Functional Monomers and Polymers CLXXVIII.* Specific Interaction of Nucleic Acid Analogs in HPLC System

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ABSTRACT: Selective separation of oligoethyleneimine derivatives having pendant adenine or thymine bases was found in high performance liquid chromatography (HPLC) using nucleic acid base, such as thymine or adenine, bonded silica gel columns. The separation behavior of the oligomers was concluded to be caused by the specific interaction between the oligomers and the nucleic acid base on silica gel.
    KEY WORDS   Nucleic Acid Base / Thymine / Adenine / Silica Gel / Selective Separation / Intermolecular Interaction / High Performance Liquid Chromatography /

Synthetic nucleic acid analogs have recently received much attention, and numerous studies have been devoted to the preparations and the properties of these analogs, which may find a number of applicability as polymeric drugs, photosensitive polymers, and other high functional materials.[1-5]

Hitherto, polyethyleneimine derivatives with nucleic acid bases were synthesized by Overberger and his co-workers,[6] and their interactions were studied in solution.[7] Interactions of the oligomer models were also studied in solution for the polyethyleneimine derivatives, where amino acids were incorporated as the side chain of the polyethyleneimine derivatives.[8-10] The intermolecular interactions (b in Figure 1) of the polyethyleneimine derivatives were found to be largely affected by the intramolecular interactions (a and c in Figure 1) between nucleic acid bases in oligomers and in polymers. For the intramolecular interactions of nucleic acid bases in the oligomers and polymers, two factors should be considered: the distance between neighboring

X(Spacer); none
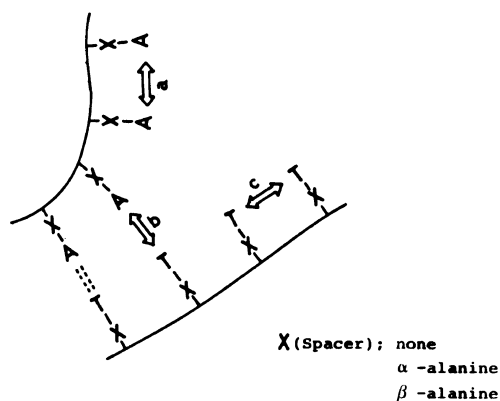   α-alanine
            β-alanine

Figure 1.   Intramolecular (a, c) and intermolecular (b) interactions in the formation of complex between nucleic acid analogs.

pendant groups along the main chain and that between the pendant base and main chain, namely the length of the side chain.[5]

In previous reports, we indicated that chemically and thermally stable nucleic acid derivatives bonded on silica gel can be applied to HPLC systems for the specific separation of nucleic acid base derivatives.[11-13] The HPLC

