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A RPLC C_{30} Column for the Separation of Oligonucleotides

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In reversed-phase high-performance liquid chromatography of large oligonucleotides, synthesized chemically and subsequently deblocked, a silica-based column prepared with triacontyldimethylchlorosilane, has been found to be superior to those with octadecylsilyl ligand.

In gene manipulation, preparation of the targeted gene fragments is one of the most important steps. Chemical synthesis is one of the methods for this purpose.[1] Single-stranded oligonucleotides, obtained by this method, can be purified as a function of the number of bases by an anion-exchange chromatography (AEC).[2] In order to remove the impurities that cannot be separated by AEC, the application of reversed-phase high-performance liquid chromatography (RPLC) has been explored and it has been reported that this method can differentiate the base sequences of large oligonucleotides.[3,4] In the present study, in order to improve the RPLC separation technique, the effect of the alkyl groups bonded to silica has been investigated.

Oligonucleotides used as samples were prepared by the liquid-phase triester method.[5,6] Base sequences of the samples are dTCAAATC (7b), dCGGATTTGA (9b), dCCIAAITCCATCCAICCITAIGC (23b), and dCCIAAITCCATCCAICCCATITAITC (26b). The HPLC system used consisted of a HLC-803D chromatograph, GE-4 gradient system, and UV-8 Model II variable-wavelength UV detector (all from Toyo Soda, Tokyo,

Japan). A commercially available column tested was μ-Bondapak C_{18} (Waters Associates, Milford, MA, USA). Experimental columns were prepared from TSK silica (Toyo Soda) by the method described previously.[3,7] A parameter, HETP', used for the evaluation of column efficiency for oligonucleotides, was obtained according to the procedure described previously.[3]

Our previous paper demonstrated that the column efficiency for oligonucleotides increases with increasing chain length of the modifying reagent. In this study, therefore, silica bonded with a triacontyl alkyl chain was examined for oligonucleotides separation. Triacontyldimetylchloro- (MC-C30) and triacontyltrichlorosilane (TC-C30) were used for the column preparation and the results obtained for the oligonucleotides with these columns were compared. Table 1 summarizes the HETP' values obtained with both columns for 7b, 9b, 23b, and 26b.

Table 1. Column Efficiencies for Oligonucleotides, Obtained with C_{30} Columns, Prepared with Different Alkylating Reagents

No.	Alkylating reagent	HETP'(μm)			
		7b	9b	23b	26b
1	MC-C30	0.8	1.2	6.6	5.1
2	TC-C30	1.4	1.4	44	50

This result indicates that only MC-C30 leads to good peak shapes. The superiority of the monochloro reagent to the trichloro may be due to the same reason as that previously discussed for C_{18} columns:[3] the surface coverage of 15 %, obtained by use of octadecyldimethylchlorosilane, produces desired results. Therefore, a C_{30} column, prepeared with triacontyldimetylchlorosilane (No.1), was used in the following experiments.

The separations of oligonucleotides, produced with this C_{30} column, were compared with those obtained with C_{18} columns. Shown in Fig.1 are the chromatograms obtained. The commercially available C_{18} column gave broad peaks for all the samples. The experimental C_{18} column produced sharp peaks, even for 23b and 26b although the samples were eluted closely to each other. The separation of oligonucleotides was improved by the C_{30} column without changing the peak shape.

The C_{30} column was also used for mixtures of oligonucleotide. A 9-mer (dAGTTGTCCA), containing impurities formed during the synthesis, was

