Spectroscopic properties of various 2'(3')-O-aminoacyldinucleoside phosphates analogous to the 3' terminus of AA-tRNA.

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ABSTRACT

Hypochromicity and circular dichroism data are reported for the 2' and 3'-O-aminoacyldinucleoside phosphates cytidyl-(3'-5')-2'(3')-O-L-phenylalanyl-adenosine, cytidyl-(3'-5')-2'-deoxy-3'-O-L-phenylalanyl-adenosine, cytidyl-(3'-5')-2'-deoxy-3'-O-glycyl-adenosine, and cytidyl-(3'-5')-3'-deoxy-2'-O-L-phenylalanyl-adenosine, all of which can act as analogs of the 3' terminus of AA-tRNA in various partial reactions of protein biosynthesis. Although all these systems have a 2'-OH group in the furanose of the 3'-residue, differences exist in the extent and/or mode of base-base overlap for most of them, except for cytidyl-(3'-5')-2'(3')-O-L-phenylalanyl-adenosine and cytidyl-(3'-5')-3'-deoxy-2'-O-L-phenylalanyl-adenosine. It is concluded that the biological activity of the above analogs is affected both by the position of the aminoacyl group and the stacking properties of the bases.

INTRODUCTION

The 2' and 3'-O-aminoacyldinucleoside phosphates C-A-Phe, C-A(2'H)Phe, C-A(2'H)Gly and C-A(2'Phe)H¹ act as analogs of the 3' terminus of AA-tRNA in various partial reactions of protein biosynthesis.^{2,3} Accordingly, it was of particular interest to examine the optical properties of these compounds in an effort to gain important insights as to the significance of conformation of the 3' terminus of AA-tRNA in protein biosynthesis. In this paper we studied the hypochromicity and CD of these aminoacylated oligonucleotides in order to evaluate the effect of aminoacylation and sugar modification on the geometry of the dinucleoside phosphates.

All systems studied contained the 2'-OH group in the furanose of the 3'-residue, whereas the furanose of the 5'-residue was either a ribose, a 2'-deoxy- or 3'-deoxyribose. There had been some evidence that the influence of the 2'-OH group on the conformation of a dinucleotide is only significant provided the 2'-OH group is located next to a 3'-phosphodiester linkage.⁴ Actually it had been proposed^{4,5} that the 2'-OH group in the rNp portion of the dimer may provide a steric hindrance in forcing the bases in a particular mode of stacking. A study on 2'-O-methyl derivatives had previously revealed that possible hydrogen bonding of the 2'-OH group to some acceptors has little in-

fluence on the conformation or stability.^{6,7}

It was reasonable to assume that all the various aminoacyldinucleoside phosphates studied here would exhibit similar base-base stacking geometries, since they only differ in substituents which are located outside of the stack. However, we present experimental evidences which clearly indicate that this assumption is not always adequate. Subtle differences exist in the extent and/or mode of base-base overlap for most of these derivatives except for C-A-Phe and C-A(2'Phe)H. For the latter two compounds it is proposed that their base stacking conformation is similar.

MATERIALS AND METHODS

The aminoacyldinucleoside phosphates utilized in this study were prepared according to a previously described method.² Ultraviolet CD measurements were made on a Cary Model 6002 CD attachment to a Cary 60 recording spectropolarimeter at 28°C. Results are reported in terms of differential dichroism absorption ($\epsilon_L - \epsilon_R$) and are given on the basis of the molar oligomer concentration. The molar extinction coefficients of C-A, C-A-Phe, C-A(2'H)Phe, C-A(2'H)Gly and C-A(2'Phe)H were obtained by phosphorous analysis.⁸

RESULTS AND DISCUSSION

In Table I the molar extinction coefficient and hypochromicity of C-A and some of its aminoacylated derivatives are reported. The hypochromicity values were calculated according to Warshaw and Tinoco⁹ using, however, phosphate based molar extinction coefficients for the oligomers and literature values for the molar extinction coefficients of equimolar mixtures of the corresponding 5'-nucleotide, nucleoside or deoxynucleoside and amino acids. Our determined hypochromicity value for C-A is close to that reported earlier.⁹ From Table I it can be seen that 2'(3') aminoacylation of the 5'-bound adenosine moiety with a Phe group will not change the hypochromicity significantly. Aminoacylating the 5'-bound deoxyadenosine in position 3' with Phe gives a hypochromicity similar to that obtained with Gly. However, the data in Table I clearly indicate that both 2'-dA derivatives, C-A(2'H)Phe and C-A(2'Phe)H, have a greater per cent hypochromicity than C-A or C-A-Phe. Thus, the extent of base-base overlap is greater in the former two cases and smaller in the latter two. It is interesting to note that rAprA has a slightly smaller hypochromicity than rApdA;⁴ however, the difference was smaller than in our case and not seriously considered by the authors. Possibly, the presence of aminoacyl groups in the 2'-deoxyribose ring will even further increase the extent of base-base overlap.

Table I

Molar Extinction Coefficient and Hypochromicity of C-A and 2'(3')-O-Aminoacyl-dinucleoside Phosphates^{a)}

	Molar Extinction coef. at 260 nm (10 ³) ^{b)}	% Hypochromicity at 260 nm ^{c)}
C-A	20.6 ± 2%	8.8%
C-A-Phe	21.0 ± 2%	7.7%
C-A(2'H)Phe	20.2 ± 2%	12.4%
C-A(2'H)Gly	20.0 ± 2%	12.7%
C-A(2'Phe)H	20.5 ± 2%	-- ^{d)}
a) determined at room temperature in a 0.05M Tris (HCl), 0.1M NH ₄ Cl, 0.01M MgCl ₂ , pH 7.0 buffer.		
b) are per phosphate and refer to the oligomer.		
c) for the hypochromicity determination literature values for the molar extinction coefficients of the corresponding 5' nucleotide, nucleoside or deoxynucleoside and amino acids were used.		
d) not determined, because the molar extinction coefficient of p3'dA is not yet known.		

