*Carbohydrate Polymers* **21** (1993) 289–293

# Transport of nucleic acid bases against their concentration gradients through quaternized chitosan membrane

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A water-insoluble anion exchange membrane was prepared by crosslinking with ethyleneglycol diglycidylether, a membrane made of quaternized chitosan and poly(vinyl alcohol). The transports of nucleic acid bases such as uracil, cytosine, adenine, and guanine were investigated as one side of the membrane in a diaphragm cell was acidic and the other basic. Uracil was transported against its concentration gradient from the basic side to the acidic side regardless of the pH on the basic side. Cytosine, adenine, and guanine were also transported against their concentration gradients, but the direction of their transport depended upon the pH on the basic side. In particular, the transport directions for adenine and guanine were switched during identical transport experiments. Mechanisms for the transport of these nucleic acid bases against their concentration gradients through the quaternized chitosan membrane are discussed.

## INTRODUCTION

Transport against their concentration gradients of organic compounds such as aniline (Uragami *et al.*, 1982) amino acids such as L-phenylalanine and glycine (Uragami *et al.*, 1982), nucleic acid bases such as adenine and uracil (Uragami *et al.*, 1986*a*, 1991) through cation exchange membranes with carboxylic acid and sulfonic acid group as fixed functional carrier have been studied and mechanisms for their transport have been discussed. Similar transport for amino acids such as *l*-phenylalanine and organic acids such as benzensulfonic acid and benzoic acid (Uragami *et al.*, 1988) through anion exchange membranes having amino groups as fixed functional carriers have been reported and also the transport of amino acids through synthetic polymer membranes containing pyridium cationic charge sites (Yoshikawa *et al.*, 1987).

In this paper, in order to reveal mechanisms for transport against a concentration gradient of organic compounds, the transport of nucleic acid bases such as uracil, cytosine, adenine, and guanine through a chitosan membrane with quaternary ammonium groups as fixed functional carriers were studied under various conditions and the mechanisms for their transport are discussed in detail.

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## EXPERIMENTAL

### Materials

Chitosan with a degree of deacetylation of 99·1% and an average molecular weight of $5 \times 10^4 \sim 1 \times 10^5$ was produced by the Bioscience Laboratory of the Katokichi Co. Quaternized chitosan (q-chitosan), synthesized by the method reported in a previous paper (Uragami *et al.*, 1986*b*) and having a degree of quaternization of 76·1%, and poly(vinyl alcohol) (PVA) produced by the Kurary Co., having an average degree of polymerization of $1725 \pm 25$ and a degree of saponification of $88 \pm 1$ mol%, were employed as the membrane substances. All reagents used in this study were supplied from commercial sources.

### Preparation of the membrane

Casting solutions were prepared by mixing aqueous solutions of 2·5 wt % q-chitosan and 5·0 wt % PVA to

Carbohydrate Polymers 0144-8617/93/$06.00

become q-chitosan/PVA in the ratio 3:7. Membranes were made by pouring the casting solution onto a rimmed glass plate and allowing water to evaporate at 60°C for 8 h in an oven. Since the membranes obtained were soluble in water, water-insoluble membranes were made by heat-treating the membranes at 120°C for 3 h in an oven, crosslinking with ethyleneglycol diglycidyl ether (500 $\mu$mol) in an aqueous solution of $2 \times 10^{-2}$ M NaOH and washing with 2 M HCl in an aqueous solution of 90 wt % methanol. An ion-exchange capacity of the q-chitosan membrane was 3·7 mequiv/g.

### Apparatus and measurements

Transport experiments were carried out at 25°C under magnetic stirring in a diaphragm cell (Uragami *et al.*, 1982). The effective membrane area in the cell was 8·0 $cm^2$. The concentrations of nucleic acid bases such as uracil, cytosine, adenine and guanine in both initially acidic (A) and initially basic (B) sides were determined by UV spectroscopy. The concentration of $K^+$ ions in both sides was determined by atomic adsorption photometry. The pH in the two sides was measured using a pH meter (Hitachi-Horiba F-7SSII type).

