Physical consequences of chemical modification: circular dichroism of the kethoxalated oligoribonucleotides

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ABSTRACT.

Alterations in CD spectra are found in G-containing oligoribonucleotides after modification with kethoxal (β -ethoxy- α -ketobutyraldehyde). Stacking interactions in kethoxalated oligomers are followed by temperature dependence of their CD amplitudes. It is shown that for oligomers with nucleosides in anti-conformation adduct formation destroys the stacking interaction with 3'-neighbour but not with a 5'-neighbour. For nucleosides in non-standard conformation (i.e. syn-conformation of guanine in GpGpCp) the physical alterations may be seen in those cases, when the substituting group affects the initial conformation or the interplane base contacts via, for instance, blocking NH_2 -group of guanine in GpUp.

The results demonstrated that even a single monomer modification in a polymer chain could not be considered as a local event having no influence on the three-dimensional structure. The degree of conformational disorders depends both on the conformation of single nucleotides in the stack and on the nature of the nearest neighbours of the modified base.

INTRODUCTION.

Chemical modification is now widely applied for structural and functional investigations of nucleic acids¹. However, with a very few exceptions physical consequences of the modifications used are not known and, therefore the conclusions made may be in many cases misleading since they do not take into account a contribution of "secondary" effects produced in the polynucleotides by modification.

Evidence in favour of this viewpoint was obtained in this work with kethoxal (β -ethoxy- α -ketobutyraldehyde) known to react selectively with guanine bases in polynucleotides²⁻⁶. As was shown earlier⁷ by means of CD measurements adduct formation with G bases located in single stranded regions of nucleic acid may affect the stacking interactions.

The shape of CD spectra is highly sensitive to the conformation of nucleic acids⁸⁻¹¹ and oligonucleotides in particular^{12,13}. There are two sources of optical activity in polynucleotides. First, optical asymmetry is induced in planar heterocyclic bases by covalently attached sugar residues and depends on the mutual orientation of the base and the sugar moieties which is characterized with a torsion angle φ_{CN} , angle of rotation around glycosidic bond¹⁴. Second, the interaction of optical transitions of neighbouring bases (exciton mechanism) causes an additional dichroism¹⁵ the value and shape of which depend on the mutual orientation of bases¹⁶. Thus in both cases CD depends on the conformation of single nucleotides. At high temperatures where polynucleotides lose their ordered structures CD reflects simply the sum of contributions of various configurations of non-interacting nucleotides which form the polymer chain. The CD amplitude is reduced after heating and the degree of reduction reflects the degree of the initial regularity¹⁷.

Thus, the influence of the chemical modification on the stacking interactions of bases and in general on the structure of nucleotides may be studied by means of temperature dependence of the CD spectra.

MATERIALS AND METHODS.

Kethoxal was synthesized as described¹⁸. The purity of the preparation was checked according to Litt⁴. The purification and control of the purity of oligoribonucleotides was done by means of thin layer chromatography on cellulose plates in isobutyric acid: 0.5 M NH_3 , pH 3.7 (10:6 v/v). All oligonucleotides used were homogeneous in this system and UV spectra were found to be identical with those published.

Non-standard abbreviations: A-RNA - A form of double-helical RNA with 11 base pairs per turn, G^* - kethoxalated guanosine.

Oligonucleotides (5.10^{-7} M) were incubated with 1% kethoxal in 0.05 ml of sodium phosphate buffer 0.01 M, pH 6.7 for 18 hrs. The excess of kethoxal was removed by gel-filtration through Sephadex G-25 (fine) column. Elution was carried out with water and fractions containing modified oligonucleotides were concentrated and their UV spectra recorded.

CD spectra were measured in a Jouan II CD-185 in 0.01 M cacodylate buffer containing 0.05 M boric acid, pH 7.0 in a cell with 1 cm pathlength. Sample temperature was adjusted ($\pm 0.5^\circ$) by a thermostated cuvette holder and read by means of a thermistor fixed in the solution under investigation. The temperature dependence of CD amplitudes was obtained with continuous recording of the dichroism values at fixed wavelength. The heating time in the 0-80° interval was about 150 min.

The following molar extinction coefficients were used for calculations: GpCp, 9,100; ApGp, 11,500; CpGp, 9,000; GpUp, 10,400; UpGp, 9,800; GpGpCp, 9,200^{15,16}. No changes in molar extinctions at the given wavelength were found after kethoxalation. The influence of the modification on hypochromicity values of oligonucleotides was not taken into consideration. All spectra shown in the figures are normalized to molar concentrations of the nucleotides.

RESULTS.

The structural formula of the guanine base modified with kethoxal and the external view of the molecular model of this adduct are shown in fig.1. It is evident from the fig.1b that the molecule of adduct at variance with the natural base is not flat due to the OH-groups projecting from one side of the base plane and the massive substituting radical at the other. It is natural to suppose that these structural peculiarities may influence the stacking interactions between the modified base and neighbouring nucleotides.

