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Optical resolution with membranes derived from marine polymers

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ABSTRACT

Novel polyion-lipid complexes were prepared from quaterinized chitosan (QCh), which was a derivative of marine (natural) polymer, and three types of anionic amphiphile, such as sodium 1-dodecanesulfate (C12SNa), sodium 1-tetradecanesulfate (C14SNa), and sodium 1-hexadecanesulfate (C16SNa). Those complexes gave durable self-standing membranes. The QCh–lipid complex membranes prepared in the present study showed chiral separation ability; in other words, they selectively transported L-Lys from racemic mixture of Lys adopting a concentration gradient as a driving force for membrane transport. Permselectivity of QCh-C12S membrane toward L-Lys was determined to be 3.31 under the concentration difference of 1.0×10^{-3} mol dm^{-3}. From transport experiments and adsorption studies, it was revealed that the permselectivity was dominantly determined by diffusivity selectivity. It is expected that the present study would open a door to novel materials.

Keywords: Chiral separation; Chitosan; Green polymers; Membranes; Optical resolution; Permselectivity

1. Introduction

Chemical industries dealing with pharmaceuticals, agrochemicals, food additives, fragrances, and so forth, need enantiomerically pure compounds. Industrial-scale resolution of racemic mixtures by liquid chromatography is regarded as an efficient separation technique, but it is a batch separation process. By adopting simulated moving bed chromatography (SMB), optical resolution can be carried out continuously. However, the operation of SMB is complicated and requires optimization study. Apart from continuously operating SMB, chiral separation with membranes is regarded as a promising method to obtain optically pure compounds [1–3]. Comparing with optical resolution with SMB chromatography, membrane-based chiral separation would have following advantages, such as easy and continuous operation, energy-saving, low cost, and easy scale-up. Contrary to these, membrane-based chiral separation has been hardly emerged in industries, because of the lack of suitable membrane materials. To this end, to develop novel chiral separation membranes can be thought to be one of desirable research subjects in membrane science and technology.

Chiral separation membranes are divided into two types of membrane system, such as liquid [4–7] and polymeric (solid) [8–26] membranes. As is well known, excepting optical activity, each enantiomer shows the same physicochemical properties [27]. From this, there can be found chiral environment or chiral recognition sites in those chiral separation membranes [4–26], even though the membrane system for chiral separation was different. Among a large numbers of polymeric

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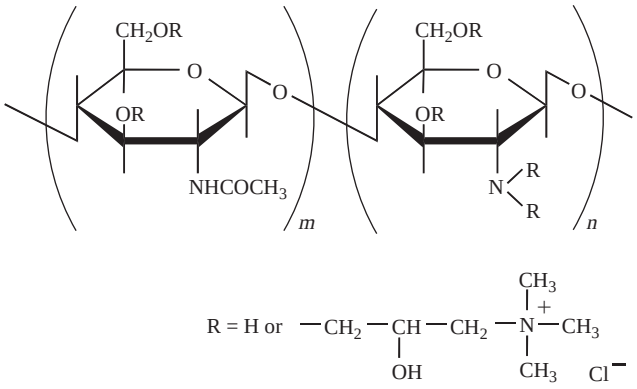

Fig. 1. Chemical structure of QCh having 2-hydroxypropyltrimethylammonium moiety.

materials, natural polymers are promising candidate materials for chiral separation membranes because they are expected to have chiral recognition sites or chiral environment. Based on this, chiral separation has been studied by adopting DNA [17,19,20,22] and chitosan [16,26], which are an environmentally-friendly 'green (marine)' polymer and a derivative of that, respectively. Chiral separation with DNA has been studied as a form of polyion complex membranes [20,22] since DNA is considered as a polyanion with a huge molecular weight [27]. Instead of DNA, in the present study, quaterinized chitosan (QCh), which is thought to be a polycation, was adopted as a membrane material for chiral separation. In addition to the above study [16,26], chitosan and its derivative have been intensively studied as a membrane material [28–36]. In the present study, QCh, having 2-hydroxypropyltrimethylammonium moieties as cationic charge sites, was adopted as a polycation for the raw material of chiral separation membranes. As shown in Fig. 1, cationic charge sites in QCh had Cl^- as counter ions. Novel polyion amphiphile complexes were prepared from QCh and anionic amphiphile of three types of 1-alkanesulfate. In the present paper, the film (membrane) formation abilities of those complexes newly obtained and their chiral separation abilities of those membranes will be described.