## RESULTS AND DISCUSSION

### Transport of uracil

Changes in the concentration of uracil and $K^+$ ions, and pH changes with time on the A side and the B side of the q-chitosan membrane in the diaphragm cell are shown in Fig. 1. The concentration of uracil on the B side increased up to a maximum and then decreased; in contrast, that on the A side decreased and then increased with time. Since the initial concentration of uracil was the same on both sides, these results suggest that uracil is transported through the q-chitosan membrane from the acidic side to the basic side against its concentration gradient between the two sides of the membrane. Also $K^+$ ions are transported by an antiport in the transport against a concentration gradient. The transport of uracil from the acidic side to the basic side against its concentration gradient and that from the basic side to the acidic side showed similar behavior, when the pH on the A side was 1·0 and the pH on the B side was changed. Such transport against the concentration gradient is significantly related to the pH changes on both sides. Table 1 shows a state of the solution on both sides across the q-chitosan membrane after 5 h when the pH of the initially acidic side was 1·0 and the pH of the initially basic side was changed. Under the conditions of runs 1 and 2, both sides became acidic after 5 h, and for runs 3 and 4 both became basic. The charged state of uracil and the interface on both sides of the q-chitosan membrane are shown in Fig. 2. The $pK_b$s of uracil are 9·5 and 13. Under the initial conditions of transport, state (a) in Fig. 2 was maintained, i.e. uracil on the A side is kept to a neutral molecule and that on the B side is negatively charged. On the other hand, the interfaces with the A and the B side of the q-chitosan membrane are charged positively and negatively, respectively. Therefore, the neutral molecule of uracil on the A side is incorporated into the membrane on the A side by an electrostatic interaction with the quaternary ammonium cation on the interface of the membrane in the A side. On the B side, however, uracil cannot be incorporated from the B side into the membrane, because a strong electrostatic repulsion occurs between the negatively charged uracil and the negatively charged membrane. The transport of uracil against the concentration gradient through the q-chitosan membrane in Fig. 1 can be understood by such a transport mechan-

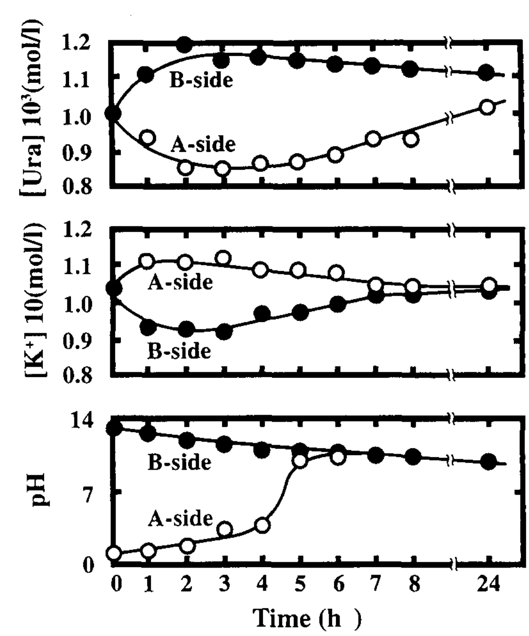


**Fig. 1.** Changes of the concentrations of uracil and $K^+$ ions, and the pH changes on both sides with time through the q-chitosan membrane. Initial concentration on the B side: $1 \times 10^{-3}$ mol liter$^{-1}$ of uracil and $1 \times 10^{-1}$ mol liter$^{-1}$ of KOH in aqueous solution. Initial concentration on the A side: $1 \times 10^{-3}$ mol liter$^{-1}$ of uracil, $1 \times 10^{-1}$ mol liter$^{-1}$ of HCl in aqueous solution.