The CD spectrum of the adduct is nearly indistinguishable with the non-modified Gp. As shown separately the UV spectra of the modified oligonucleotides are also similar with the spectra of the same oligonucleotides before adduct formation. Thus modification of the guanine base does not disturb signi-

ificantly the electronic structure and configuration of the nucleotide.

Fig.1. The spatial molecular model of kethoxalated guanine

CD spectra of GpCp before and after kethoxalation are presented in fig.2,A. The modification causes changes both in the shape of the spectrum and in its amplitude. The temperature dependence of CD amplitude (fig.2,B) shows that on heating GpCp has lost its ordered structure and its dichroism is reduced. On the contrary, in the case of GpCp there is no such effect demonstrating the absence of stacking interactions in this oligonucleotide even near 0°. Analogous data are shown in fig. 2,C,D for dinucleotides CpGp and GpGp. Both the CD spectra and the temperature dependences of the CD amplitudes are similar and, therefore, prove the absence of changes in stacking interactions in the kethoxalated dinucleotide.

The CD spectra for ApGp and ApGp are similar (fig.2,E), however in the latter case an insignificant decrease in the CD amplitude is observed pronounced better in the positive longwave band. The temperature dependences of $\Delta \epsilon$ for the ApGp and for the ApGp are similar although the degree of their reduction slightly differs: 60% for the ApGp and 50% for the ApGp (fig.2,F).

The GpUp and GpUp have significant differences in their CD spectra that indicates great alterations in the structure of the dinucleotide GpUp after modification (fig.2,G).

The temperature dependence of $\Delta \epsilon$ is consistent with the view that stacking interactions are practically absent in GpUp in the temperature range investigated both before and after modification.

Fig.2. CD spectra and temperature dependences of their amplitudes ($\Delta\epsilon$) for kethoxalated(--- and ●) and non-modified (— and ○) oligoribonucleotides. Ordinates represent the relative values of CD amplitudes measured at maximum: B, 267 nm; D, 280 nm; F, 265 nm; H and L, 260 nm, J, 280 nm. A and B, GpCp; C and D, CpGp; E and F, ApGp; (continued over)

Fig.2 (continued). G and H, GpUp; I and J, UpGp; K and L, GpGpCp.

The CD spectra for dinucleotides UpGp and UpGp^{*} show a diminution in the CD amplitude in the positive longwave band and changes in the shape of spectrum in the shorter wavelength region (fig.2,I,J). Thus adduct formation is accompanied with definite alterations in the UpGp structure. UpGp is also less ordered in the temperature interval studied as may be seen from the temperature dependence of $\Delta\epsilon$: kethoxalation reduces the absolute value of the thermal reduction of $\Delta\epsilon$ (fig.2,J).

In the case of trinucleotide GpGpCp a longwave shift in the positive maximum of the CD spectrum is observed after modification (fig.2,K). The temperature dependence of CD shows also the difference: on heating the fall of $\Delta\epsilon$ for the GpGpCp stops at 40°C whereas for the GpGpCp only at 60°. The $\Delta\epsilon$ value for the GpGpCp diminished by 35% after heating as compared with 60% for the GpGpCp (fig.2,L). Thus it is clear from the data presented in fig.2,L that modified trinucleotide is partially organized by stacking interactions however they are significantly weakened due to kethoxalation.

DISCUSSION.

Examination of the molecular models of the adduct reveals the existence of 4 possible orientations of the side groups in the imidazolydinic cycle appeared after modification (fig.3), namely, two with massive side radical above and under the base plane and the other two with cis- and trans-positions of OH⁻ groups. The side groups of the imidazolydinic cycle of the adduct in all the orientations hinder the formation of normal stack between kethoxalated guanine and its 3' neighbour as may be seen on the models of oligonucleotides. (Under "normal base stacks" we mean structures known after X-ray crystallographic work^{19,20}). The stacking interaction with a 5'-neighbour is not disturbed since the additional heterocycle with the massive side group is out of the overlapping region. In the model of dinucleotide (fig.4) conformational parameters of A form of RNA²⁰ have been used assuming that the majority of nucleotides in RNA molecule has the same structure as in A-RNA. However, the model used may be inadequate for the structure of the dinucleotide in solution since it is known that conformation of

Fig.3. Possible orientations of the side groups in the additional cycle of the adduct.
View from N_1 - C_2 side of the guanine base.

Fig.4. Backbone model of the modified GpCp.
O - oxygen, N - nitrogen.

dinucleotides in solution in a number of cases differs with that in the crystal or in the double-stranded polymer²¹. Later on in discussion this statement will be illustrated further.

In the case of GpCp we presumably deal with the structure shown in the fig.5 where the massive OH⁻ groups outside the

Fig.5. The scheme for overlapping base planes in A-RNA (taken from 19). O, oxygen, ●, nitrogen, —, ribose

base plane hinder the formation of a normal base stack and lead to a complete loss of the ordered structure even at 0°.