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* For Part CLXXVII, see T. Wada, Y. Inaki, and K. Takemoto, *J. Bioactive and Compatible Polymers*, in press.
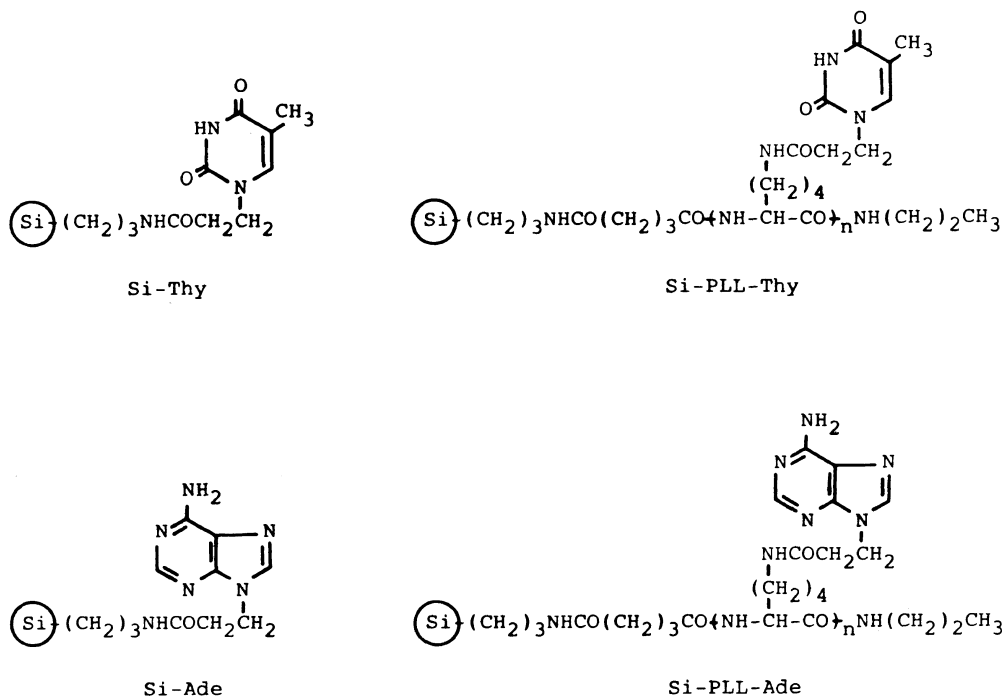
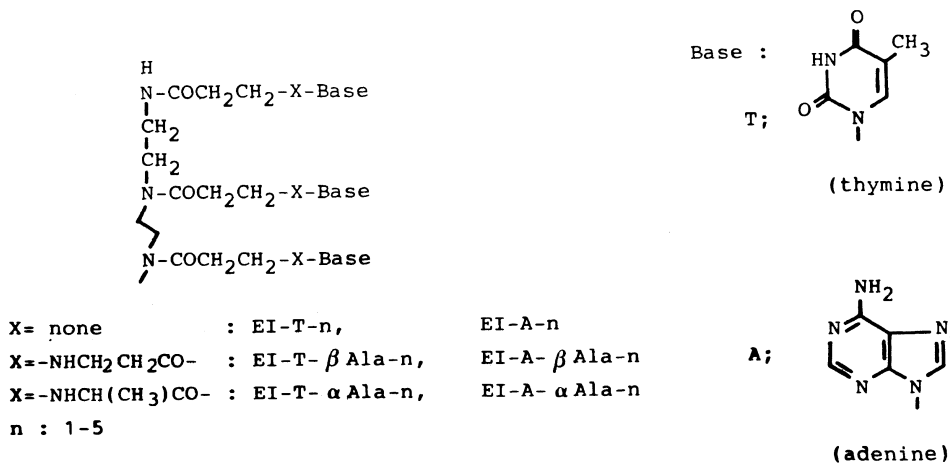**Figure 2.** Nucleic acid base immobilized silica gels.

Figure 3. Oligomer models.

systems of the nucleic acid base derivatives gave information about intermolecular interactions of synthetic nucleic acid analogs. Therefore, evaluation of the interactions between the synthetic nucleic acid analogs may be possible from the data of these HPLC systems.

The present paper deals with the relationship between the separation behavior in the HPLC system and intermolecular interactions in solution for the nucleic acid analogs based on polyethyleneimine. The columns of the

HPLC system used here were the silica gel derivatives of nucleic acid bases (Figure 2). The synthetic nucleic acid analogs used were the oligomer models of polyethyleneimine derivatives with spacer-separated thymine and adenine bases in which α-alanine or β-alanine was used as a spacer (Figure 3).

EXPERIMENTAL

HPLC Procedure

Columns. The specific separation of nucleic acid oligomer models was studied using the nucleic acid base bonded 3-aminopropyl-silanized silica (APS-silica) (Si-Thy and Si-Ade), poly-L-lysine derivatives bonded APS-silica (Si-PLL-Thy and Si-PLL-Ade) (Figure 2),[11−13] and a commercially available reverse-phase column (ODS column, TSK-gel, Tosoh). All resins were packed into stainless steel tubes (15 cm in length, 4.6 mm i.d.).