2. Experimental

2.1. Materials

QCh with 2-hydroxypropyltrimethyl-ammonium cationic charge sites, of which degree of quaterinization being 1.3, was kindly supplied by Dainichiseika Color & Chemicals Mfg. Co. Ltd. and used as received. Three types of anionic amphiphile, such as sodium 1-dodecanesulfate (C12SNa), sodium 1-tetradecanesulfate (C14SNa), and sodium 1-hexa decanesulfate (C16SNa) were used without further purification. Dimethyl sulfoxide (DMSO), D-glutamic acid (D-Glu), L-glutamic acid (L-Glu), D-lysine (D-Lys), L-lysine (L-Lys), D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), and sodium azide (fungicide) were used without further purification. Water purified with an ultrapure water system (Simpli Lab, Millipores S. A., Molsheim, France) was used.

2.2. Preparation of QCh–lipid complexes

The preparation of QCh–lipid complex was simple like that of DNA–lipid complex [37,38]: a charge equiv. of an aqueous solution of QCh and aqueous anionic amphiphiles (C12SNa, C14SNa, or C16SNa) were mixed at a prescribed temperature (ambient temperature for C12SNa, 40°C for C14SNa, and 50°C for C16SNa). The formed precipitate was washed with water by filtration, centrifugation, or Soxhlet extractor and dried *in vacuo* at 80°C for 1 d.

The ^{1}H NMR spectra of QCh–lipid complexes were recorded on a BRUKER AV300 with the residual partially protonated DMSO (DMSO-d_6) as an internal standard (2.49 ppm), using DMSO-d_6 as a deuterated solvent.

2.3. Membrane preparation

A 0.4 g of QCh–lipid complex was dissolved in 7.6 g of DMSO. The DMSO solution thus prepared was poured into a TeflonPFA 75 mm diameter laboratory dish and the solvent was allowed to evaporate at 80°C for 24 h. The thickness of the membrane (film) was ca. 63 μm for QCh-C12S, ca. 69 μm for QCh-C14S, and ca. 57 μm for QCh-C16S membranes, respectively.

2.4. Characterization of QCh–lipid complex membranes

Moisture sorption experiments were made at the constant temperature of 20°C by using desiccator, of which humidity was adjusted to a relative humidity of 15%, 39%, (56) 58%, or 100% by using an aqueous sulfuric acid solution. The membrane sample was dried *in vacuo* at 50°C. The sorption amount of moisture was evaluated by a weight increase. Water content (%) is defined as:

$$\text{Water Content} = 100 \text{ x } (W_s - W_d)/W_d, \quad (1)$$

where W_d means weight of dry membrane and W_s is that of water swollen membrane, respectively.

Tensile stress–strain of QCh–lipid complex membrane was measured by SHIMADZU Autograph AGS-H under the relative humidity of 58% at the constant temperature of 20°C.

2.5. Enantioselective transport

A membrane (area 3.0 cm^2) was fixed tightly with Parafilm between two chambers of a permeation cell. The volume of each chamber was 40.0 cm^3. An aqueous solution of racemic mixture of amino acid was placed in the left-hand chamber and an aqueous solution in the right-hand chamber. Each concentration of racemic amino acid was fixed at 1.0×10^{-3} mol dm^{-3}. In both chambers, 0.02 wt.% of sodium azide was added as a fungicide. All experiments were carried out at 40°C. The amounts of the D-and L-isomers that permeated through the membrane were determined by liquid chromatography (LC) [JASCO UV 1580, equipped with a UV detector (JASCO 1570)] employing a CROWN-PAK CR(+) column (250 × 4.0 (i.d.) mm) (Daicel Chemical Ind., Ltd.) and aqueous perchloric acid solution as eluent.

The flux, J (mol cm^{-2} h^{-1}), is defined as:

$$J = Q/At, \tag{2}$$

where Q (mol) is the amount of permeated amino acid, A (cm^2) is the effective membrane area and t (h) is the time.