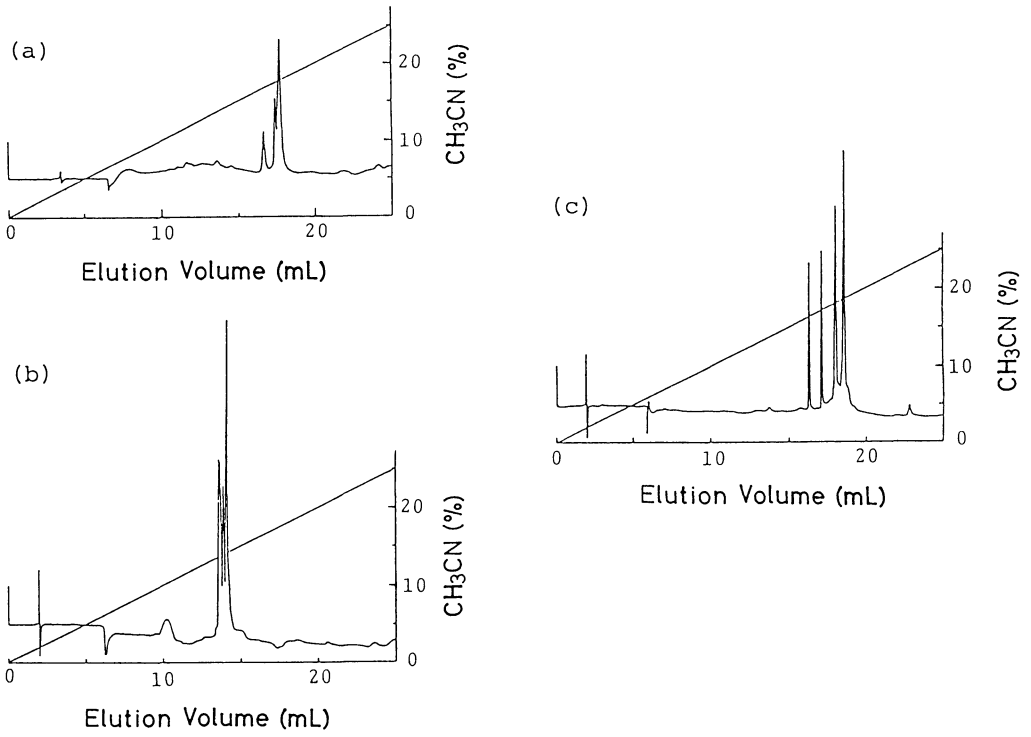

Fig.1. Separation of oligonucleotides (7b, 9b, 23b, and 26b) obtained with (a) a commercially available C_{18} column, (b) an experimental C_{18} column, and (c) an experimental C_{30} column. Conditions: flow rate, 0.5 mL/min; eluent, MeCN gradient in 0.1 M aq. $MeCOONH_4$ (profiles of MeCN gradient are shown in the Figures); detection, UV at 260 nm; temperature, ambient.

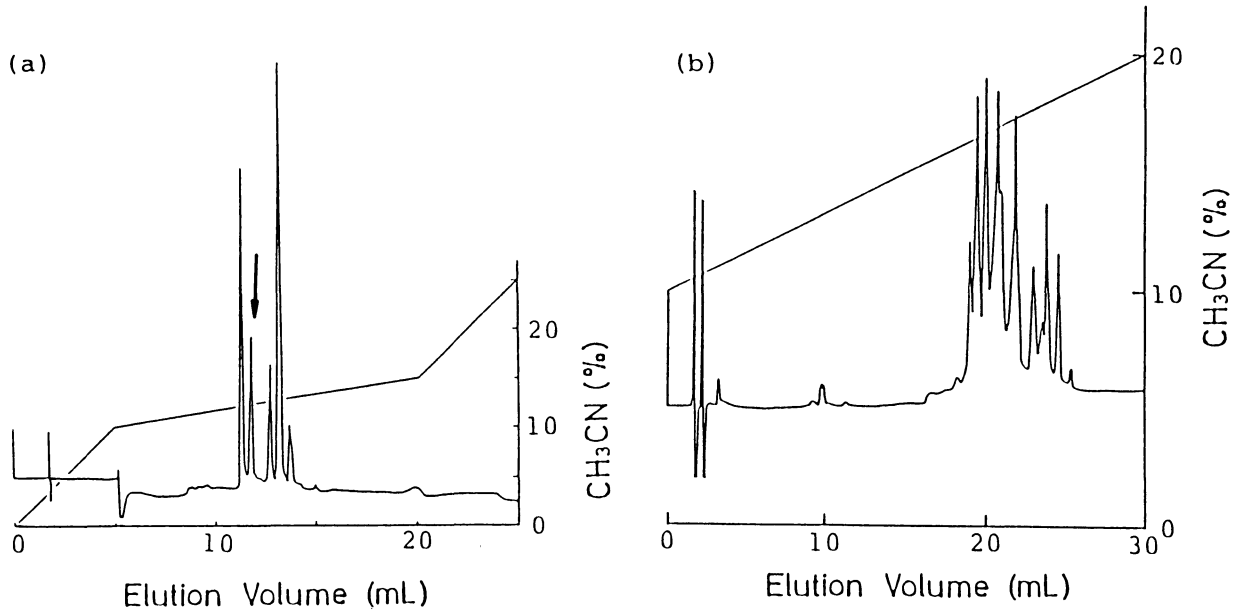

Fig.2. Elution profiles of (a) 9-mer (dAGTTGTCCA), containing impurities and (b) 15-mers (dGTCXAYYTAZTCCAT, X=A, G, C, and T, Y=A and G, and Z=T and C), obtained with an experimental C_{30} column. Profiles of MeCN gradient in 0.1 M $MeCOONH_4$ are shown in the Figures. Other conditions are the same as those given in Fig.1.

chromatographed on the C_{30} column. The targeted oligomer, indicated by an arrow, could be isolated from the by-product, as shown in Fig.2a. Also, the resolution of dGTCXAYYTAZTCCAT (X=A, G, C, and T, Y=A and G, and Z=T and C), which consists of 32 isomeric oligonucleotides having the same base numbers but different base sequences, was tested on this C_{30} column. The chromatogram obtained is shown in Fig.2b. In this chromatogram, well-resolved peaks, arising from the 32 isomeric large oligonucleotides, are observed, demonstrating the high efficiency of this column for oligonucleotides.

In order to see the difference in the separating ability of the C_{18} and C_{30} test columns for other compounds, the elution behavior of n-alkyl alcohols was compared. The log k' values obtained for n-alkyl alcohols were plotted against the carbon number of the samples. The plotted lines, obtained with the C_{18} and C_{30} test columns, were similar to each other. It was also determined that other series of compounds such as proteins, barbiturates, organic acids, and dinitrobenzene isomers, gave similar elution profiles on both the columns. These results show the unique retention of oligonucleotides on RPLC columns.

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