Modification of the dinucleotide with a reverse base sequence, CpGp causes no essential changes in the CD spectrum (fig.2,C) and in temperature dependence of $\Delta\epsilon$ due to the reason already mentioned: the massive side groups are located outside the region of contacts between neighbouring bases. The same behaviour is observed also with ApGp since in this case the side group of Gp does not overlap with its 5' neighbour, either. Small spectral differences between ApGp and GpGp may be accounted for with insignificant changes in the conformation of ApGp or in electric and/or magnetic transition moments of bases.

In other oligonucleotides investigated, i.e., GpUp, UpGp and GpGpCp the structural alterations produced by modification are more complicated and could not be interpreted by a simple way. We believe this difficulty reflects the fact that conformation of these oligonucleotides before modification differs in solution and in double-stranded polymers. For example, ac-

cording to Guschlbauer et al.²¹ the GpUp possesses structure in which Ura base is oriented normally to the Gua base, GpUp has an altered shape of the CD spectrum that reflects the distortion of the initial three-dimensional structure. Since modification blocks the NH₂ group of the Gua participating in the stabilization of the normal orientation of Ura to Gua in the GpUp it might be reflected inter alia in the value of φ_{CN} angle followed by CD spectral changes in the GpUp.

According to NMR data²² the UpGp has no ordered structure at room temperature. This conclusion contradicts with our measurements since we have found a strong temperature dependence of the $\Delta\epsilon$ at $< 30^\circ\text{C}$ attributed undoubtedly to some type of ordered structure.

The drop of the initial CD amplitude (fig.2,I) reflects the decrease in degree of the UpGp packing or an alteration of its conformation. Moreover, this effect accounts for the less pronounced decrease of $\Delta\epsilon$ with temperature for UpGp then with UpGp (fig.2,J).

Several examples are known^{23,24} when G containing dinucleotides have an unusual structure in solution including syn-conformation of Gua residue. If this is also the case for the UpGp the modification might produce restrictions in the interplanar base interactions as it follows from the molecular model studies. These spatial limitations give satisfactory explanation for the observed alterations in the amplitude and shape of the CD spectrum as well as for decrease in the thermal decline of the $\Delta\epsilon$.

In principle the same considerations are applicable for the data with GpGpCp since it is known²⁴ that GpGp may contain nucleotides in syn-conformation. If to assume that all three bases in GpGpCp are in a usual anti-conformation it might be predicted that the GpGpCp should be fully disordered. However this is not the case since only partial loss of ordered structure was observed after modification. Hence the ordered structure is stabilized in dinucleotides mainly due to nearest-neighbour interactions²⁵ this residual packing should be attributed solely to the interaction in the GpGp pair due to

the fact that no ordered structure in the $\overset{*}{\text{GpCp}}$ was found (fig. 2,B). Thus in order to explain the preservation of some ordered structure in the $\overset{*}{\text{Gp}}\overset{*}{\text{GpCp}}$ one might assume the syn-conformation of single or both Gua bases in the modified trinucleotide. In this case stacking interactions between Gua bases remain while planar interactions between Gua and Cyt are destroyed leading to partial disordering observed experimentally.

Kethoxalation of guanine occurring in the usual anti-conformation might be followed by destruction of the interplanar interactions with the next 3' base, not with the 5' neighbour, as might be seen on the scheme of overlapping bases in double stranded fully complementary RNA¹⁹.

As shown here this regularity is proved experimentally for those cases when oligonucleotides in solution have conformation analogous with that presented in fig.4.

When chemical modification is used in structural-functional studies of nucleic acids, it is commonly accepted that selective chemical treatment of the polymer may be considered as a "point mutation". For example, when chemical modification causes inactivation of acceptor function in the investigation of tRNA recognition sites it is generally believed that those particular bases which undergo modification are directly or indirectly involved in recognition. The data presented here show that this view is oversimplified at least in the case of kethoxal modification of nucleic acids.

The physical consequences of the modification are different depending on (a) conformation of the given nucleotide in the polymeric chain and (b) nature of the 3' and 5' nearest neighbours. Kethoxalation may cause practically complete loss of vertical packing like in $\overset{*}{\text{GpCp}}$, or change the conformation in those cases when the stacking interactions have been already destroyed before modification. Finally, kethoxalation may have no strong influence on the conformation, i.e. as for example in the case of syn-conformation of G in GpGp .

Thus in any particular case physical consequences of chemical modification depend on local primary and three-dimensional structure of the polynucleotide. Presumably analogous

complicated picture will be found with other chemical modifications especially when massive radicals are introduced into polymer. In the light of these considerations one might be cautious in interpretation the data on functional inactivation of the tRNA^{Phe} (yeast) with kethoxal^{6,26}.

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