**Table 1. Initial pH and the state of the solution after 5 h on both sides across the q-chitosan membrane**

| No. | Initial pH | | After 5 h | |
|---|---|---|---|---|
| | A | B | A | B |
| 1 | 1·0 | 10·5 | Acid | Acid |
| 2 | 1·0 | 12·0 | Acid | Acid |
| 3 | 1·0 | 13·0 | Base | Base |
| 4 | 1·0 | 13·5 | Base | Base |

A is for 'initially acidic', B is for 'initially basic'.

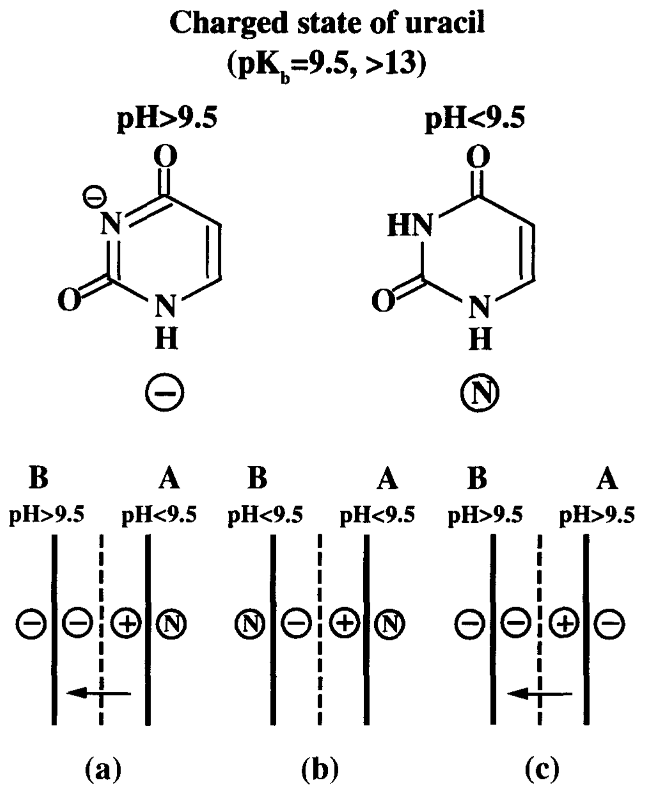


**Fig. 2.** Charged state of uracil and interface on both sides of the q-chitosan membrane under various conditions. Arrows indicate the transport direction. ⊕: positively charged, ⊖: negatively charged, Ⓝ: neutral.

