STACKING INTERACTIONS IN CHEMICALLY MODIFIED tRNA's AND OLIGONUCLEOTIDES

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1. Introduction

Chemical modification is now widely used as a tool for structural and functional investigations of nucleic acids (cf. [1]). However, in most cases modification of nucleic acid is not followed by analysis of possible conformational changes produced in a polymer. Therefore, for unambiguous interpretation of the functional and structural changes one needs information about possible "secondary" effects which may be produced by the modification.

Kethoxal (β-ethoxy-α-ketobutyraldehyde) selectively reacts with guanine bases [2, 3] which are non-hydrogen bonded [4, 5]. Since this agent has been applied [5, 6] for analysis of the functional role of the looped G's in tRNA molecules it is reasonable to study possible changes in stacking interactions in the neighbourhood of the modified G's which mainly contribute to the maintenance of a three-dimensional structure of the looped regions of the tRNA.

Study of circular dichroism and its changes with temperature seems to be the most appropriate technique for this purpose.

It was shown in this work by means of CD spectra measurements at various temperatures with tRNA and G-containing oligonucleotides that kethoxalation profoundly reduced the stacking interaction of the modified G with neighbouring pyrimidine base. On the other hand, if the neighbouring base belongs to purines the stacking is reduced insignificantly.

2. Experimental

2.1. *Materials*

Kethoxal was synthesized as described previously [7]. The purity of the preparation was checked according to Litt [4]. The mixed tRNA preparation was isolated from commercial baker's yeast. The purification and control of the purity of oligoribonucleotides GpCp, ApGp, GpGpCp was done by means of thin-layer chromatography on cellulose plates in isobutyric acid: 0.5 M NH_4OH, pH 7.0 (10:6 v/v). GpCp and GpGpCp were homogeneous in this system, in ApGp ~ 15% of impurities were found. The spots were eluted with water and UV spectra were measured with a Specord UV VIS and found to be identical with those published.

2.2. *Kethoxalation of oligonucleotides and tRNA*

Oligonucleotides (5×10^{-7} M) were incubated with 1% kethoxal in 0.05 ml of sodium phosphate buffer, 0.01 M, pH 6.7. Products were purified on TLC cellulose plates in isopropanol: H_2O (7:3 v/v). Spots were eluted and UV spectra recorded.

Two preparations of modified tRNA were prepared, $tRNA_1^*$ and $tRNA_2^*$ which contained 5 and 19 moles of kethoxal per mole of tRNA, respectively. To prepare $tRNA_1^*$, a solution of 4×10^{-5} M tRNA, 0.18 M sodium cacodylate buffer, pH 7.0, 1 M KCl, 0.05 M $MgCl_2$ and 0.07 M kethoxal was incubated for 3 hr at

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Abbreviations: CD, circular dichroism; G* and tRNA*, guanine and tRNA respectively, modified with kethoxal; tRNA, transfer ribonucleic acid; TLC, thin-layer chromatography.

20°. For preparing $tRNA_2^*$, a solution of 4×10^{-5} M tRNA, 0.18 M sodium cacodylate buffer, pH 7.0, 5×10^{-3} M ethylene diamine tetraacetate and 0.07 M kethoxal was incubated for 2 hr at 37°. After incubation $tRNA^*$ was precipitated by addition of 2 vol of ethanol and 0.1 vol of 20% CH_3COOK, pH 5.0 at 0°. The precipitate formed was dissolved in 0.01 M cacodylate buffer, pH 7.0 and passed through a Sephadex G-25 column containing the same buffer.

2.3. *CD measurements*

CD spectra were measured in a Roussel–Jouan dichrographe II CD-I85 in 0.01 M cacodylate buffer containing 0.05 M boric acid, pH 7.0. Sample temperature was regulated by a thermostated cuvette holder and read (± 0.25°) 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: tRNA 7,200; GpCp, 9,100; ApGp, 11,500; and GpGpCp, 9,200 [8, 9]. No changes in molar extinctions at the given wavelength were found after kethoxalation. The influence of the modification on the hypochromicity values of oligonucleotides was not taken into consideration.

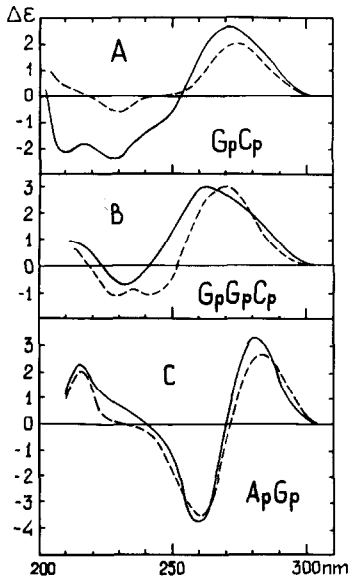

Fig. 1. CD spectra of oligonucleotides before (——) and after (---) kethoxal modification measured at 20°.

