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PREPARATION OF PVA MEMBRANE CONTAINING NUCLEIC ACID ANALOGS AND STUDIES ON SEPARATION OF NUCLEOSIDES AND DINUCLEOTIDES USING THIS MEMBRANE.

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Abstract PVA membranes containing nucleic acid analogs were prepared. Separation of nucleosides and dinucleotides were studied using these membranes. In the case of PVA membranes containing thymine base, selective diffusion of adenosine, which is the complementary Watson-Crick base pair of thymine, was observed. This may be caused by specific base-base interaction between thymine and adenine.

Keywords: Specific Interaction, Hydrogen Bonding, Nucleic Acid, Separation, Nucleotide, Membrane

INTRODUCTION

The chemistry of nucleic acid model polymers has recently received much attention. A number of synthetic polymers containing nucleic acid bases have been prepared and their properties have been studied. We previously prepared water soluble polyethyleneimine and poly-L-lysine derivatives containing cytosine, hypoxanthine, thymine, uracil, 5-fluorouracil and adenine, and interactions of these polymers with polynucleotides in aqueous solution were studied.^{1,2,3,4}

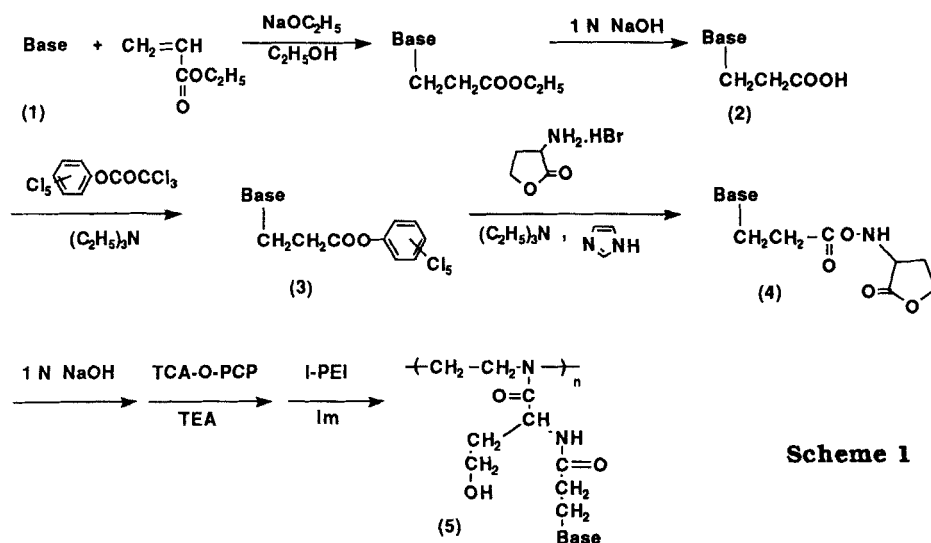
These nucleic acid analogs were found to form stable polymer complexes with polynucleotides by specific base-base interactions in neutral aqueous solutions.

On the other hand, analysis and separation of nucleic acid components has recently received much attention. It is especially important to develop a separation method that is able to separate nucleic acid components by specific interactions between nucleic acid bases. For this purpose, we previously reported the synthesis of silica gel derivatives containing nucleic acid bases or nucleosides and application of these analogs to HPLC of specific separations of nucleic acid components.^{5,6}

In the present study, water soluble nucleic acid analogs are applied to membrane separation of nucleic acid components.

EXPERIMENTAL

The synthesis of water soluble nucleic acid analogs is shown in

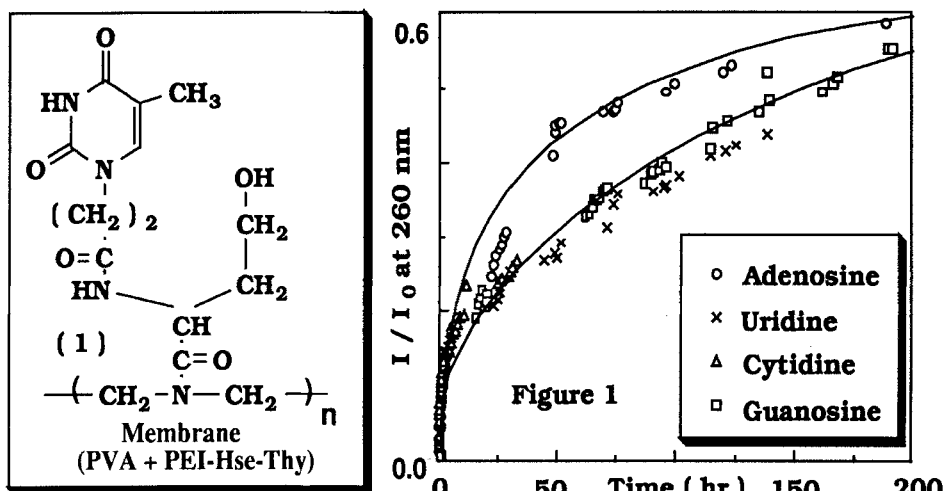


Scheme 1

Scheme 1^{1,2,3}. The nucleic acid base of the water soluble nucleic acid analog used in this study was thymine (PEI-Hse-Thy) (1). Preparation of membranes were carried out by the solution casting method. Various mixing ratios of PVA with PEI-Hse-Thy were used. Glutaric dialdehyde was used as the cross-linking agent. Separation of nucleosides and dinucleotides were carried out using U-tube cell containing these membranes between the sample solution and the buffer solution. Kolthoff Buffer solution of nucleosides and dinucleotides (pH = 6.98) were used as sample solutions. The diffusion of nucleosides and dinucleotides through these membranes were detected by the increase in UV absorbances of buffer solutions.

Result and Discussion

The best condition for the preparation of the membrane was PVA : PEI-Hse-Thy = 45 : 1 (wt%) using glutaric dialdehyde as cross-linking agent and baking at 40 °C. Figure 1 shows the diffusion curves of some nucleosides using this membrane. In this figure, selective diffusion of adenosine was observed. In the case of PVA membranes, this selective diffusion was not observed. So, this selective diffusion was caused by the effect of the nucleic acid



analogs of thymine. It may be caused by specific base-base interactions between thymine and adenine. The parameters of these diffusions are summarized in Table 1. In this table, the diffusion parameter (D_m) of adenosine is high compared with other nucleosides. The partition coefficient of adenosine is, however, similar to that of cytidine. In other words, the affinity of adenosine for this membrane is like that of cytidine. Therefore, selective diffusion of adenosine using this membrane depends on diffusion processes. In this process, thymine bases in the membrane may be acting as carriers of adenosine. The data in this table show that uridine has the lowest affinity for this membrane. This may be caused by a low affinity between thymine and uridine.

Table 1

	Adenosine	Uridine	Cytidine
$D_m [\times 10^5 \text{ cm}^2/\text{min}]$	5.69	4.50	4.23
K	87.03	161.5	86.83
$S [\times 10^6 \text{ mol/cm}^3]$	1.9	1.2	1.7

※ D_m : Diffusion Parameter [$D_m = (V_1 V_2 d / 2 A V_0 t) \ln(1 - 2C_2/C_0)$],
 K : Partition Coefficient [$K = I_e / (I_0 - I_e)$]

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