pH-Sensitive Gating by Conformational Change of a Polypeptide Brush Grafted onto a Porous Polymer Membrane

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Abstract: Benzyl glutamate NCA was graft-polymerized onto a porous poly(tetrafluoroethylene) membrane in order to study the effects of pH and ionic strength on permeation rate. The membrane was first glow-discharged in the presence of ammonia in order to produce amino groups on the surface. Following graft polymerization the graft chains were hydrolyzed to yield poly(glutamic acid). The rate of water permeation through this poly(glutamic acid)-grafted polymer membrane was pH-dependent and found to be slow under high-pH conditions and fast under low-pH conditions. Under high-pH conditions, randomly coiled graft chains extend to close the pores. The chains form a helix structure and open the pores under low-pH conditions. The magnitude of the permeation rate was dependent upon the length and density of graft chains. Ionic strength also affected the permeation rate.

Introduction

Polypeptides are unique among polymeric materials. Their structure is stabilized *via* hydrogen bond formation, as well as hydrophobic and electrostatic interactions. Various types of synthetic proteins with precisely folded three-dimensional structures[1−10] have been designed and synthesized. In addition, ionizable polypeptides[11,12] undergo a helix−coil structure transition; this transition may be useful to aid in the design and synthesis of stimuli-responsive materials. Polypeptide derivatives have already been used to successfully synthesize these kinds of materials.[13−17]

We have previously synthesized glucose-[18] and pH-responsive[19,20] and photoresponsive[21] permeation-control systems using porous membranes grafted with stimuli-sensitive vinyl polymers acting as "polymer brushes". The rate of permeation was regulated by external stimuli. In the present study, an ionizable polypeptide, i.e., a polypeptide brush, was grafted onto a porous polymer membrane and the rate of water permeation was regulated by changing the pH of the water.

Materials and Methods

Synthesis of a Grafted Membrane. The synthetic scheme of polymer brushes is shown in Figure 1. A porous membrane (Omnipore membrane, a tetrafluoroethylene/ethylene copolymer: diameter, 47 mm; thickness, 80 μm; pore size, 10 μm) was purchased from Millipore Co. This membrane was chosen because the organic solvents used in the polymerization of the α-amino acid *N*-carboxanhydride do not swell the membrane. The membrane was held at a pressure of 0.05 Torr in the presence of ammonia gas and glow-discharged using a high-frequency modulator (400 W). The number of amino groups produced on the membrane surface was determined by ninhydrin. The calibration was performed using *n*-octylamine.

The treated porous membrane was incubated for 2 days at room temperature in a *N*,*N*-dimethylformamide (DMF) solution containing various concentrations of γ-benzyl L-glutamate *N*-carboxanhydride, which was synthesized as previously reported.[22] The grafted membrane was washed with DMF until infrared absorptions arising from NCA and the polypeptide became undetectable in the washing liquid.

In order to hydrolyze the graft chains, the poly(γ-benzyl L-glutamate) (PBLG)-grafted membrane was then incubated overnight at 0 °C in an

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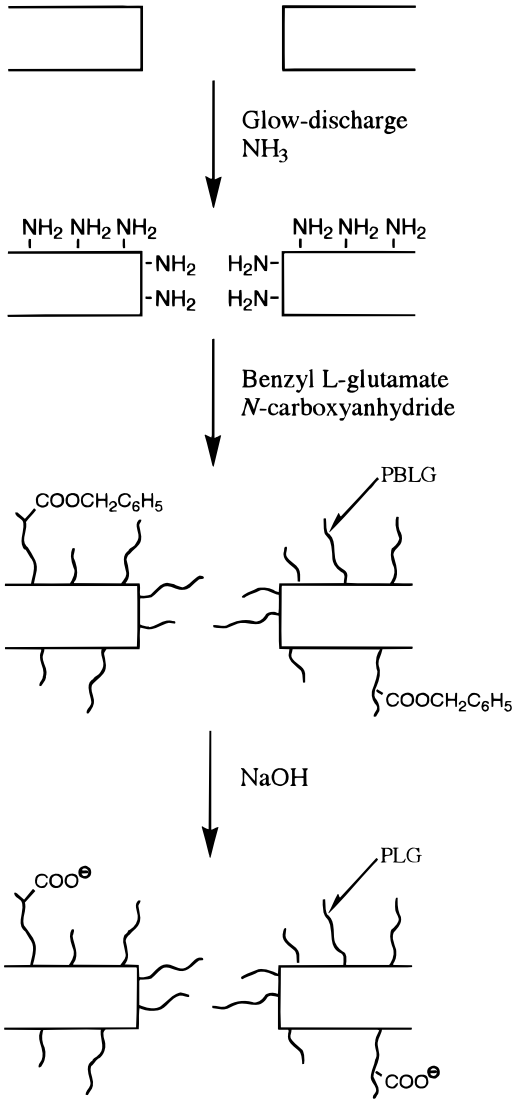