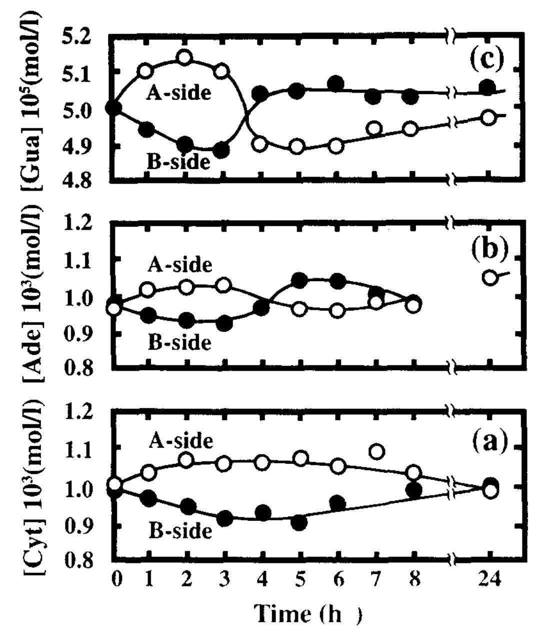


**Fig. 3.** Changes in the concentrations of cytosine, adenine and guanine on both sides with time through the q-chitosan membrane. (a) Initial concentration on the B side for cytosine: $1 \times 10^{-3}$ mol liter$^{-1}$ of cytosine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KOH in aqueous solution. Initial concentration on the A side for cytosine: $1 \times 10^{-3}$ mol liter$^{-1}$ of cytosine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KCl and $1 \times 10^{-1}$ mol liter$^{-1}$ of HCl in aqueous solution. (b) Initial concentration on the B side for adenine: $1 \times 10^{-3}$ mol liter$^{-1}$ of adenine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KOH in aqueous solution. Initial concentration on the A side for adenine: $1 \times 10^{-3}$ mol liter$^{-1}$ of adenine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KCl and $1 \times 10^{-1}$ mol liter$^{-1}$ of HCl in aqueous solution. (c) Initial concentration on the B side for guanine: $5 \times 10^{-5}$ mol liter$^{-1}$ of guanine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KOH in aqueous solution. Initial concentration on the A side for guanine: $5 \times 10^{-5}$ mol liter$^{-1}$ of guanine, $1 \times 10^{-1}$ mol liter$^{-1}$ of KCl and $1 \times 10^{-1}$ mol liter$^{-1}$ of HCl in aqueous solution.

ism. Under the conditions of runs 1 and 2 in Table 1, state (a) in Fig. 2 is changed to state (b) with time. Under the conditions of runs 3 and 4, state (a) becomes state (c). The arrows in states (a) and (c) indicate the direction of the transport of uracil against the concentration gradient. Uracil is transported from the A side to the B side against the concentration gradient under every condition in Table 1. The degree of change from state (a) to state (c) is higher than that from state (a) to state (b).

**Transport of cytosine, adenine and guanine**

Figure 3 shows the concentration changes of cytosine, adenine and guanine on both sides through the q-chitosan membrane. These results were obtained under the same conditions for the transport of uracil shown in Fig. 1. The transport behaviors of cytosine, adenine, and guanine were significantly different from that of uracil. For the transport of cytosine, the transport direction was reversed, i.e. from the B side to the A side; adenine and guanine were initially transported against their concentration gradients from the B side to the A side through the q-chitosan membrane, but the transport direction was reversed after a certain length of time.

Next, the transport direction for nucleic acid bases such as uracil, cytosine, adenine, and guanine, and $K^+$ ions was investigated by keeping the initial pH at 1·0 on the A side while changing the initial pH on the B side. The results obtained are summarized in Table 2, in which the arrows indicate the transport direction and the values in parentheses are the $pK_a$ and $pK_b$ for nucleic acid bases. The $K^+$ ion is always transported from the B side to the A side and uracil is transported from the A side to the B side regardless of the pH on the B side. However, in the transport of cytosine, which is a pyrimidine base like uracil, the cytosine molecule is transported from the B side to the A side against its concentration gradient for a pH on the B side of up to 13·0, but the transport direction is reversed as the pH on the B side reaches 13·5. Such transport phenomena for cytosine with the change of initial pH on the B side can be explained by six states between the q-chitosan membrane and cytosine, as shown in Fig. 4. Since the $pK_a$ and $pK_b$ values of cytosine are 4·5 and 12·2, respectively, cytosine is negatively charged at a pH higher than 12·2, positively charged at a pH lower than

**Table 2. Transport direction of uracil (Ura), cytosine (Cyt), adenine (Ade), guanine (Gua) and $K^+$ ions in the transport against the concentration gradient through the q-chitosan membrane**

| Transporting species | Initial pH on B side | | | |
|---|---|---|---|---|
| | 11·0 | 12·0 | 13·0 | 13·5 |
| $K^+$ | B → A | B → A | B → A | B → A |
| Ura (9·5) | B ← A | B ← A | B ← A | B ← A |
| Cyt (4·5, 12·2) | B → A | B → A | B → A | B ← A |
| Ade (4·15, 9·8) | B → A | B → A | B → A<br>B ← A | B ← A |
| Gua (3·2, 9·6, 12·4) | B → A | B → A | B → A<br>B ← A | B ← A |

Arrows indicate the transport direction.
Values in parentheses are $pK_a$ and $pK_b$ values for nucleic acid bases.
The initial pH on the A side was kept at 1·0 and the initial pH on the B side was changed.

