Introduction of Peptide Functions into DNA by Nucleic Acid Peptides, NAPs

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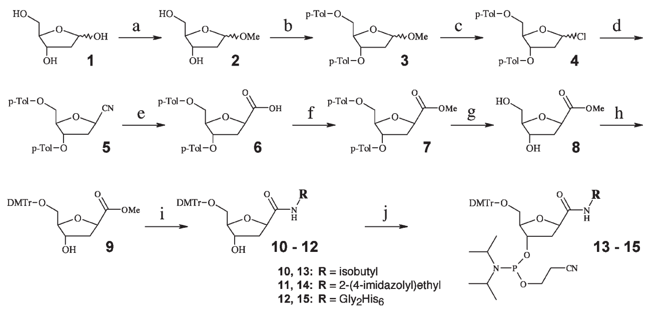
Nucleic acid peptides (NAPs) with a mimetic amino acid side residue at the base position of the nucleotide via an amide bond were synthesized from 3-deoxy-6-O-(4,4′-dimethoxytrityl)allonic acid methyl ester as the common precursor. Furthermore, an NAP with an octapeptide at the C1′ position was synthesized. The peptide-linked NAP exhibits both functions of the oligopeptide part and of the oligonucleotide part.

Investigations of functional nucleic acids have rapidly progressed since the discovery of ribozymes[1] and many enzymatic functions and molecular recognition functions were developed.[2] However, nucleic acids have potential limitations, that is, any functional nucleic acids should express their functions only by using four bases, a sugar, and a phosphate group, while proteins could utilize the various functional groups in the natural 20 amino acids. Actually, almost all the complex functions in living organisms are borne by proteins.[3] Thus, modified nucleic acids with amino acid side chains would be useful for the construction of novel functional molecules.[4] Especially, nucleotide analogues modified at their C1′ position would be a candidate for preparing functional nucleic acids because many C1′ modified nucleotides are applicable to enzymatic procedures.[5] However, C1′ modified nucleotides reported so far are prepared under rather severe condition.[4–6] Furthermore, common synthetic route for varied amino acid side chains is hard to realize, thus, the uncomplicated C1′ modification with common precursor is desired.

In this study, we synthesized a nucleic acid peptide (NAP) with a mimetic amino acid side residue at the base position of the nucleotide via an amide bond as shown in Scheme 1. This NAP is the molecule at the contraposition of the peptide nucleic acid (PNA) with nucleobases and a peptide backbone derived by Nielsen et al.[7] By using NAPs, any amino acid side chains can be introduced into a DNA strand easily in common procedure by standard DNA synthesizer. Moreover, our procedure is applicable to C1′ modification using any other molecules with an amino group. Therefore, the NAP strategy would realize many new artificial functional nucleic acids. 3-Deoxy-allonic acid methyl ester (**8**) that was used for the preparation of the DNA labeling reagents by Nelson et al. or its dimethoxytritylated form (**9**) was chosen as the common precursor for preparation of NAPs.[8,9] Compound **5** was prepared as previously reported from 2-deoxy-D-ribose (**1**). Here we used a modified process with shorter time than the procedure by Nelson et al. Acid hydrolysis of the ditoluoyl nitrile **5** provided the carboxylic acid **6**, and was methylated to **7**. The toluoyl groups of **7** were removed by treatment with $NaOCH_3$ to get the product **8**, then it was tritylated to afford **9**. The compound **9** was an important precursor for preparation of its functionalized derivatives.

The compound **9** was then coupled with the free amino



Scheme 1. Synthetic route of the phosphoramidite **13–15**.[9] (a) HCl, CH_3OH; (b) *p*-toluoyl chloride, pyridine; (c) HCl, diethyl ether; (d) trimethylsilyl cyanide, CH_2Cl_2; (e) HCl, dioxane; (f) CH_3OH, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 4-dimethylaminopyridine, dioxane; (g) CH_3ONa, CH_3OH; (h) dimethoxytrityl chloride, pyridine; (i) an amin, CH_3CN; (j) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, CH_3CN, 1H-tetrazole.

group. The Leu-NAP and His-NAP that have the mimetic residue of the aliphatic leucine and aromatic histidine side chain, respectively, were prepared as examples. Compound **10**, the nucleoside analogue of Leu-NAP was synthesized by the coupling of **9** and isobutyl amine.[10] Similarly, compound **11** was synthesized by the coupling of **9** and histamine. Compounds **10** and **11** were phosphitylated to produce the β-cyanoethyl phosphoramidites **13** and **14** in order to introduce it into a DNA strand with the same chemistry as the normal DNA synthesis. The reactions were analyzed by TLC and confirmed by MALDI-TOF mass and ^1H NMR spectra. For DNA synthesis, a normal solid-phase synthetic procedure was used except that the coupling step for phosphoramidites **13** or **14** proceeded for 45 min to ensure sufficient reaction with the 5′-OH of the oligodeoxynucleotide. After the synthesis, the trityl group at the 5′ end was removed directly in the last coupling step, then deprotecton and cleavage from the solid support was achieved by 25% ammonia:ethanol = 3:1 (v/v) solution at room temperature.