Samples. Six kinds of oligoethyleneimine derivatives containing pendant thymine or adenine bases (Figure 3) were prepared by the methods described in literature.[8,9] The compounds abbreviated as EI-T-*n* for the thymine derivatives and EI-A-*n* for adenine derivatives have no amino acid spacer groups between the nucleic acid bases and the main chain, where *n* is the number of units. The compounds abbreviated as EI-T-αAla-*n* for the thymine derivatives and EI-A-αAla-*n* for the adenine derivatives have an α-alanine unit as the spacer group between the main chain and nucleic acid base. The other compounds abbreviated as EI-T-βAla-*n* for the thymine derivatives and EI-A-βAla-*n* for the adenine derivatives have a β-alanine unit as the spacer group.

Apparatus. The experiments were performed with a TOYO SODA high speed liquid chromatograph series HLC 803D equipped with a thermostated column oven and a UV detector operating at 254 nm. The column-oven was set at the desired temperature. Methanol, water, and ethylene glycol, used as chromatographic solvents, were of analytical-reagent grade. The amount of sample injected was 1.67 to 2.67 μg, dissolved 1 μl of water–methanol mixture.

RESULTS AND DISCUSSION

Selective Separation of Thymine and Adenine Derivatives

In the previous study, the specific interaction of the oligoethyleneimine derivatives of the nucleic acid bases in solution was evaluated by hypochromicity value of the UV spectra.[5,8−10] The oligomers investigated were oligoethyleneimine derivatives containing adenine (EI-A-X-*n*) and thymine (EI-T-X-*n*) as shown in Figure 3, where X means the kind of spacer (none, α-alanine, and β-alanine) units and *n* means the number of nucleic acid base units in the oligomer. The results showed molecular weight dependency of the intermolecular interaction: that is, the interaction between complementary oligomers (EI-T-*n* and EI-A-*n*) was small, and the interaction between the oligomer and the polymer (EI-T-*n* and PEI-A, and EI-A-*n* and PEI-T) was remarkable. The polymer effect observed in the intermolecular interaction was assumed to be caused by an entropy factor; the polymer had many nucleic acid base units in one molecule.