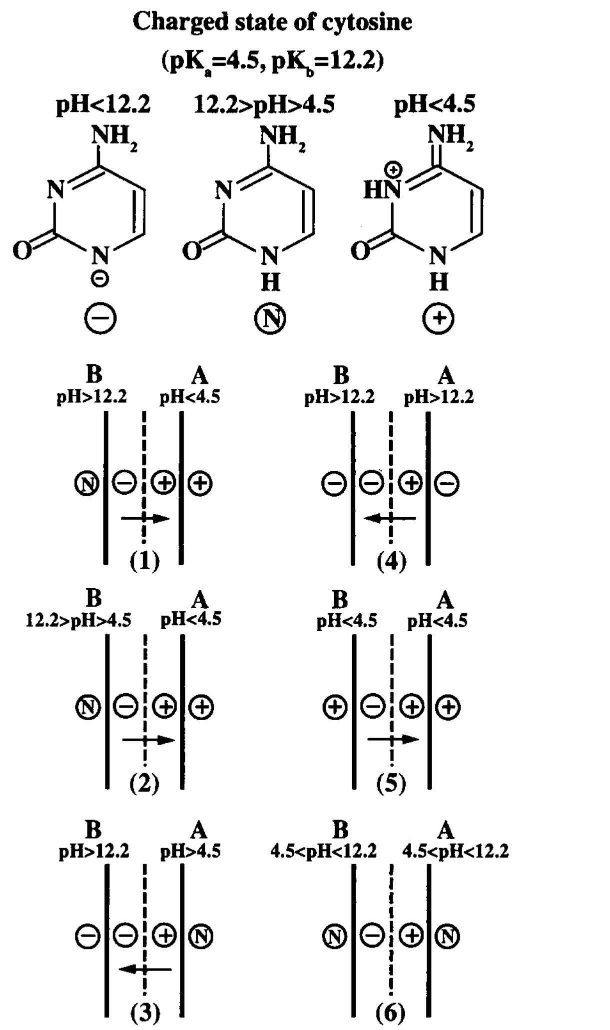


**Fig. 4.** Charged state of cytosine and interface on both sides of the q-chitosan membrane with the pH changes. Arrows indicate the transport direction. ⊕: positively charged, ⊖: negatively charged, Ⓝ: neutral.

4·5, and neutral in the region between these pHs. Therefore, when the initial pHs on the B side are 11·0 and 12·0, state (2) in Fig. 4 is set up and shifted immediately to state (5) as a consequence of the pH changes on both sides. Cytosine on the A side is positively charged and cannot be incorporated into the membrane because of strong electrostatic repulsion between the positively charged cytosine and the quarternary ammonium group, but since cytosine on the B side becomes positively charged, it can be incorporated into the membrane and transported from the B side to the A side against the concentration gradient through the q-chitosan membrane. On the other hand, when the initial pH on the B side is 13·5, state (1) in Fig. 4 is initially set, changes to state (3) and then to state (4). State (1) cannot be maintained for long because the pH on both sides immediately changes and rapidly becomes state (3). Since both states (3) and (4) are held for a long time, a weak interaction between neutral cytosine and quarternary ammonium cation and an interaction between quarternary ammonium cation and negatively charged cytosine occur, and consequently cytosine is transported against its concentration gradient from the A side to the B side through the q-chitosan membrane.

In Table 2, it can be seen that the transport behavior for the purine bases such as adenine and guanine are significantly different from those for the pyrimidine bases such as uracil and cytosine. Thus, the transport directions for adenine and guanine are switched during an identical transport experiment as shown in Fig. 3. This switching phenomenon in the transport against the concentration gradient is very strange. The charged state of the q-chitosan membrane and adenine is shown in Fig. 5. Since the $pK_a$ and $pK_b$ values for adenine are 4·15 and 9·8, respectively, adenine is negatively charged at pH values higher than 9·8, positively charged at pH values lower than 4·15, and neutral in the pH region in between. As the initial pHs on the B side are 11·0 and 12·0, state (1) transport is initially set up, changes immediately to state (4) and then to state (5), because $H^+$ ions on the A side exist in excess compared with $OH^-$ ions on the B side. Thus, an adenine molecule on the B side successively changes from being negatively charged, to neutral and finally positively charged by the pH change on the B side. The positively charged adenine on the interface of the membrane on the B side is incorporated into the membrane on the B side by an electrostatic interaction, transferred to the A side due to an electrical potential gradient, and then released by the $H^+$ ion on the A side. Consequently, adenine can be transported against its concentration gradient from the B side to the A side. When the pH on the B side is 13·0, adenine is first transported from the B side to the A side, changed to neutral in state (2) due to the pH

