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Adsorption and elution of bovine γ-globulin using an affinity membrane containing hydrophobic amino acids as ligands

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ABSTRACT

A hollow-fibre affinity membrane containing hydrophobic amino acids as ligands was prepared by the radiation-induced grafting of glycidyl methacrylate onto a porous polyethylene hollow fibre and subsequent coupling with phenylalanine (Phe) or tryptophan (Trp). The densities of the Phe and Trp ligand of the resulting affinity membrane were 0.4 and 0.4 mol/kg, respectively. The Trp-containing affinity membrane exhibited a higher amount of adsorbed bovine γ-globulin (BGG) than the Phe-containing membrane. To evaluate the adsorption behaviour of the membrane, the BGG-containing buffer solution was permeated from the inside to the outside of the Trp-containing hollow-fibre affinity membrane through the ligand-immobilized pores. The breakthrough curves as a function of effluent volume coincided irrespective of the flow-rate, *i.e.* the residence time (55–220 s) of the solution across the membrane (thickness 0.83 mm), as a result of negligible mass transfer resistance. A series of chromatographic procedures, (adsorption–washing–elution) was repeated twice and a satisfactory quantitative elution was attained. The reproducible profile of the flux and the protein concentration assured a quantitative cycle of chromatography using the affinity membrane containing Trp as a ligand.

INTRODUCTION

Affinity ligands can be classified into biospecific and pseudobiospecific ligands [1]. Amino acids, especially hydrophobic amino acids, belong to the group of pseudobiospecific ligands. Phenylalanine (Phe)- or tryptophan (Trp)-containing immunoadsorbents based on polyvinyl alcohol beads have been used for rheumatism and myasthenia gravis therapy [2,3]. In addition, Phe has been used as a ligand for the purification of serum proteins [4,5] and enzymes [4,6].

Membrane-based affinity chromatography was proposed by Brandt *et al.* in 1988 [7]. The technique has advantages over conventional bead-based chromatography for the following two reasons: (1) the module charged with hollow fibres requires a much lower operating pressure than a bead-packed bed; and (2) as the biomolecule to be determined can be directly transported by convection to the ligand immobilized on the inner surface of the microporous membrane, faster adsorption onto the affinity membrane can be attained. Brandt *et al.* [7] demonstrated the efficient isolation of fibrinogen and IgG with gelatin- and protein A-containing affinity membranes. However, details of the preparation of affinity membranes and properties such as ligand density and pore structure were not described.

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Functional materials have been developed by applying radiation-induced graft polymerization. These include a packing material for the selective recovery of uranium from sea water [8], a chelating hollow-fibre membrane for the collection of cobalt ultrapure water [9] and affinity membranes for the separation of biomolecules from biological fluids [10]. Radiation-induced grafting is a useful technique as it is applicable to various kinds and arbitrary shapes of polymers.

In this study, affinity membranes based on polyethylene hollow fibres were prepared and hydrophobic amino acid–bovine γ-globulin (BGG) were selected as an affinity pair. The objectives of the study were three-fold: (1) to compare the effectiveness of the Phe and Trp ligands with respect to the adsorption isotherm and kinetics; (2) to clarify the breakthrough features by varying the inlet concentration and flow-rate of the feed; and (3) to examine the adsorption–elution cycle using an affinity membrane.

EXPERIMENTAL

Preparation of Phe- and Trp-containing affinity membranes

Hollow-fibre affinity membranes containing Phe and Trp as ligands were prepared by the radiation-induced grafting of glycidyl methacrylate (GMA) onto a porous polyethylene hollow fibre, followed by coupling of the epoxide group produced with L-Phe and L-Trp. The commercially available hollow fibre (Asahi Chemical Indusry, Japan) was used as a base polymer for grafting. This hollow fibre has been used industrially for microfiltration. The inner and outer diameters of this hollow fibre are 2.02 and 3.28 mm, respectively. The hollow fibre is made of polyethylene with a nominal pore diameter of $2 \cdot 10^{-7}$ m and a porosity of 71%. The details of grafting and coupling have been described elsewhere [11]. The resulting hollow fibres containing Phe and Trp as ligands were referred to as Phe-T and Trp-T fibres, respectively; T denotes tubular. The degree of GMA grafting and coupling efficiency were about 120 and 12–13%, respectively:

$$\text{degree of GMA grafting} = 100[(W_1 - W_0)/W_0]$$

Coupling efficiency for Phe-T fibre (X_p)

$$W_2 = W_0 + (W_1 - W_0)[X_p(142 + 165) + (1 - X_p)(142 + 18)]/142$$
$$X_p = [142(W_2 - W_0)/(W_1 - W_0) - 160]/147$$