Figure 4 shows chromatograms of EI-T-*n* and EI-A-*n* with water–methanol (1 : 1, v/v) mobile phase at 40°C on nucleic acid analog HPLC systems (Si-Ade and Si-Thy) and on reverse-phase column (ODS). The nucleic acid bases are known to be hydrophobic and are separated by reverse-phase chromatography.[14−16] It is also known that the hydrophobicity of purine bases is higher than that of pyrimidine bases. Therefore, the retention time of purine derivatives (EI-A-*n*) is longer than that of pyrimidine derivatives (EI-T-*n*) on reverse-phase chromatography. The oligomers were slightly separated on the ODS column, but EI-T-1 was the latest elution in the EI-T-*n* oligomers. Therefore, it should be considered that the separation of the oligomers
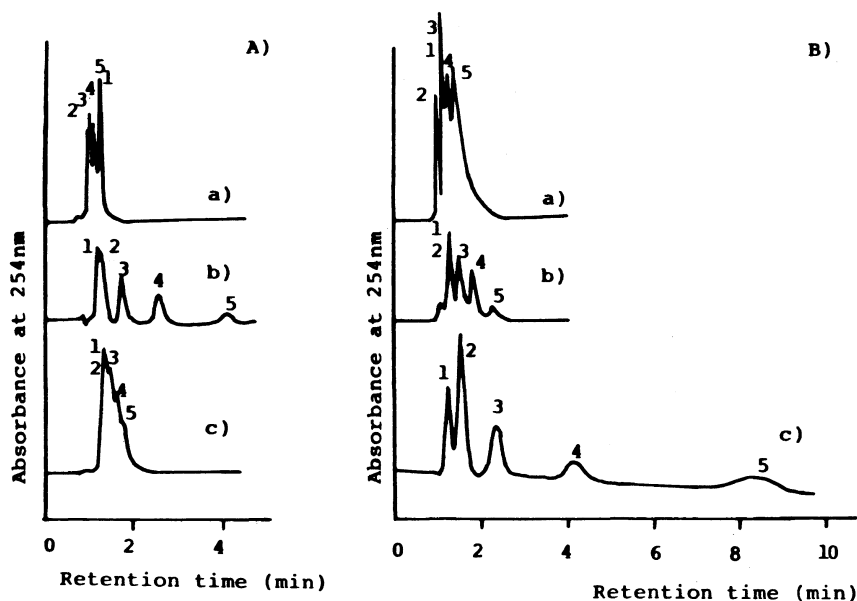
Figure 4. Chromatograms of A) EI-T-*n* and B) EI-A-*n*. Column: a) ODS; b) Si-Ade; c) Si-Thy. Mobile phase, water–methanol (1 : 1, v/v); flow rate, 1.0 ml min^{-1}; temperature, 40°C; detector, 254 nm. Peaks: A) 1 = EI-T-1; 2 = EI-T-2; 3 = EI-T-3; 4 = EI-T-4; 5 = EI-T-5. B) 1 = EI-A-1; 2 = EI-A-2; 3 = EI-A-3; 4 = EI-A-4; 5 = EI-A-5.
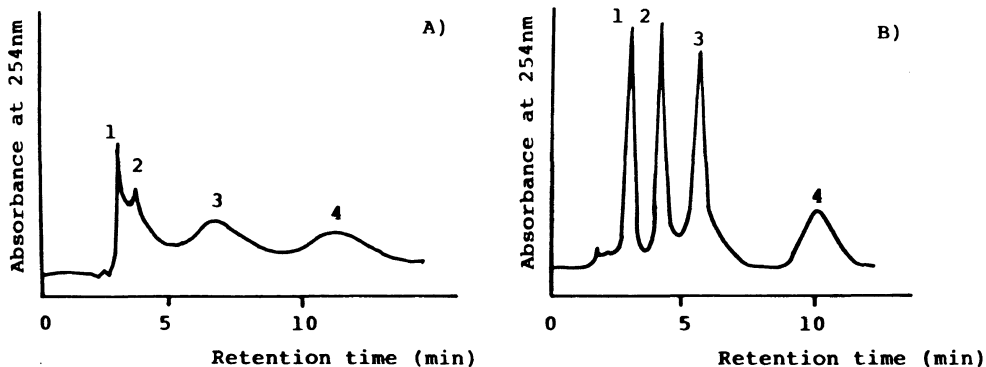

Figure 5. Chromatograms of A) EI-A-αAla-*n* and B) EI-A-βAla-*n* on Si-Thy column. Mobile phase, water–methanol (1 : 1, v/v); flow rate, 1.0 ml min^{-1}; temperature, 30°C; detector, 254 nm. Peaks: A) 1 = EI-A-αAla-1; 2 = EI-A-αAla-2; 3 = EI-A-αAla-3; 4 = EI-A-αAla-4. B) 1 = EI-A-βAla-1; 2 = EI-A-βAla-2; 3 = EI-A-βAla-3; 4 = EI-A-βAla-4.

on the ODS column was caused by their hydrophobicity.

On the other hand, the EI-T-*n* oligomers showed good separation from each other on Si-Ade, and the retention times were longer than those of the EI-A-*n* oligomers on Si-Ade. Behavior of separation of the EI-A-*n* oligo-

