Nuclear magnetic resonance of nucleotide-amidates. Investigation of the secondary structure of methylester of deoxyadenylyl - (5' - 3') - deoxyadenylyl - (5'→N) - L - phenylalanine

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ABSTRACT

The secondary structure of methyl ester of deoxyadenylyl - (5' - 3') - deoxyadenylyl - (5'→N) - L-phenylalanine / L- PheOMe - d(pApA)/ was investigated using proton magnetic resonance. The folded conformation stabilized by hydrophobic interactions in aqueous solution was shown for this compound.

The investigation of the chemical shifts of aromatic protons of L- PheOMe - d(pApA) permitted us to construct the conformational model of this compound.

INTRODUCTION

The formation of the specific complexes between proteins and nucleic acids very likely involves direct interactions between amino acid residues of the protein and bases of the nucleic acid. The aromatic rings of phenylalanine, tyrosine and tryptophan could interact with purine or pyrimidine rings of nucleic acid bases to form stacked complexes. Aromatic amino acids are often included into the active sites of enzymes involved into the nucleic acid metabolism /1-3/.

It is useful to study non-covalent interactions between monomeric units of nucleic acid and protein to elucidate the problem of nucleic acid-protein "recognition". Studies of nucleotidyl-(5'→N) - amino acids, containing phenylalanine, tyrosine and tryptophan by optical rotatory dispersion, circular dichroism /4-5/ and nuclear magnetic resonance (n m r) spectroscopy /6-8/ have shown the aromatic side chains of amino acids and heterocyclic bases in these compounds to be folded. For these compounds the conformational models have been constructed /9/.

In this paper we have investigated the secondary structure of phenylalanine derivative of deoxyadenylyl - (5'-3')- - deoxyadenylyl - 5' - phosphate by n m r . Using this model compound we intended to detect the influence of phenylalanine - adenine interactions on adenine-adenine stacking.

MATERIALS AND METHODS

Methyl ester of deoxyadenylyl - (5' - 3') - deoxyadenylyl - (5'→N) - L-phenylalanine was prepared by the carbodiimide method /10/. This compound was identified using the acid hydrolysis and UV - spectroscopy data.

Prior to the spectral measurements L-PheOMe - d(pApA) had been purified by paper electrophoresis which was carried out for 60 min with a voltage gradient of 30 V/cm on Watman No 3 paper in triethylammonium bicarbonate buffer (pH 8.5, 0.05 M). Then paper chromatography was carried out on Watman No. 1 paper in 2-propanol - conc. ammonia-water (7:1:2 V/V) solvent system.

Varian XL-100-15 nuclear magnetic resonance spectrometer was used for n m r studies. All the compounds were dissolved in deuterium oxide (D₂O, 99.8%), and then evaporated twice with D₂O to reduce the concentration of HDO. In some experiments we used dimethyl sulfoxide-d₆ (Me₂SO-d₆). Chemical shifts were monitored relative to an internal t-butanol standard. The chemical shifts were always measured to be better than 0.3 cps. The pD values were calculated using the pD=pH-0.4 equation /11/. The concentration in the experiments was 5.10⁻³M. This concentration was chosen to minimize intermolecular stacking processes /8/.

RESULTS AND DISCUSSION

The n m r spectrum of L-PheOMe-d(pApA) is present in Figure 1. The chemical shifts of protons of different groups of L-PheOMe - d(pApA) and equimolar mixture of methyl ester of phenylalanine + d(pApA) are listed in table I.

The spectral assignment of d(pApA) adenine protons was made using the data of the previous paper /12/. The signal of d(pApA) H-8(3') proton was identified by compar-

Figure I. The n m r spectrum of methyl ester of deoxyadenylyl-(5'-3') - deoxyadenylyl - (5'→N)-L-phenylalanine in D₂O. (8.9. . 10⁻³ M, pD 6.5, T=28°C).

ing the spectra of d(pApA) at pD 6.5 and pD 8.8 /13/. The assignment of aromatic proton's signals of L-PheOMe-d(pApA) was made by comparing the spectrum L-PheOMe-d(pApA) with the spectrum of the equimolar mixture: L-PheOMe+d(pApA). The validity of spectral assignment of signals H-8 protons was proved by deuterium-exchange /14/.

Comparison of the chemical shifts of aromatic protons of L-PheOMe-d(pApA) with the chemical shifts of the corresponding protons in equimolar mixture of L-PheOMe + d(pApA)

Table I

Chemical shifts (δ) of protons of different groups of methyl ester of deoxyadenylyl-(5'-3')-deoxyadenylyl-(5'→N)-L-phenylalanine and equimolar mixture-deoxyadenylyl-(5'-3')-deoxyadenylyl-5'-phosphate+methyl ester of L-phenylalanine,

D₂O, pD 6.5, T=28°C.

Compound	concentration, M	H-8 (3')	H-8 (5')	H-2 (3')	H-2 (5')	aromatic protons of phenylalanine	-OCH ₃
L-PheOMe-d(pApA)	$4.6 \cdot 10^{-3}$	8.24	8.42	7.97	8.12	7.09	3.67
L-PheOMe+d(pApA)	$4.3 \cdot 10^{-3}$	8.47	8.44	8.02	8.13	7.44	3.88

(Table I) has revealed:

a) upfield chemical shifts of H-8(3') H-2(3') adenine protons and aromatic protons in L-PheOMe-d(pApA); this phenomena due to the ring-current effect of amino acid aromatic radical on adenine base protons, or to the similar effect of 5'-adenine ring-current on phenylalanine aromatic protons /15, 16/.

b) the change in line resonances profiles of aromatic protons of phenylalanine resulting from the magnetic field gradient of 5'-adenine ring-current /6/,

c) the chemical shifts of H-2(5') and H-8(5') protons of L-PheOMe-d(pApA) are the same as in equimolar mixture: L-PheOMe+d(pApA); the phenomena due to stacking interactions between adenine bases in L-PheOMe-d(pApA) are the same as in d(pApA).