Coupling efficiency for Trp-T fibre (X_t)

$$W_2 = W_0 + (W_1 - W_0)[X_t(142 + 204) + (1 - X_t)(142 + 18)]/142$$
$$X_t = [142(W_2 - W_0)/(W_1 - W_0) - 160]/186$$

where W_0, W_1 and W_2 are the weights of a starting fibre, GMA-grafted fibre and the Phe-T or Trp-T fibres, respectively. The values 142, 165, 204 and 18 are the molecular weights of GMA, Phe, Trp and water. The ligand density was calculated as:

$$\text{ligand density for Phe-T fibre} = X_p(W_1 - W_0/142/W_2$$

$$\text{ligand density for Trp-T fibre} = X_t(W_1 - W_0/142/W_2$$

The chemical structures of the Phe-T and Trp-T fibres are illustrated in Fig. 1.

The dimensions of the resulting hollow fibres were measured using a microscope and a scale. The specific surface area of the dried hollow fibre was determined using Quantasorb (Yuasa Ionics, Japan) according to the BET method. The apparent density of the hollow fibre was calculated by dividing the dry weight by the wet volume.

Adsorption isotherm

The adsorption isotherm of BGG onto the Phe-T and Trp-T fibres was measured by the batchwise

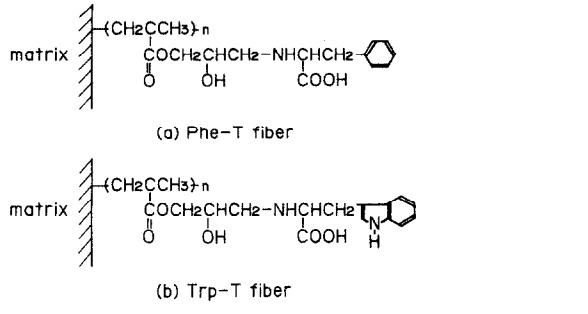

Fig. 1. Chemical structure of the affinity membrane containing Phe or Trp as a ligand.

method. The prescribed weight of each hollow fibre was immersed in the protein solution. BGG was purchased from Sigma (St. Louis, MO, USA; No. G5009, Cohn Fraction II, III) and was used as received. A phosphate-buffered saline (PBS) solution was prepared by dissolving powdered Dulbecco's PBS (Nissui Pharmaceutical, Japan) in water. BGG was dissolved in the PBS buffer solution [pH 7.4; ionic strength (I) = 0.19], the concentration of which ranged from 1 to 4 g/l. Contact for 24 h allowed the system to attain equilibrium. The amount of BGG adsorbed onto the hollow fibre was calculated from the decrease of BGG concentration in the solution. BGG was determined by UV spectroscopy (at 280 nm).

Membrane affinity chromatography

Fig. 2a shows the experimental apparatus for the adsorption and elution of proteins using the hollow-fibre affinity membrane. The 12-cm-long hollow fibre was set in a U-shaped configuration. The protein solution in the syringe was fed at a constant flow-rate ranging from 0.010 to 0.040 l/h. The BGG solution was applied to the inside of the hollow-fibre affinity membrane and was permeated to the outside through the pores around which the ligand was immobilized. The effluent dropping from the outside of the membrane was collected continuously and the BGG concentration of each fraction was determined. After 30 ml of the effluent had crossed the membrane, the feed solution was switched to the buffer solution to rinse the membrane. After rinsing with 20 ml, 20 ml of the eluate were fed through at the same flow-rate. The eluate was a mixture of 1 M sodium chloride solution and 50% ethylene glycol [4]. This adsorption–washing–elution cycle was repeated once more. In each procedure, the concentration of BGG was determined.