Figure 1. Schematic illustration of grafting of ionizable polypeptide onto porous membrane.

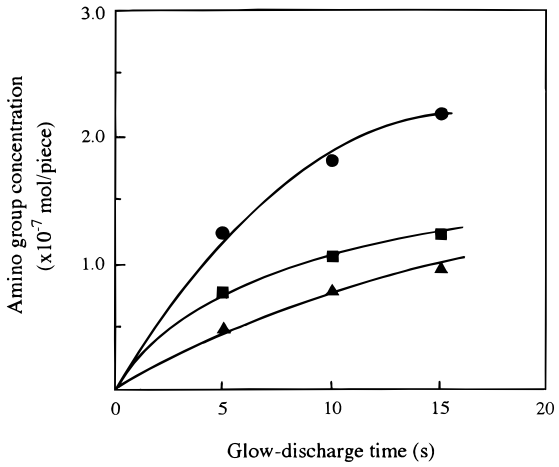

Figure 2. Time course of amino group introduction onto surface of porous membrane. Electric power was (●) 400 W, (■) 200 W, and (▲) 100 W.

organic solvent containing 3.3 mL of dioxane/1.3 mL of methanol/1 mL of 4 N NaOH. The treated membrane was then washed with distilled water until the pH of the washing liquid became constant.

The number of amino groups on the membrane surface was determined using the ninhydrin reaction according to the method described by Sarin et al.[23]

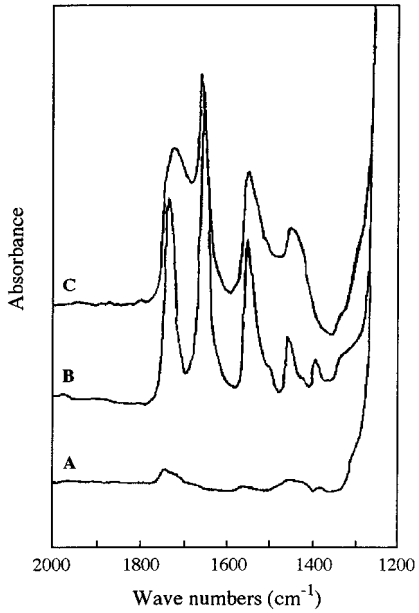

Figure 3. FT-ATR-IR spectra of (A) untreated, (B) poly(benzyl L-glutamate)-grafted, and (C) poly(L-glutamic acid)-grafted porous membranes.

The amount of amino groups consumed during the graft polymerization was estimated from the difference between the amino group concentration before and after the reaction with *tert*-butoxycarbonyl-L-glutamic acid γ-benzyl α-*N*-hydroxysuccinimide ester [Boc-L-Glu(OBzl)-OSu], which is an activated ester of γ-benzyl L-glutamate and is assumed to be similar to the benzyl glutamate NCA. The treated membrane was incubated for 2 days at room temperature in a 3 wt % Boc-L-Glu(OBzl)-OSu solution in DMF. The membrane was then washed with DMF until Boc-L-Glu(OBzl)-OSu could not be detected by ultraviolet absorption. The concentration of amino groups was measured by the ninhydrin method.