An Leu-NAP was introduced into a DNA strand to produce d(CTTTC(Leu-NAP)TTCTT). The amide bond of the Leu-NAP was quite stable during the DNA synthesis and deprotection procedures. In the same way, a His-NAP was introduced in a DNA to produce d(TTGT(His-NAP)ATCCATT). In the case of His-NAP, the amide bond cleavage was observed during the purification step of the DNA and mild condition above mentioned was indispensable to avoid the cleavage.[11] As the result, it was clearly demonstrated that amino acid side chains could be introduced into DNAs at desired positions.

Any compounds with a free amino group can be coupled with the common precursor **9** and the corresponding phosphoramidite could be prepared similar for the synthesis of **13** or **14**.

This general characteristic of **9** indicates that any polypeptides and proteins would be able to be introduced into the desired and specified region of a DNA via the branched-type NAPs. When the connection of a peptide strand and a nucleotide strand is required, all one has to do is just to couple compound **9** or its phosphoramidite derivative with free polypeptides or free proteins. However, when the coupling at the amino terminus of a peptide strand is required, the side residues of the polypeptide may have to be protected.[12] Especially, for the successive synthesis of a DNA strand, the protecting group of the polypeptide must not be removed by acidic treatment during the 5′ deprotection of the oligonucleotide on the solid support. In this study, we have also synthesized an NAP (His_6-NAP, **12**) coupled with the octapeptide, glycine-glycine-histidine-histidine-histidine-histidine-histidine-histidine (Gly_2His_6). Compound **12** was converted to the corresponding phosphoramidite **15** and introduced into a DNA strand. To show one of the applications of the functional linkage of the polypeptide and oligonucleotide, a modified oligonucleotide, namely His_6-NAP-T_{20} with a His_6-NAP at the 5′ end of the eicosathymidylic acid was prepared. This His_6-NAP-T_{20} compound is the result of the linkage of two functions: one is the oligopeptide function to bind to the imino-diacetic acids via divalent metal ions[13] and the other is the oligonucleotide function to bind to the polyadenylic acids (poly(A)) by Watson–Crick base pairing. By using this molecule, one of the most useful columns in molecular biology, that is the column to pick up messenger RNA with the poly(A) tail from the total cell extract, should be easily prepared.[14]

The results of the poly(A) trapping experiment by the His_6-NAP-T_{20} are shown in Figure 1.[15] As shown in Figure 1a, the His_6-NAP-T_{20} was bound to a column in which the imino-diacetic acids were immobilized while T_{20} without Gly_2His_6 did not bind and all the T_{20} passed through the column (Figure 1b). This phenomenon implies the exhibition of the His_6-NAP-T_{20} function at the peptide part. When the poly(A) was loaded to the column, the poly(A) molecules were then trapped on the column in which the His_6-NAP-T_{20} was immobilized while no trapping of poly(A) was observed in the column with immobilized Gly_2His_6 without the T_{20} (Figure 1c). This result indicates the exhibition

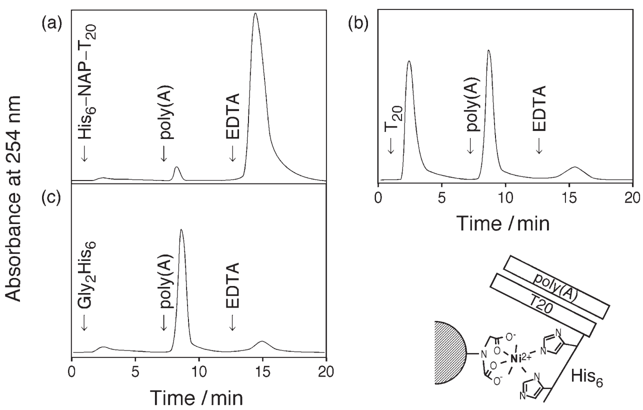


Figure 1. Poly(A) trapping experiment.[15] Three samples, (a) NAP-DNA (His_6-NAP-T_{20}), (b) DNA (T_{20}), and (c) peptide (Gly_2His_6) were used for the analysis. Arrows indicate the time for loading of the solutions to the column. The UV absorption at 254 nm indicates the elution of nucleic acids (T_{20} or poly(A)) from the column.

of the His_6-NAP-T_{20} function at the oligonucleotide part. Once the histidines were dissociated from the column resin by chelation of the Ni^{2+} ions, the trapped complex of His_6-NAP-T_{20} and poly(A) was eluted from the column as shown Figure 1a. Thus, we have succeeded in introducing an oligopeptide function into the DNA via linkage of the oligopeptide strand and the DNA strand by an NAP. Though the coupling of the peptide function might be achieved by other methods previously reported,[4–6] our NAP strategy should be most common and easiest way to introduce varied functions of desired amino acids or oligopeptides into a DNA.

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9 Synthetic protocols are available in supporting information (S.info) at http://fiber.konan-u.ac.jp/support/CL_NAP/S.info.pdf
10 The molecular model of the sugar moiety in compound **10** is shown in S.info as Figure S1. The torsion angles of product **10** are also listed in Figure S1 along with the values of **8**.
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15 Detailed procedure and a schematic illustration of the poly(A) trapping experiments are shown in S.info as Figure S2.

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