The permselectivity $\alpha_{L/D}$ is defined as the flux ratio J_L/J_D divided by the concentration ratio of racemic amino acids [L-AA]/[D-AA]:

$$\alpha_{L/D} = (J_L/J_D)/([\text{L-AA}]/[\text{D-AA}]). \tag{3}$$

2.6. Adsorption selectivity

The membranes were immersed in racemic mixture of Lys, similar to the mixtures studied in enantioselective transport, and the membranes were allowed to equilibrate at 40°C. Aliquots of the solution at the initial stage and after equilibrium had been reached were used for quantitative estimation by LC.

The amount of Lys in the supernatant subtracted from the initial amount in the solution gave the amount of Lys adsorbed by the membrane.

The adsorption selectivity $S_{A(L/D)}$ is defined as:

$$S_{A(L/D)} = ((\text{L-AA})/(\text{D-AA}))/([\text{L-AA}]/[\text{D-AA}]), \tag{4}$$

where (L-AA) and (D-AA) are the amounts of racemic amino acids adsorbed in the membrane, and [L-AA] and [D-AA] are the concentrations in the solution after equilibrium had been reached.

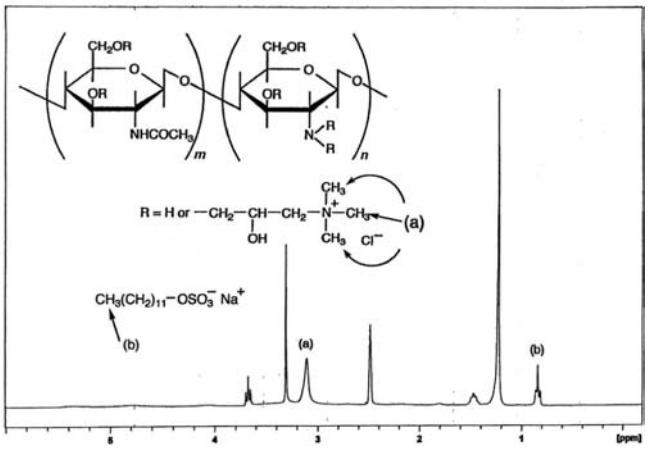

Fig. 2. ^{1}H NMR spectrum of QCh–C12S complex (300 MHz, DMSO-d_6.)

3. Results and discussion

3.1. Characterization of QCh–lipid complex membranes

As an example of ^{1}H NMR spectra, that of QCh–C12S complex is shown in Fig. 2. The compositions of QCh–lipid complexes were determined by using intensity of methyl protons for anionic amphiphiles (0.92 ppm) and that for QCh (3.17 ppm) in the ^{1}H NMR spectra. The ratios of the amount of anionic site to that of cationic site were determined to be 1.45 for QCh–C12S and QCh–C14S complexes and 1.46 for QCh–C16S one, respectively. Even though QCh–lipid complexes were washed with water thoroughly, anionic amphiphile existed in excess in every complex. It is an unsolved problem to obtain complexes with the ratio of sulfate anions to ammonium cations of unity. It can be thought that some anionic amphiphiles existed as a form of micelle in the QCh–lipid complex, though, there is, at the moment, no supporting data.

In Fig. 3, moisture sorption isotherms of the membrane at 20°C are shown. Each membrane gave a similar dependence on the relative humidity and the water content was increased over the relative humidity of around 60%.

The QCh–lipid complexes obtained in the present study gave durable self-standing membranes. Stress–strain curves of those three types of QCh–lipid complex membranes are shown in Fig. 4. Mechanical properties of those complex membranes are summarized in Table 1 together with common polymers [39]. The mechanical properties were not strengthened linearly by changes in carbon numbers of 1-alkanesulfate.