mers on Si-Thy resembled that of the EI-T-*n* oligomers on Si-Ade. The retention times of the EI-A-*n* oligomers on the Si-Thy column were the longest of the three column systems. Hence, the separations of oligomers for EI-T-*n*/Si-Ade system and for EI-A-*n*/Si-Thy system were assumed to be mainly caused by spe-
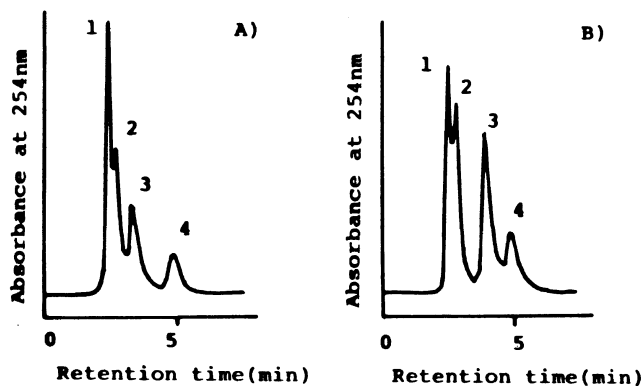
Figure 6. Chromatograms of A) EI-T-αAla-*n* and B) EI-T-βAla-*n* on Si-Ade column. Mobile phase, water–methanol (1 : 1, v/v); flow rate, 1.0 ml min⁻¹; temperature, 30°C; detector, 254 nm. Peaks: A) 1 = EI-T-αAla-1; 2 = EI-T-αAla-2; 3 = EI-T-αAla-3; 4 = EI-T-αAla-4. B) 1 = EI-T-βAla-1; 2 = EI-T-βAla-2; 3 = EI-T-βAla-3; 4 = EI-T-βAla-4.

cific hydrogen bonding interactions between adenine and thymine. The results also suggest that the more the base-pairing sites, the later the retention times. The hydrophobic interaction, however, was not excluded in the case of the EI-A-*n*/SI-Ade system.

For the other oligomers which have spacer units such as α-alanine or β-alanine, the separation of oligomers was good on complementary base immobilized silica (Figures 5 and 6). The retention times, however, were different in the three types of oligomers.

Effect of Mobile Phase

It has been known that the complex formation of the synthetic nucleic acid analogs is influenced by the condition used; temperature and solvent. The formation of the polymer complex between polymethacrylate derivatives of nucleic acid bases could not be observed in a good solvent for the nucleic acid bases such as dimethyl sulfoxide. On the other hand, the complementary polymer complex was formed by the addition of a poor solvent for nucleic acid bases such as ethylene glycol into the solution in dimethyl sulfoxide.[1,3,5]

Studies on the polyethyleneimine derivatives of nucleic acid bases were carried out in mixtures of water and ethylene glycol (1 : 1 and
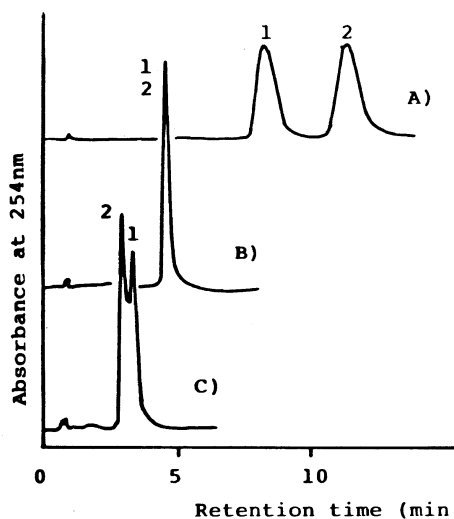

Figure 7. Chromatograms of EI-T-3 and EI-A-3 mixture on Si-Ade. Mobile phase: A) water–ethylene glycol (4 : 1, v/v); B) water–ethylene glycol (4 : 2, v/v); C) water–ethylene glycol (4 : 3, v/v); flow rate, 1.0 ml min⁻¹; temperature, 30°C; detector, 254 nm. Peaks: 1 = EI-T-3; 2 = EI-A-3.

2 : 1, v/v).[5] The complementary complex was formed at a low composition of ethylene glycol to water (about 1 : 2, v/v), but the polymer precipitated with excess water. In the present study, the HPLC analysis of the oligoethyleneimine derivatives was also studied in the water–ethylene glycol solvent system which

was used for polymer complex formation in solution.