The amount of grafted polypeptide was determined by measuring the amount of amino acids produced by hydrolytic decomposition of the polypeptide graft.[24] The grafted membrane was placed in a test tube and immersed in a solution of 0.5 mL of 6 N hydrochloric acid and 0.5 mL of propionic acid. The mixture was heated to 110 °C and held at that temperature for 24 h. The mixture was diluted with 1.0 mL of 4 N sodium acetate buffer (pH 5.5) and mixed with 750 μL of ninhydrin reagent, which was prepared by dissolving 0.67 g of ninhydrin, 0.1 g of hydrindantin, and 25 mL of methylcellosolve in 100 mL of acetate buffer. The resulting mixture was heated in boiling water for 15 min. Next, ethanol (50 wt %) was added to the solution, and the mixture was stirred for 30 s. The absorbance at 570 nm of the final solution was measured. The calibration was carried out using L-glutamic acid solution of known concentrations.

Measurement of Water Permeation. Water permeation was measured as previously described.[19,20] The prepared membrane was mounted on a cell and placed below a water reservoir; water was allowed to flow through the membrane under a nitrogen atmosphere of 0.2 kg/cm^2. The pH and ionic strength of the permeated water were adjusted using HCl, NaOH, and NaCl. The rate of water permeation was calculated by measuring the mass of water that was able to pass through the membrane per minute.

FT-ATR-IR and UV Spectroscopy. FT-ATR-IR and UV spectra were obtained using a Digilab FTS-15E/D and a Hitachi spectrometer, respectively. The ATR spectra were obtained using a KRS-5 prism with an incident angle of 60°.

Results

Graft Polymerization. The density of amino groups on the surface of porous membranes increased with increasing power and time of the glow-discharge treatment (Figure 2).

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Table 1. Concentration of Amino Groups Produced in Glow-Discharge Treatment and after Reaction with Activated Ester [Boc-L-Glu(OBzl)-OSu]

glow-discharge time (s)	concentration of amino groups (nmol/cm^2) after glow-discharge treatment	concentration of amino groups (nmol/cm^2) after reaction	reactive amino groups (%)
5	25.1	15.5	38
10	36.3	20.8	42
15	43.6	24.5	44

Table 2. Average Degree of Polymerization of PBLG Graft in PBLG-Grafted Porous Membranes Prepared under Different Conditions[a]

monomer concn (wt %)	concn of graft chains (μmol/cm^2)	av degree of polymerization
0.5	0.22	12
1	1.46	79
2	2.73	148
3	3.75	203

[a] The concentration of reactive amino groups was 19.6 nmol/cm^2.

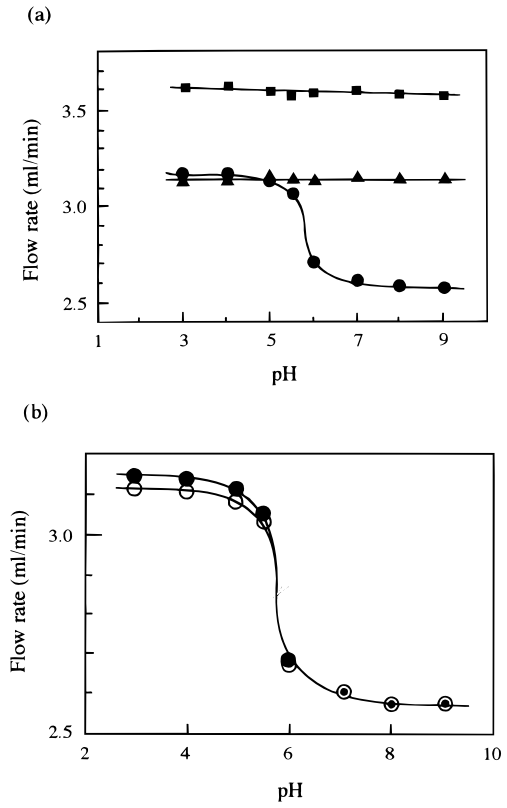

Figure 4. (a) pH dependence of water permeation through (■) untreated, (▲) poly(benzyl L-glutamate)-grafted, and (●) poly(L-glutamic acid)-grafted porous membranes. (b) Reversible change in water permeation rate by pH adjustment from pH 3 to 9 (●) and from pH 9 to 3 (○). Degree of polymerization and surface concentration of graft chains were 79 and 19.2 nmol/cm^2, respectively.