To examine the specificity of the Trp-T fibre to serum proteins, a mixture of BGG and bovine serum albumin (BSA) in PBS buffer was permeated across the membrane at a constant flow-rate of 0.030 l/h. The inlet concentrations of BGG and BSA were both 1 g/l. Each concentration in the effluent was determined by liquid chromatography with a Asahipak GS-520H column (Asahi Chemical Industry, Japan). All experiments were performed at 303 K.

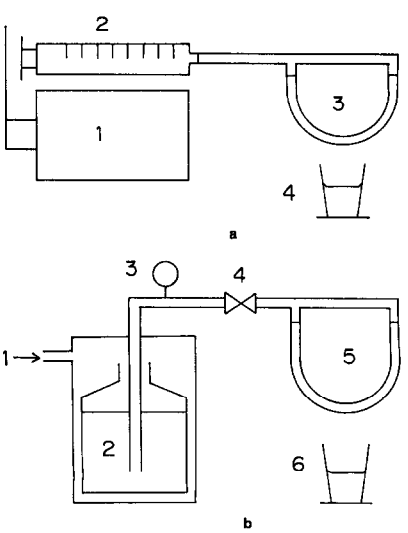

Fig. 2. Experimental apparatus for membrane chromatography. The solution was forced to permeate across the hollow-fibre membrane. (a) At constant flow-rate: 1 = syringe infusion pump; 2 = syringe; 3 = hollow-fibre membrane; 4 = measuring cylinder. (b) At constant filtration pressure: 1 = nitrogen cylinder; 2 = feed tank; 3 = pressure gauge; 4 = valve; 5 = hollow-fibre membrane; 6 = measuring cylinder.

Flux measurement

The initial filtration pressure of the Trp-T fibre was $3.0 \cdot 10^3$ N/m^2 at a flow-rate of 0.040 l/h. The flow-rate changes during the chromatographic procedures as a result of protein adsorption and elution. To examine the change in flow-rate of the hollow-fibre affinity membrane, a series of solutions, *i.e.* the BGG-containing buffer, the buffer and the eluate, were permeated across the Trp-T fibre at a constant filtration pressure of $1.0 \cdot 10^4$ N/m^2. The experimental apparatus is shown schematically in Fig. 2b. The flow-rate of each solution dropping from the outside of the membrane was measured. The flux, u_i, was calculated by dividing the flow-rate by the inner surface area of the membrane:

$$u_i = (\text{flow-rate})/\pi d_i L$$

where L is the length and d_i the inner diameter of the hollow fibre.

RESULTS AND DISCUSSION

Properties of Phe-T and Trp-T fibres

The physical properties of Phe-T and Trp-T fibres are summarized in Table I. The physical properties of the two affinity membranes were similar. The pure water flux and specific surface area of the Trp-T fibre were about 75 and 60% of those of the starting hollow fibre, respectively. The 120% degree of GMA grafting and 12.3% coupling efficiency of Trp gave a ligand density of 0.40 mol/kg. As the apparent density was 0.35 kg (dry)/l (wet), the ligand density per kg was converted into 0.14 mol/l (wet), which is two orders of magnitude greater than that of commercial affinity beads. For example, the ligand density of the commercial affinity bead containing Trp is 0.003 mol/l [12]. The remaining epoxide group is quantitatively converted on coupling into a diol group [13]. The diol group renders the polymer surface hydrophilic and prevents non-selective adsorption [13], whereas the coupled Phe and Trp adsorb BGG pseudospecifically. Both the inner and outer diameters of the hollow-fibre affinity membrane increased by chemical modification compared with the starting hollow fibre. The membrane thickness corresponds to the bed height when the hollow-fibre affinity membrane is regarded as a packed bed charged with affinity beads.

Adsorption isotherms

Fig. 3 shows the adsorption isotherms of BGG onto the Phe-T and Trp-T fibres. The Trp-T fibre bound more BGG than the Phe-T fibre. The reason for this difference is unclear. The data were correlated with a Langmuir-type equation and the saturation capacities of the Phe-T and Trp-T fibres were calculated to be 36 and 49 g/kg, respectively. The saturation capacity of the affinity membrane was determined from the specific surface area when the ligand was in excess of the protein [11].