Figure 7 shows chromatograms for a mixture of trimer models (EI-T-3 and EI-A-3) on Si-Ade with different mixing ratios of water–ethylene glycol (EG) mobile phases; the H_2O–EG ratios were 4:1 (7A), 4:2 (7B), and 4:3 (7C) (v/v). In the H_2O–EG (4:1, v/v) mobile phase (Figure 7A), the trimer model of thymine was eluted faster than adenine trimer. Using H_2O–EG (4:2, v/v) as a mobile phase (Figure 7B), EI-T-3 and EI-A-3 showed the same retention times. On the other hand, EI-T-3 which had the complemental thymine bases of adenine in Si-Ade was eluted slower than EI-A-3 with H_2O–EG (4:3, v/v) mobile phase (Figure 7C). Consequently, it is clear that the kind of interaction changed by a mixing ratio of water–ethylene glycol as the mobile phase.

The interaction of the nucleic acid bases is affected by three main factors; that is, hydrophobic, hydrogen bonding, and electrostatic interactions. For the neutral nucleic acid analogs, the electrostatic interaction may be neglected. The latest elution of EI-A-3 on Si-Ade column with H_2O–EG (4:1, v/v) mobile phase (Figure 7A) can be explained by the hydrophobic interaction and/or by the specific base pairing; however the hydrophobic interaction may be the main factor in aqueous solution. With increasing content of ethylene glycol to water, the hydrophobic interaction decreases, which causes the decrease in the retention as shown in Figure 7C. At high composition of ethylene glycol, therefore, the specific hydrogen bonding interaction becomes a main factor of retention, so EI-T-3 was eluted later than EI-A-3 in Figure 7C.

Effects of Spacer Units in the Oligomer Models

The effects of the spacer groups of polyethyleneimine derivatives on interactions were studied in a solution system.[7–10] The spacer group (α-alanine or β-alanine units) separates the nucleic acid base from the ethyleneimine chain (Figure 3), and affects the contribution of both the intramolecular and intermolecular interactions of the nucleic acid bases (Figure 1). The β-alanine spacer in EI-A-X-n promotes the intermolecular interaction of the adenine bases with the thymine derivative. However, the same spacer group in EI-T-X-n inhibits the intermolecular interactions of the thymine bases with the adenine derivatives. This is due to the influence of the intramolecular interaction on the intermolecular interaction: the higher the intramolecular interaction, the less the intermolecular interaction. On the other hand, the α-alanine spacer both in EI-A-X-n and EI-T-X-n inhibited the intermolecular interaction of the nucleic acid bases with the complementary bases. This is due to the steric effect of the α-alanine unit.

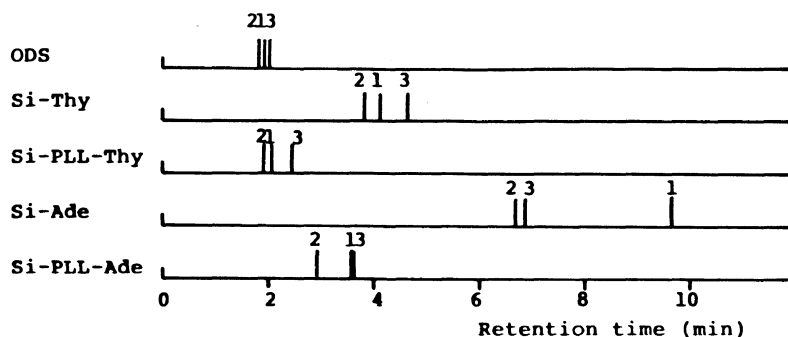Figure 8 shows the retention times for three