The infrared ATR spectra of porous membranes are shown in Figure 3. Infrared absorption by the PBLG-grafted membrane peaked at 1735 cm^{-1}, arising from the ester linkage, and at 1650 and 1550 cm^{-1}, which correspond to amides I and II, respectively. Surface hydrolysis of the grafted membrane resulted in a new absorption at 1715 cm^{-1}; this absorption arose from the carboxylic acid group. The marked decrease in absorption at 1735 cm^{-1} following alkaline treatment most likely indicates extensive hydrolysis of the benzyl groups of the graft chains.

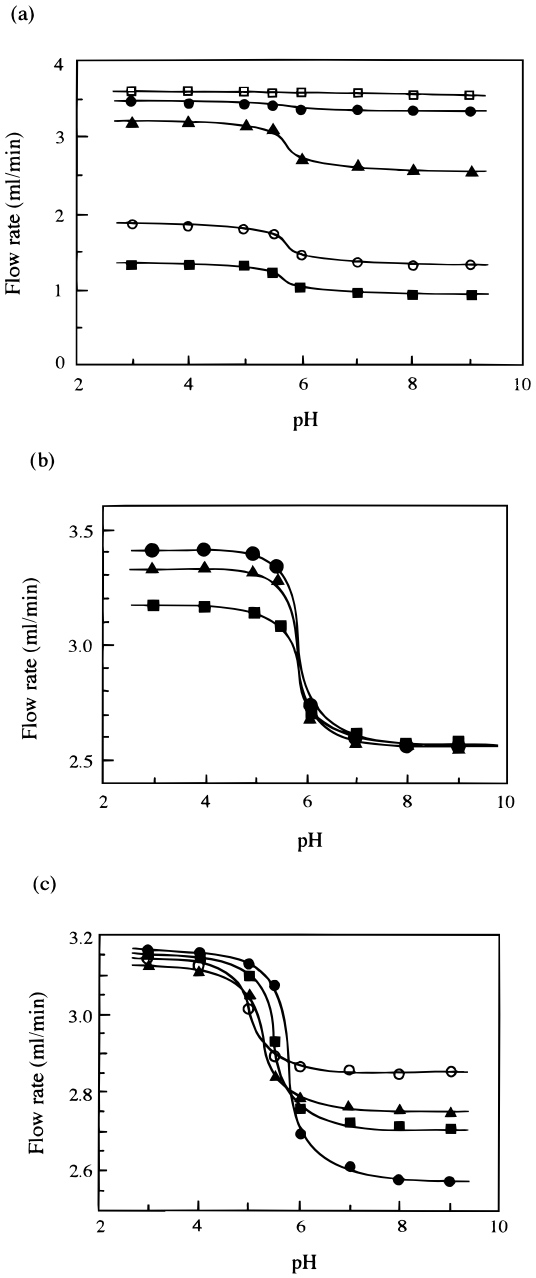

Figure 5. (a) pH dependence of water permeation through porous membranes grafted with poly(L-glutamic acid) grafts of various degrees of polymerization. Degree of polymerization of graft chains are none (□), 12 (●), 79 (▲), 148 (○), and 203 (■). Surface concentration of graft chains was 19 nmol/cm^2. (b) pH dependence of water permeation rate through porous membranes grafted with poly(L-glutamic acid) of various surface densities. Surface concentration of graft chains are 9.6 nmol/cm^2 (●), 16 nmol/cm^2 (▲), and 19 (■) nmol/cm^2. Degree of polymerization is 79. (c) pH dependence of the water permeation rate through a porous membrane grafted with poly(L-glutamic acid) under various ionic strengths. Ionic strengths were 0 (●), 0.05 (■), 0.10 (▲), and 0.20 (○). Degree of polymerization and surface concentration of graft chains are 79 and 19 nmol/cm^2, respectively.

The amino groups produced by glow-discharge treatment and those consumed during graft polymerization were quantified using the ninhydrin test. These results are shown in Table 1. The number of amino groups consumed during the graft polymerization was estimated from the difference in amino group concentrations before and after their reaction with Boc-L-Glu(OBzl)-OSu. An average of 40% of the initial amino groups reacted with the activated ester.