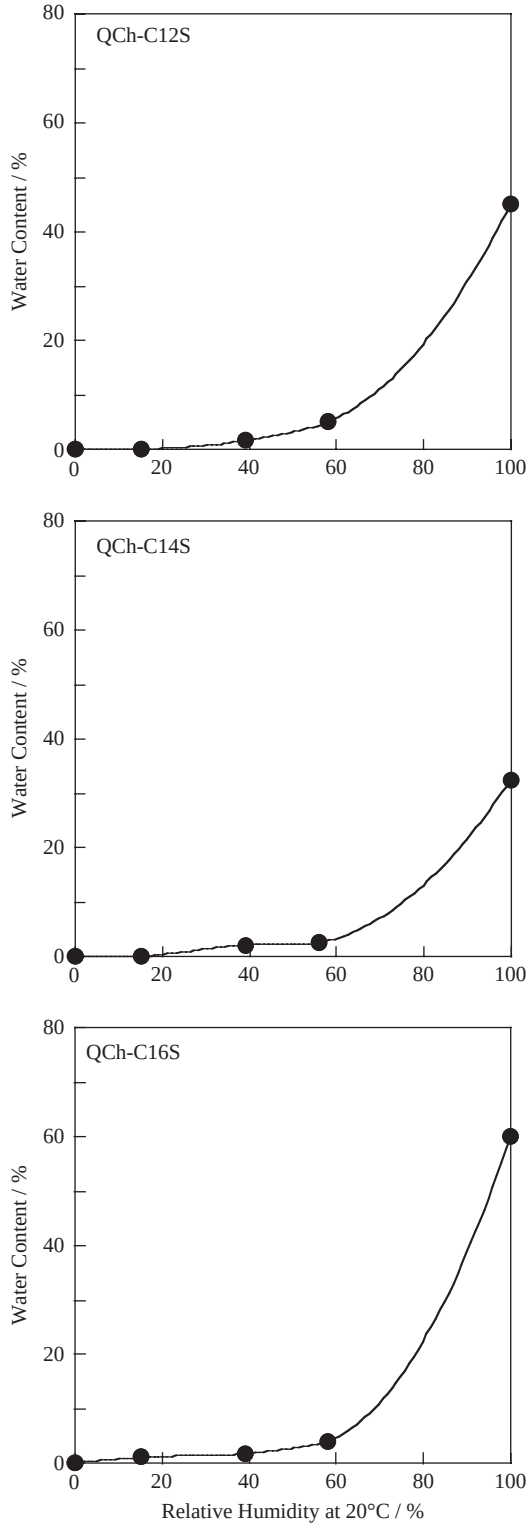

Fig. 3. Moisture sorption of QCh–lipid complex membranes at various values of relative humidity at 20°C.