Figure 8. Retention times of the tetramer models containing thymine base on ODS, Si-Thy, Si-PLL-Thy, Si-Ade, and Si-PLL-Ade. Mobile phase, methanol; flow rate, 1.0 ml min^{-1}; temperature, 30°C. Peaks: 1 = EI-T-4; 2 = EI-T-αAla-4; 3 = EI-T-βAla-4.
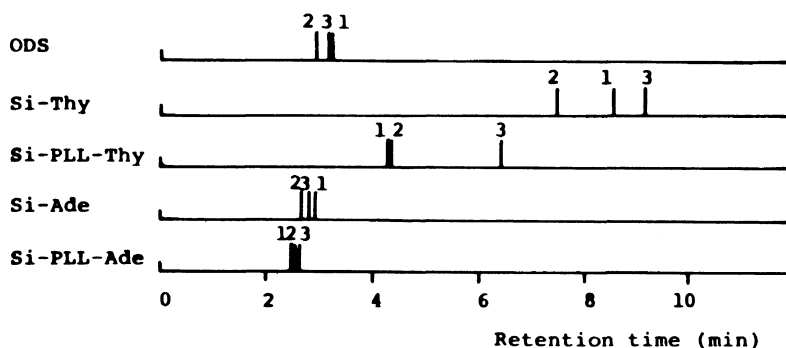
Figure 9. Retention times of the dimer models containing adenine base on ODS, Si-Thy, Si-PLL-Thy, Si-Ade, and Si-PLL-Ade. Mobile phase, methanol; flow rate, 1.0 ml min^{-1}; temperature, 30°C. Peaks: 1 = EI-A-2; 2 = EI-A-αAla-2; 3 = EI-A-βAla-2.

kinds of tetramers which have different spacer groups using five kinds of columns systems in the methanol mobile phase. Using methanol as the mobile phase, hydrophobic interactions may be neglected from the separation factors. The tetramer models of thymine gave excellent separation on Si-Ade under this condition, and the retention times were in the order, EI-T-αAla-4 $\leq$ EI-T-βAla-4 $\ll$ EI-T-4. The data exactly corresponded to the ability of intermolecular interactions of thymine derivatives to occur with the adenine derivative in solution (both α-alanine and β-alanine spacer groups in EI-T-X-n inhibit the intermolecular interaction of thymine base).

Retentions of the thymine tetramers on the polymer bound silica gel (Si-PLL-Ade) were also observed, but their order differed from the case on Si-Ade: EI-T-αAla-4 $\ll$ EI-T-4 $\leq$ EI-T-βAla-4. Distribution of nucleic acid bases is irregular but all over the surface of silica gel in Si-Ade (monomeric nucleic acid base bonded). On the other hand, distribution of the nucleic acid bases is regular on the helical poly-L-lysine derivative in Si-PLL-Ade (polymeric nucleic acid base bonded), and the polymer is in part on the surface of the silica gel. Therefore, the absolute value of the retention time cannot be compared between Si-Ade and Si-PLL-Ade, but the order of retention time may be a reflection of interactions of nucleic

acid bases. The behavior of the separation of the tetramer model of thymine on Si-PLL-Ade was different from that on Si-Ade or on the silica gel containing thymine base. The retention of EI-T-βAla-4 on Si-PLL-Ade was similar to EI-T-4 and stronger than EI-αAla-4. This may be caused by the well matching state between the thymine base units in EI-T-βAla-4 and the adenine base units on helical poly-L-lysine of Si-PLL-Ade.

Similar results were obtained for the separation of EI-A-X-n as shown in Figure 9, where the adenine dimer models were used because the longer oligomer eluted too late. The dimer models of adenine can be separated successively, EI-A-αAla-2, EI-A-2, and EI-A-βAla-2 on the Si-Thy column. On using the Si-PLL-Thy column, EI-A-βAla-2 can be separated from the other dimer models, while the separation is negligible with ODS, Si-Ade, and Si-PLL-Ade columns. The data for EI-A-X-n also corresponded to the ability of intermolecular interactions of adenine derivatives to occur with the thymine derivative in solution (the α-alanine spacer inhibits the intermolecular interaction, and the β-alanine spacer promotes the intermolecular interaction).

The separation behavior of the nucleic acid base oligomers on nucleic acid base bonded silica gels can thus be concluded to be similar to the intermolecular interactions of the oli-

gomers with the polymer in solution. Hence, these HPLC systems are useful for estimation of interactions between nucleic acid analogs.

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