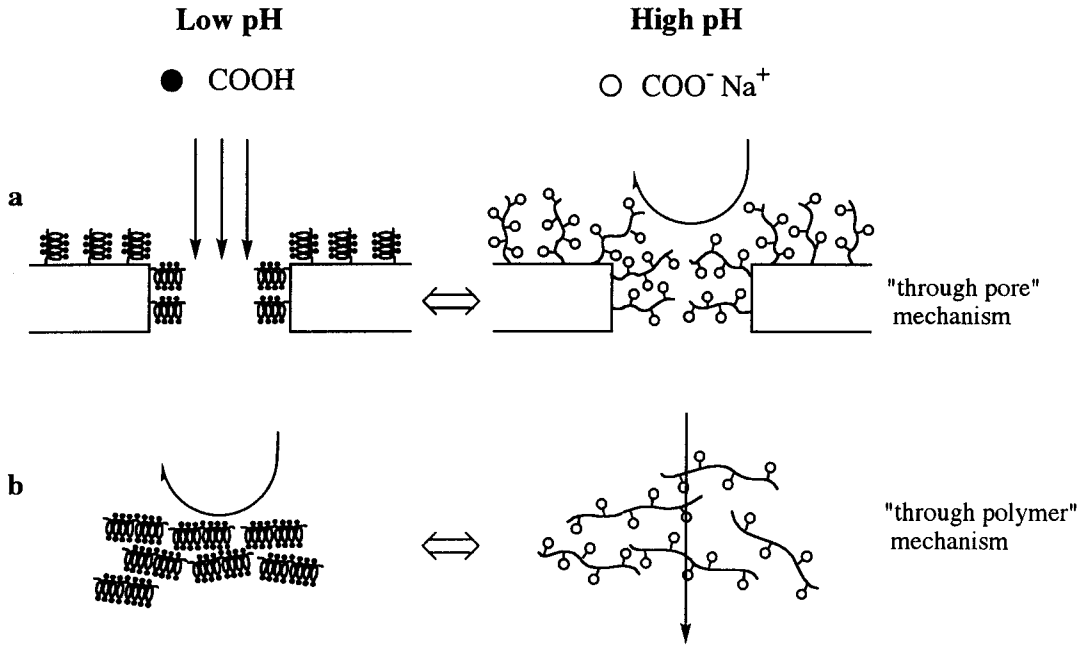

Figure 6. Explanation of pH-dependence water permeation through (a) porous membrane grafted with ionizable polypeptide and (b) dense membrane composed of ionizable polypeptide.

Because the primary-amine-initiated polymerization of γ-benzyl L-glutamate NCA proceeds without termination, the amino groups are considered to be quantitatively consumed during the initiation step.[25] Therefore, the degree of polymerization (DP) of the graft chains was calculated by dividing the amount of PBLG by the amount of consumed amino groups (Table 2). As expected, the DP increased as monomer concentration in the feed increased.

Water Permeability. Figure 4a shows the pH dependence of water permeation rate for aminated, PBLG-grafted, and poly(L-glutamic acid) (PLG)-grafted membranes. The permeation rate of the PBLG-grafted membrane was significantly lower than that of the plasma-treated membrane, and both were independent of pH. In contrast, the rate of permeation through the PLG-grafted membrane was strongly dependent on pH. At a lower pH, the permeation rate closely resembled that of the PBLG-grafted membrane, but decreased dramatically beyond pH 5.5. This trend was reversible and can be seen in Figure 4b.

The dependence of water permeation rate upon pH through various PLG-grafted membranes is shown in Figure 5a. The permeation rate decreased with increasing DP of the graft chains. However, the dependence was sharpest with the membrane that possessed PLG grafts of DP 79. Longer graft chains appeared to reduce the sensitivity of the membrane to changes in pH.

The density of the graft chains also affected the pH-dependent water permeation as shown in Figure 5b. The difference in permeation rate between low- and high-pH regions decreased with increasing surface density. Under high-pH conditions, the permeation rate was nearly independent of PLG graft density. However, under low-pH conditions, membranes with PLG grafts of lower surface density showed a higher permeation rate.

The effect of ionic strength on permeation is shown in Figure 5c. Under high-pH conditions, permeability is strongly dependent on ionic strength. However, the effect is quite small under low-pH conditions. As ionic strength was increased, the rate of permeation became less sensitive to changes in pH. In addition, the inflection point of each curve moved to a lower pH region as ionic strength was increased.