TABLE I

PROPERTIES OF THE PHE-T AND TRP-T FIBRES

Property	Phe-T	Trp-T	Starting hollow fibre
Degree of GMA grafting (%)	120	120	–
Coupling efficiency (%)	12.8	12.3	–
Ligand density (mol/kg)	0.40	0.40	–
Inner diameter (mm)	2.43	2.43	2.02
Outer diameter (mm)	3.96	4.08	3.28
Specific surface area (m^2/g)	13.0	13.9	23.9
Apparent density (kg/l)	0.36	0.35	0.24
Porosity (–)	0.60	0.60	0.71
Pure water flux[a] (m/h)	0.090	0.090	0.12

[a] At a filtration pressure of $1.0 \cdot 10^4$ N/m^2.

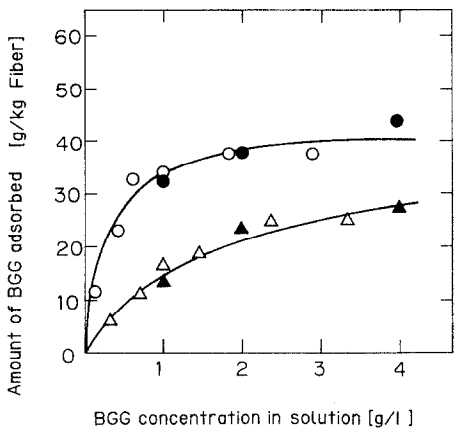

Fig. 3. Adsorption isotherms of BGG on the affinity membranes. The Phe-T (triangles) or Trp-T (circles) fibre was contacted with the BGG-containing buffer solution at 303 K by the batch mode (open symbols) and the breakthrough mode (closed symbols).

Affinity chromatography with hollow-fibre membrane

By varying the BGG concentration of the feed, the breakthrough curves (BTCs) were determined. Fig. 4a and b show the BTCs for the Phe-T and Trp-T fibres, respectively. As expected from the isotherm results, the Trp-T fibre exhibited more favourable characteristics than the Phe-T fibre. The amount of BGG adsorbed onto the membrane in equilibrium with the inlet concentration can be calculated by the following integration:

$$q_0 = \int_0^{V_s} (C_0 - C) \mathrm{d}V/W$$

where C_0, C and q_0 are the concentrations of the feed and effluent and the amount of the protein adsorbed in equilibrium with C_0. V and W are the effluent volume and the weight of the membrane, respectively. V_s is the effluent volume for which concentration reaches C_0. The results of this integra-

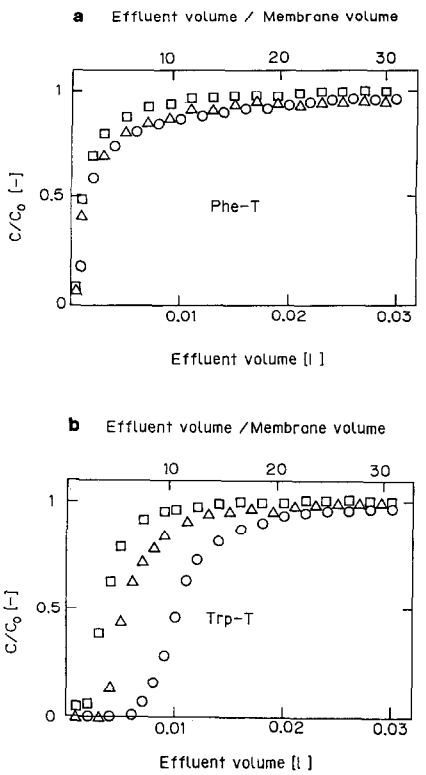

Fig. 4. BTCs as a function of inlet concentration. (a) Phe-T fibre; (b) Trp-T fibre. The BGG solution was applied to the inside of the 12-cm-long hollow fibre at a constant flow-rate of 0.02 l/h. The BGG concentration of the effluent dropping from the outside across the hollow fibre was determined as a function of the effluent volume. C_0: (○) 1; (△) 2; and (□) 4 g/l.

tion were inserted into Fig. 3. The isotherm data calculated from the BTC analysis agreed with the data determined by the batchwise method. Although the flow-rate can interfere with adsorption on the polymer surface due to the deformation of protein by shear stress [14,15], the flow-rate in the pores did not affect the adsorption capacity in this instance. The shear stress on the surface of the straight pore can be calculated from the following [16]:

$$\text{shear stress} = \Delta P r_p/(2L_p)$$

where ΔP is the filtration pressure and r_p and L_p are the radius and length of the pore, respectively. When the filtration pressure was $3.0 \cdot 10^3$ N/m^2, the shear stress was calculated to be about 0.2 N/m^2 at most, assuming that r_p was $1.0 \cdot 10^{-4}$ mm and L_p is the thickness of the Trp-T fibre (0.83 mm). This stress is too small to damage the protein.