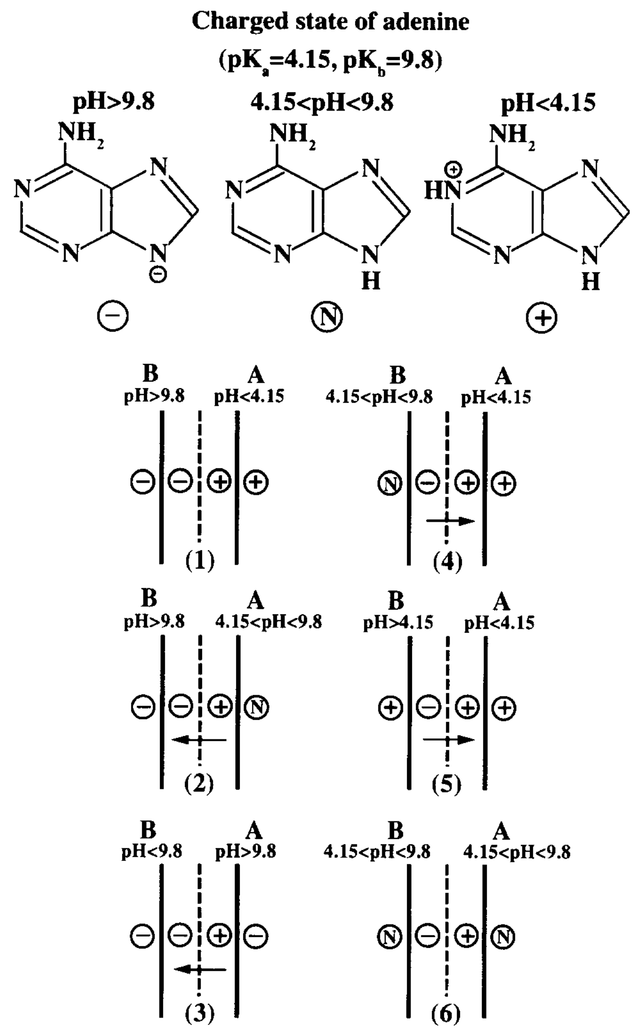


**Fig. 5.** Charged state of adenine and interface on both sides of the q-chitosan membrane with the pH changes. Arrows indicate the transport direction. ⊕: positively charged, ⊖: negatively charged, Ⓝ: neutral.

change on the A side, and then positively charged in state (3). Therefore, in states (2) and (3), adenine is transported from the A side to the B side. The direction of transport of adenine under this condition is changed with time, i.e. it is dependent upon the pH change on the A side. When the initial pH on the basic side is raised to 13·5, the state is successively changed from (1) to (2) to (3), and reversed transport of adenine should be observed at an initial pH of 13·0 on the B side. Such a reversed transport under this condition, however, cannot be found, owing to the following phenomenon. Since $OH^-$ ions on the basic side exist, a rise in pH on the acidic side is immediately observed after the start of transport due to a diffusion of $OH^-$ ions from the B side to the A side, and states (2) and (3) are kept for a long time. Consequently, neutral and negatively charged adenine are transported from the A side to the B side.

The $pK_a$ of guanine is 3·2, and its $pK_b$s are 9·6, and 12·4; values similar to those of adenine. Hence, the transport mechanism for guanine through the q-chitosan membrane with pH change can be explained in the same way as for adenine.

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