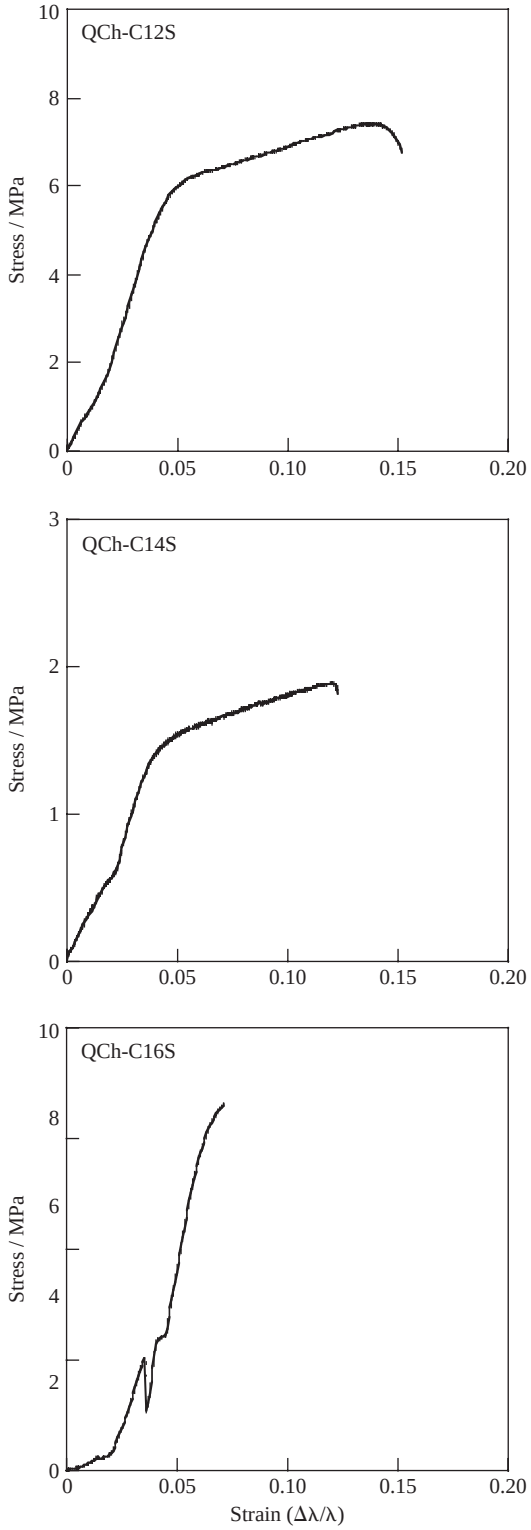

Fig. 4. Stress–strain curves of QCh–lipid complex membranes at 20°C. (Relative humidity, 58%.)

This might be due to the fact that the present QCh–lipid complexes were not charge equivalent complexes.

In the present study, tensile stress–strain of QCh–lipid complex membrane was measured at the relative

Table 1
Ultimate mechanical properties of QCh–lipid complex membranes

Membrane	Tensile strength at break/ MPa	Elongation at break	Tensile modulus/ MPa
QCh–C12S	6.8	0.152	139
QCh–C14S	1.8	0.123	40
QCh–C16S	16.5	0.071	258
Polystyrene*	50	0.025	3,400
Poly(methyl methacrylate)*	65	0.100	3,200
Nylon 66*	80	2.000	2,000
Nylon 6*	75	3.000	1,900

*Cited from ref. [39]

humidity of 58% at 20°C. Tensile modulus of QCh–lipid complex membrane would be decreased and the membrane would be softened with the increase in relative humidity as observed in DNA–lipid complex membranes [40].

3.2. Chiral separation of racemic mixture of amino acid

In the present study, three types of racemic amino acid were adopted as model racemates, such as racemic mixture of Glu, which has a very polar anionic side chain, that of Phe, having an aromatic side chain, and that of Lys with a very polar cationic side chain, respectively. In the present study, a concentration gradient was adopted as a driving force for membrane transport. Among those three types of racemic mixture, chiral separation of racemic Lys was observed, while optical resolution of racemic mixture of Phe and Glu through those three types of membrane were hardly observed. Time–transport curves of racemic mixture of Lys through those membranes are shown in Fig. 5. As can be seen, the L-isomer of Lys was transported in preference to the corresponding D-isomer through the membranes. The permselectivity toward the L-isomer was decreased with the increase in number of methylene moieties in anionic amphiphile. Contrary to this, flux values were increased from QCh–C12S to QCh–C16S complex membranes. Compared with other chiral separation results [8–20,22–26], relatively high flux value was observed.

In order to elucidate the mechanism for the expression of chiral separation, adsorption selectivities of those three types of membrane toward racemic mixture of Lys were studied. The results of adsorption experiments are summarized in Table 2. D-Lys was