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Discussion

The rate of water permeation through the PLG-grafted membrane was slow at a pH higher than 5.5 (Figure 4). The changes in permeation rate due to pH changes may be attributed to dissociation of the polypeptide grafts at high pH. This is schematically explained in Figure 6a. Under high-pH conditions, the dissociated PLG chains extend because of electrostatic repulsion between the side groups. The chains cover the pores and reduce the water permeation rate. PLG chains assume a helical conformation under low-pH conditions and reduce steric obstruction of the pores. This mechanism is termed a "through-pore mechanism" and was studied by Israels *et al.*[26] using a two-dimensional self-consistent mean field (SCF) theory.

Different results concerning the dependence of water permeation on pH have been reported.[14,15] Membranes composed of polypeptides carrying carboxylic groups permeated the solute at higher rates under high-pH conditions. This behavior can be explained by the mechanism shown in Figure 6b. In this "through-polymer" mechanism, solute diffuses through the interstices between swollen ionized polymer chains. These different permeation mechanisms should be taken into account when considering other stimuli-responsive vinyl polymer hydrogels.[27−36]

As was expected, the degree of polymerization (DP) and the surface concentration of the graft chains were controlled by the initial monomer concentration in the feed and the surface density

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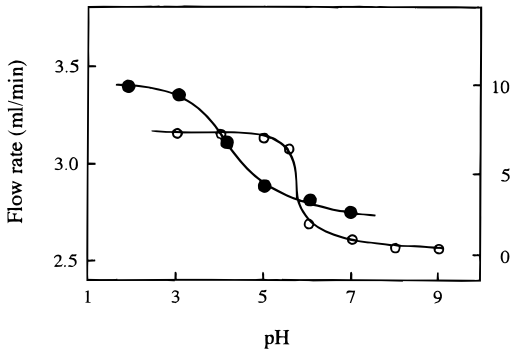

Figure 7. Comparison of the pH dependence of water permeation rate for (●) poly(acrylic acid)-grafted porous membrane (degree of polymerization and surface concentration of graft chains are 470 and 1.6 nmol/cm^2, respectively) and (○) poly(L-glutamic acid)-grafted porous membrane. Degree of polymerization and surface concentration of graft chains are 79 and 19 nmol/cm^2, respectively.

of the amino groups. Consequently the pH-dependence water permeation was regulated by the DP and the surface density of the graft chains. Increased surface density and DP of the graft chains also caused the rate of permeation to be less sensitive to changes in pH (Figure 5a,b). This may be the result of a reduction in conformational change of the graft chains accumulated near the pores.

The dependence of water permeation on ionic strength (Figure 5c) indicates the importance of electrostatic interactions. At higher ionic strengths, the decreased sensitivity of permeation rate to pH changes may be attributed to reduced electrostatic repulsion between the ionized carboxylate groups at higher pH. The shift of the inflection point to a lower pH region at higher ionic strength, as seen in Figure 5c, is most likely a result of a reduction in ionic interactions among neighboring ionic groups.

The relationship between water permeation rate and pH in the present study was compared with that of our previous poly(acrylic acid)-grafted porous membrane system (Figure 7).[19,20] Bergbreiter and Bandella[37] reported that poly(acrylic acid) graft on a surface is more sensitive than poly(acrylic acid) in solution. The pH sensitivity of the polypeptide grafts is even sharper than that of the vinyl polymer grafts. The increased sensitivity to pH change may have arisen from the fact that conformational changes in the polypeptides is exaggerated by helix−coil transition.

In the present study the inflection point of the water permeation rates, which was 5.5, was higher than the isoelectric point of PLG (DP, 620) which is 4.58 in 0.01 M NaCl.[38] However, the isoelectric point of PLG (DP, 260) is 5.65 in dioxane−0.2 M NaCl (1:2 by volume).[39] The increase of inflection point is most likely due to the presence of the PLG graft in the hydrophobic environment of the membrane surface.

In conclusion, the present study showed that various stimuli-responsive membranes may be synthesized by selection of appropriate stimuli-responsive polymers and by varying the methods of fabrication.

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