In Figures 1 and 2 the CD spectra of C-A, C-A-Phe, and C-A(2'H)Phe, C-A(2'H)Gly, and C-A(2'Phe)H are reported. Three features are readily apparent when the CD properties of the various oligomers are compared with one another. First, the magnitude of the molecular ellipticities of the oligomers with no 2'-OH group in the furanose of the 5' residue, C-A(2'H)Phe and C-A(2'H)Gly, is significantly smaller than that observed for C-A and C-A-Phe. Second, the CD spectrum of C-A(2'Phe)H shows great similarity with that of C-A and C-A-Phe. Third, aminoacylation of C-A with Phe causes a slight loss in the CD magnitude from peak to trough. Comparing the CD spectra of C-A(2'H)Phe and C-A(2'H)Gly with each other reveals that again the Phe carrying dimer has a slightly less intense CD spectrum.

It has already been pointed out that CD data may be more sensitive to differences in the mode of the base-base orientation, or in the proportion between right-handed and left-handed conformers in solution.⁴ The smaller CD amplitude for C-A(2'H)Phe and C-A(2'H)Gly is not necessarily in conflict with the higher hypochromicity values determined for these oligomers. It is generally believed that the amplitude of the CD curves is very sensitive to the angle θ of the transition dipoles. Thus, it is still conceivable that C-A(2'H)-

Fig. 1: Circular dichroism spectra of C-A-Phe (—) and C-A (--) in 0.05M Tris (HCl), 0.1M NH_4Cl , 0.01M MgCl_2 , pH 7.0 buffer at 27°.

Fig. 2: Circular dichroism spectra of C-A(2'H)Phe (—), C-A(2'H)Gly (--), and C-A(2'Phe)H (-.-) in 0.05M Tris (HCl), 0.1M NH_4Cl , 0.01M MgCl_2 , pH 7.0 buffer at 27°.

Phe and C-A(2'H)Gly have a greater base-base overlap than the other three oligomers with, however, a mode of base-base overlap which would give rise to a smaller CD intensity. It is interesting to note that A-A and A2'dA also exhibit such CD behavior.⁴

As for the small change in the CD intensity caused by the aminoacylation of C-A with Phe one could list several reasons. One likely explanation is that the Phe group exerts its influence by affecting the conformation of the furanose of the 5' residue. Such a change in the 5' sugar puckering could alter the average distance or angle between the transition dipoles of C and A which in turn should result in a change of the CD spectrum.

It is noteworthy that several investigators have observed conformational differences between tRNA and AA-tRNA (cf. ¹¹⁻¹³), while some other reports^{14,15,16} gave conflicting results. It would appear that at least some of the observed conformational changes in tRNA upon aminoacylation may occur on its 3' terminus and may be related to the stacking geometry of the 3' terminal C-C-A sequence. The changes in molecular conformation of the 3' terminus of AA-tRNA versus tRNA may thus influence its interaction with, e.g. ribosomal binding sites, elongation factor T_u and so on. The importance of stacking arrangement of the 3' end of AA-tRNA has been recently pointed out.¹⁷

In view of the previous discussion, the great resemblance between the two CD spectra of C-A(2'Phe)H and C-A-Phe suggests a similar mode of base-base overlap for these two different compounds. The structural difference between C-A-Phe and C-A(2'Phe)H consists of the presence and absence of a 3'-OH in the furanose of the 5' residue, respectively. On the other hand, both compounds have a 2'-OH group in the 3' residue which compresses the O(1') - C(1') region of the 5' residue in the dimer and it is believed that this steric interference affects the base-base overlap. Since the CD spectra suggest similar base-base overlap for both compounds we conclude that the furanose of the A(2'Phe)H and A-Phe parts are similarly compressed by the 2'-OH group of the C residue. Such a situation could arise provided the sugar puckering of A(2'Phe)H and A-Phe is similar. Proton magnetic resonance measurements on these two compounds should allow to clarify this point further.

At present it is difficult to assess how the reported differences in molecular conformation between e.g. C-A-Phe or C-A(2'Phe)H and C-A(2'H)Phe respectively would affect their biological properties. Striking differences in biological properties have been reported between C-A(2'Phe)H and C-A(2'H)Phe with the former one being recognized as substrate by elongation factor T_u and the latter being inactive.³ Ribosomal peptidyl transferase recognizes C-A-

(2'H)Phe as an acceptor substrate, whereas C-A(2'Phe)H is inactive.² The isomerizable ester C-A-Phe is active in both systems. It is conceivable, that the observed differences in the stacking properties of e.g. C-A(2'Phe)H and C-A-(2'H)Phe may play an important role in their different biological properties in addition to the position of the aminoacyl residue.

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- 1 Abbreviations used are: AA-tRNA, aminoacyl transfer ribonucleic acid; A-Phe, 2'(3')-O-L-phenylalanyladenosine; A(2'H)Phe, 2'-deoxy-3'-O-L-phenylalanyladenosine; A(2'Phe)H, 3'-deoxy-2'-O-L-phenylalanyladenosine (analogous abbreviations are used for the dinucleotide derivatives); 3'-residue, 3'-linked residue; 5'-residue, 5'-linked residue.
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