coincided with the results of protein adsorption by soaking. The adsorption-desorption process by filtration was applied to a binary protein solution of lysozyme and β-galactosidase. In the adsorption step, β-galactosidase was adsorbed more than the lysozyme, however, the absolute amount adsorbed was smaller than that with the single β-galactosidase solution since the adsorption site was partially occupied by the lysozyme. At the desorption step in the first 20 s, β-galactosidase was desorbed and concentrated by seven-fold in the permeate by changing the pH from 3 to 7, while the concentration of the lysozyme in the permeate was one-tenth of its original value.

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