Fig. 5 shows the BTCs of the Trp-T fibre as a function of the flow-rate of the feed. The upper abscissa in Fig. 5 represents the "membrane volume" corresponding to the bed volume, which is defined as:

$$\text{membrane volume} = (\text{effluent volume})/(\text{membrane volume})$$

The change in the flow-rate indicates the variation of the residence time of the solution across the membrane. The flow-rate can be converted into the mean residence time, t_r:

$$t_r = \varepsilon(d_o^2 - d_i^2)/4d_i u_i$$

where d_i and d_0 are the inner and outer diameters of the hollow-fibre affinity membrane, respectively. The porosity of the hollow fibre, ε, can be determined from measurement of its water content. When the flow-rate varied from 0.010 to 0.040 l/h, the residence time ranged from 55 to 220 s. As a result, the residence time caused almost no difference in the BTC. This was indicative of the negligible mass transfer resistance. A rectangular line for an ideal BTC is shown in Fig. 5, where the ideal breakthrough point, t_i, was calculated from the following:

$$t_i = (d_0^2 - d_i^2)\rho_a q_0/4u_i d_i C_0$$

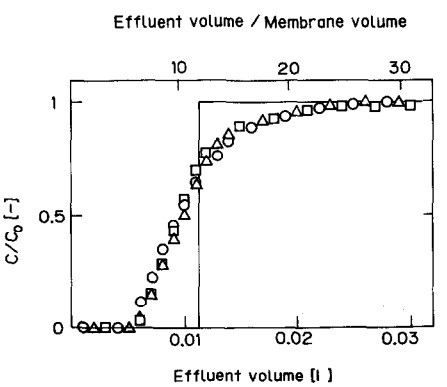

Fig. 5. BTCs of the Trp-T fibre as a function of flow-rate. The BGG solution was fed at a feed concentration of 1 g/l. Flow-rate: (○) 0.01; (△) 0.02; and (□) 0.04 l/h.

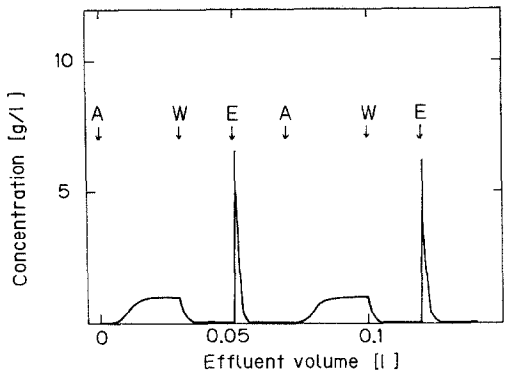

Fig. 6. Concentration change of BGG in membrane affinity chromatography. The adsorption–washing–elution cycle using the 12-cm-long Trp-T fibre was repeated twice at a constant flow-rate of 0.030 l/h. The symbols A, W and E designate adsorption, washing and elution, respectively. C_0 = 1 g/l.

where ρ_a is the apparent density of the affinity membrane. The dispersion in the BTC was obtained. This is due to the distribution in the pore length of the membrane, *i.e.* the residence time distribution of the solution across the membrane.

Fig. 6 shows the concentration change of BGG with effluent volume during the chromatographic procedures of adsorption, washing and elution. The mass balance was almost satisfied for the BGG and Trp-T fibre affinity system. Moreover, the repeated use of the affinity membrane showed a similar chromatographic performance. With an eluate of 1 *M* sodium chloride solution and 50% ethylene glycol, the peak and average concentrations of BGG were to 7 and 5.5 g/l, respectively.

A BTC for the mixture of BGG and BSA is shown in Fig. 7. BSA had a faster breakthrough behaviour than BGG at the same concentration of 1 g/l due to the higher specificity of the Trp-T fibre to BGG.