From all these facts one may conclude that L-PheOMe-d(pApA) has a folded trinucleoside diphosphate-like structure maintained by non-covalent interactions. Aromatic ring of phenylalanine interacts with 3'-adenine and does not intercalate between adenine bases.

To visualize the conformational model of L-PheOMe-d(pApA), one is elucidate the orientation of the adenine's bases with respect to the deoxyribose's residues. It is possible qualitatively to confirm that AMP spends some time in the anti-conformational state using paramagnetic label technique. It is known

that at low concentration of the nucleotide and Mn^{+2} ions, the latter becomes bound to the phosphate group of the nucleotide, causing a broadening of the resonance line of the protons localized in the close proximity of the phosphate group of the nucleotide /17-19/. In our previous papers we used this method for detecting of the existence of such conformational states, in which the bases are in the anti-orientation with respect to sugar residue in nucleotide-amidates/7,9/. The appearance of the selective broadening signal of H-8(5') and H-8 (3') protons on addition of Mn^{+2} ions (concentration $4 \cdot 10^{-5} M$) to L-PheOMe-d(pApA) (Figure 2) was interpreted as meaning that H-8 protons could be some time near the phosphate groups and hence that both adenine residues spend some time in the anti-conformation, as the difference between energy levels of syn- and anti-conformations of AMP is well-known (2 kcal/mol/20/) and it is possible to confirm that both adenine residues have preferentially the anti-conformation.

In our previous papers the conformational models of nucleotidyl-(5'→N)-amino acids (containing phenylalanine, tyrosine and tryptophan) were constructed using the ring - current effect of amino acid's aromatic radicals on nucleic acid's bases and ring current effect of nucleic acid bases on aromatic radicals of amino acids /7-9/. For the constructing of the conformational model of L-PheOMe-d(pApA) we used chemical shift differences of aromatic protons of this compound ($\Delta\delta$)*/. So for L-PheOMe-d(pApA) chemical shift - difference is equal to 5 cps for H-2(3') and 23 cps for H-8 (3') proton (Table I). Using the literature data it is possible to estimate the approximate distance from the upfielded protons to aromatic system whose ring-current produced this upfield shift. According to experimental data L-PheOMe-d(pApA) has conformation in which the aromatic ring

*/The notion of chemical shift difference is $\Delta\delta = \delta_1 - \delta_2$
 where δ_1 - chemical shift of proton of equimolar mixture;
 δ_2 - chemical shift of the corresponding proton of nucleotide-amidate.

Figure 2. The resonance lines of protons of methyl ester of deoxyadenylyl - (5'-3') - deoxyadenylyl-(5'→N)-L-phenylalanine ($1.2 \cdot 10^{-2} \text{M}$, pD 6.5, $T=28^{\circ}\text{C}$) :

a) in D_2O ; b) in D_2O , containing $4 \cdot 10^{-5} \text{M Mn}^{+2}$.

of phenylalanine is placed above 3'-adenine plane at the distance 3.4-3.6 Å. The center of phenylalanine ring disposes above the line, which links H-2(3') and H-8(3') protons. This center is nearer to H-8(3') proton. In such structure the ring-current effect of aromatic system of amino acid produced a greater influence on H-8(3') proton. The planes of 3'-adenine and aromatic phenylalanine radical are parallel. (Figure 3).

As shown above the mutual disposition of adenine rings in L-PheOMe - d(pApA) is the same as in d(pApA) /12, 21/. All representative data allowed us to construct the conformational model of L-PheOMe-d(pApA) (Figure 3).

We have also obtained the n m r spectrum of L-PheOMe-d(pApA) in dimethyl sulfoxide- d_6 for clearing up the nature of the forces stabilizing the secondary structure of the intramolecular complex L-PheOMe-d(pApA).

It is known that dimethyl sulfoxide disorders the secondary structure formed by hydrophobic interactions in dinucleotides /12/ and nucleotide amidates /9/. The chemical shift values of adenine and phenylalanine protons in this compound are similar to the values of the chemical shift of the corres-

Figure 3. Schematic presentation of the conformational model of aromatic system of methyl ester of deoxyadenylyl -(5'-3')-deoxyadenylyl - (5'→N)-L-phenylalanine as viewed in the 5' to 3' direction.

The aromatic ring of phenylalanine (dotted line) is disposed under the adenine (3'-residue) plane and adenine (3'-residue) ring (broken line) is disposed under the adenine (5'-residue) plane.

ponding protons in equimolar mixture in $\text{Me}_2\text{SO}-d_6$. The profile of multiplete of phenylalanine aromatic ring protons becomes narrower and similar to the resonance line of the corresponding protons in equimolar mixture. Thus, the structure of intramolecular complex of L-PheOMe-d(pApA) is mainly stabilized by hydrophobic interactions.

Conclusions obtained in this paper may be used in the investigation of aromatic amino acids - nucleic bases interactions in the study of nucleic acid-protein "recognition" problem.

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