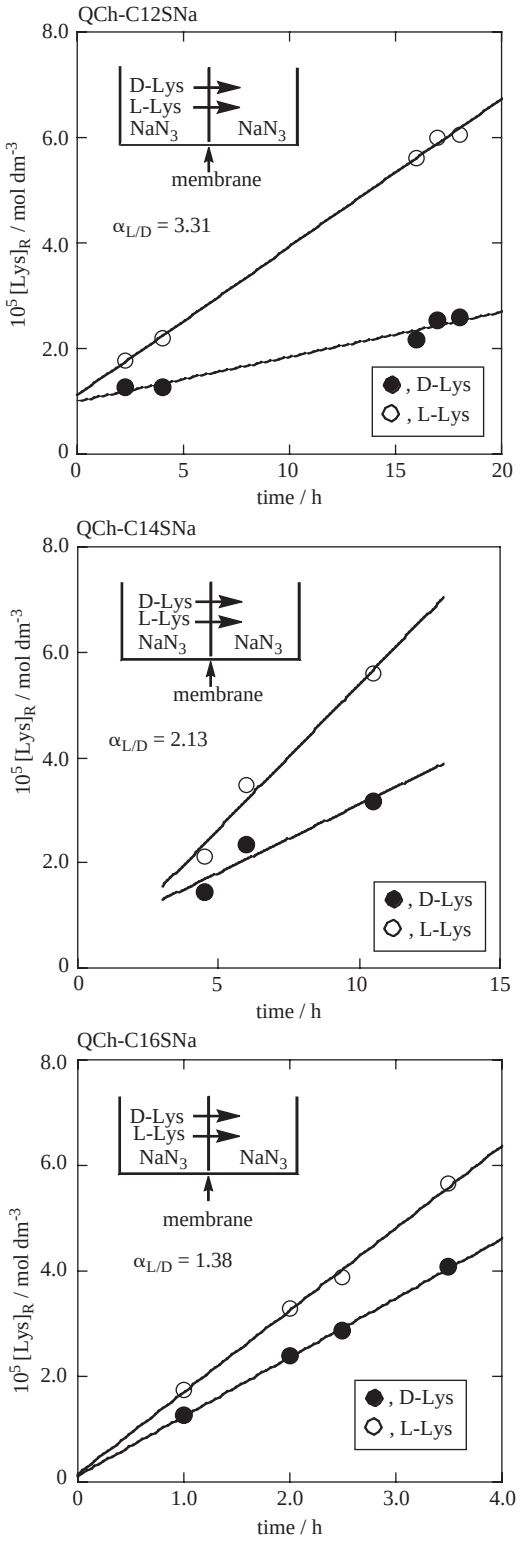

Fig. 5. Time–transport curves of racemic mixture of Lys through QCh–lipid complex membranes at 40°C.

Table 2
Chiral separation of racemic mixture of Lys through the membranes

Membrane	Permselectivity			Adsorption selectivity			
	J_D	J_L	$\alpha_{(L/D)}$	(D-Lys)/mem.	(L-Lys)/mem.	$S_{A(L/D)}$	$S_{D(L/D)}$*
	mol cm^{-2} h^{-1}	mol cm^{-2} h^{-1}		mol/g-mem.	mol/g-mem.		
QCh–C12S	1.13×10^{-8}	3.74×10^{-8}	3.31	5.32×10^{-6}	1.68×10^{-6}	0.31	10.7
QCh–C14S	3.45×10^{-8}	7.34×10^{-8}	2.13	4.39×10^{-6}	2.38×10^{-6}	0.53	4.02
QCh–C16S	1.50×10^{-7}	2.07×10^{-7}	1.38	1.47×10^{-5}	1.04×10^{-5}	0.69	2.00

*$S_{D(L/D)} = \alpha_{L/D}/S_{A(L/D)}$.

incorporated into those three types of membrane in preference to L-Lys. The adsorption selectivity toward the D-isomer decreased with the increase in methylene number of anionic amphiphile. The diffusivity selectivity $S_{D(L/D)}$ ($=D_L/D_D$, where D_L and D_D are the diffusion coefficients of the L-isomer and the D-isomer, respectively) can be determined using permselectivity ($\alpha_{L/D}$) and adsorption selectivity ($S_{A(L/D)}$). The determined diffusivity selectivities are summarized in Table 2 together with permselectivities and adsorption selectivities. Results summarized in Table 2 revealed that the chiral separation with the present membranes was dominantly determined by the diffusivity selectivity. As often observed in chiral separation [9–11,15, 22–24], in the present study, the enantiomer, which was not preferentially incorporated into the membrane, was selectively transported through the membrane. In other words, transport of the optical antipode, which was preferentially adsorbed in the membrane, was retarded. This can be explained as follows: the diffusivity of the enantiomer, in the present study, D-Lys, preferentially incorporated into the membrane was suppressed by a relatively strong interaction between the enantiomer and the membrane. As a result, the antipode, L-Lys, was selectively transported through the membranes as observed in Fig. 5.

4. Conclusions

Novel polyion–lipid complexes were prepared from QCh, which was a derivative of a marine (natural) polymer, and three types of anionic amphiphiles, such as C12SNa, C14SNa, and C16SNa. Those complexes gave durable self-standing membranes. The QCh–lipid complex membranes showed chiral separation ability; in other words, those three types of membrane selectively transported L-Lys from racemic mixture of Lys adopting a concentration gradient as a driving force for membrane transport. From transport experiments and adsorption studies, it was revealed that the permselectivity was dominantly determined by diffusivity selectivity. The results obtained in the present study would open a door to novel materials.

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