Flux consideration

Fig. 8 shows the variation of the flux under a constant filtration pressure of $1.0 \cdot 10^4$ N/m^2 during affinity membrane chromatography. As the adsorption of BGG onto the pore surface of the Trp-T fibre proceeded, the flux gradually decreased. Switching to the feed of the viscous eluate caused the flux to decrease irrespective of protein elution. The viscosities of the PBS buffer and eluate were determined to be $0.94 \cdot 10^{-3}$ and $2.81 \cdot 10^{-3}$ N/m^2 s, respectively. The viscosity ratio of the buffer to the eluate, 0.33, did not agree with the reciprocal of the flux ratio, 0.24. This is due to the difference in the interaction between the graft chain immobilized on the membrane and the solution permeating through its pores [17]; the eluate of 1 *M* sodium chloride solution and 50% ethylene glycol will cause the graft chain to swell.

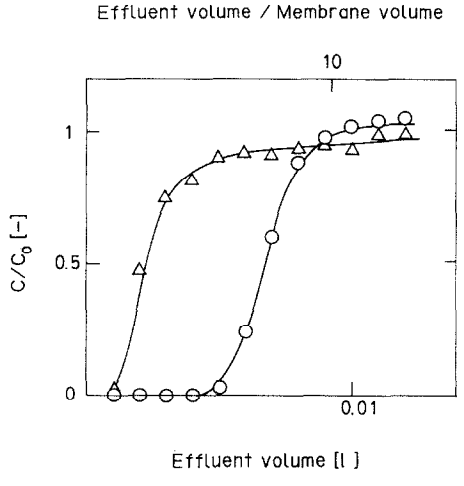

Fig. 7. BTC of the Trp-T fibre for the mixture of (○) BGG and (△) BSA. The inlet concentrations were both 1 g/l. Flow-rate = 0.03 l/h.

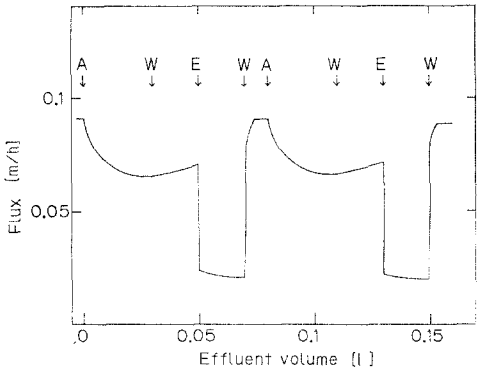

Fig. 8. Flux change in membrane affinity chromatography. The adsorption–washing–elution cycle using the 12-cm-long Trp-T fibre was repeated twice at a constant filtration pressure of $1.0 \cdot 10^4$ N/m^2. C_0 = 1 g/l. The flow-rate of the effluent was determined and was converted into the flux. The symbols are the same as those used in Fig. 6.

The affinity membrane recovered the initial flux by washing with PBS buffer. The reproducible profile in the flux and the protein concentration indicates that a stable cycle is possible in chromatography using an affinity membrane containing a hydrophobic amino acid as a ligand.

SYMBOLS

C	concentration in the effluent (g/l)
C_0	concentration in the feed (g/l)
d_i	inner diameter of the affinity hollow fibre (cm)
d_o	outer diameter of the affinity hollow fibre (cm)
L	fibre length (cm)
L_p	pore length (cm)
ΔP	filtration pressure (N/m^2)
q_0	amount of protein adsorbed per kg of the affinity membrane in equilibrium with C_0 (g/kg)
r_p	pore radius (cm)
t_i	ideal breakthrough time (s)
t_r	mean residence time (s)
u_i	flux based on the inner surface area of the hollow fibre (cm/s)
V	volume of the effluent (l)
V_s	volume of the effluent with a concentration which reaches C_0 (l)
W_0	weight of the starting fibre (kg)
W_1	weight of the GMA grafted fibre (kg)
W_2	weight of the Phe- or Trp-T fibre (kg)
X_p	coupling efficiency for Phe-T fibre
X_t	coupling efficiency for Trp-T fibre
ε	porosity of the affinity hollow fibre
ρ_a	apparent density of the affinity hollow fibre (kg/l)

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