3. Results and discussion

CD spectra of GpCp, GpGpCp and ApGp oligonucleotides before and after kethoxalation are presented in fig. 1 (A,B,C). It is evident from the data shown in fig. 1 (A,C) for the dinucleotides GpCp and ApGp that modification caused changes both in the form of the spectra, and in their amplitude. This effect may be attributed to the reduction of vertical stacking interactions in these dinucleotides [10]. In the case of GpGpCp modification, only a small shift of spectral maximum to a long wavelength region was noticed and hence the stacking interactions were not significantly disturbed.

These tentative conclusions were proved further when CD melting curves of the same oligonucleotides were studied (fig. 2). The CD amplitude of GpCp was

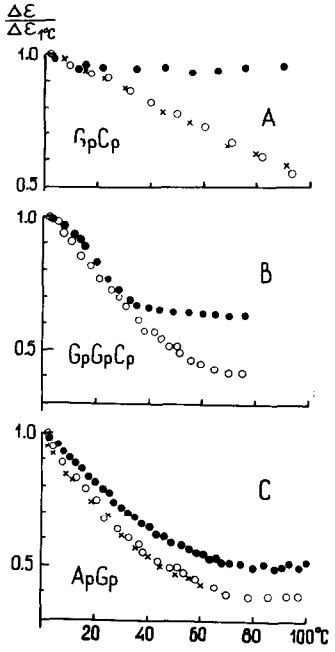

Fig. 2. Temperature dependence of the CD amplitude of oligonucleotides before (X and o) and after (•) modification. Ordinates represent the relative value of CD amplitude measured at the maximum (A, 267 nm; B, 265 nm; and C, 260 nm).

reduced on heating due to a loss of an ordered structure. On the contrary, in the case of Gp^*Cp no changes were observed after modification demonstrating the absence of the stacking interactions even at 5°.

Analogous curves for GpGpCp and Gp^*Gp^*Cp showed that modified oligonucleotide was partially organized by stacking interactions which are much less stable than in the non-modified GpGpCp: on heating the fall of $\Delta\epsilon$ for Gp^*Gp^*Cp stopped at 40° whereas for GpGpCp, only at 60°. The $\Delta\epsilon$ value for Gp^*Gp^*Cp diminished by 35% after heating as compared with 60% for GpGpCp. Thus, it is clear that stacking interactions in this case were significantly weakened by modification.

Since the spatial structure of oligonucleotides is stabilized primarily by nearest neighbour interactions [11] the observed orderedness of the Gp^*Gp^*Cp is mainly due to interactions in the Gp^*Gp^* pair since the Gp^*Cp pair as shown above has no ordered structure. From this observation one may suggest that modification destroys the vertical packing in a guanine–pyrimidine pair but has no or only a weak influence on a guanine–purine pair. This notion was proved by melting of $ApGp^*$ where an identical slope of the $\Delta\epsilon$ curve was found for both samples (fig. 2, C) with insignificant difference in absolute value of $\Delta\epsilon$ (ApGp, 60%; $ApGp^*$, 50%).

CD spectra of kethoxalated and control tRNA were shown in fig. 3 (A). The CD spectrum of untreated tRNA coincided with analogous data published earlier for similar preparations [12]. Kethoxalation

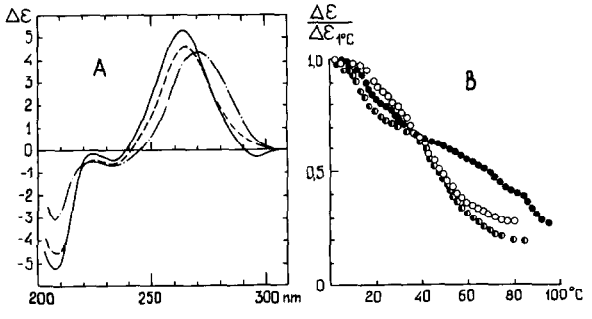

Fig. 3. CD spectra (A) and temperature dependence of their amplitudes (B) for non-modified tRNA (—— and o), $tRNA_1^*$ (--- and ◦) and $tRNA_2^*$ (·—·—·—) and •).

led to diminution of the CD amplitude from 5.2 to 4.6 and 4.3 for $tRNA_1^*$ and $tRNA_2^*$, respectively. Other changes consisted in disappearance of the long wavelength band at 296 nm, diminution of the amplitude at 210 nm and a shift of the maximum of the positive band from 264 nm for tRNA to 255.5 nm for $tRNA_1^*$ and 270.0 nm for $tRNA_2^*$. The reduction of the CD amplitude reflected the decrease of stacking interactions between bases in modified tRNA molecules. The shift of the maximum of the positive band to a long wavelength region could be interpreted either as an indication of some changes of the parameters of double-stranded regions in tRNA molecule [15] or as progressive fall of the percentage of double-stranded regions in the course of modification [10]. If to accept the second alternative then fully kethoxalated $tRNA_2^*$ contained only 20–30% of the double-helical regions; this calculation was based on the data published by others [13].

Differential melting curves shown in fig 3, B confirmed that tRNA melted relatively non-cooperatively in the absence of Mg^{2+}. The melting process was affected by tRNA modification: $tRNA_1^*$ melted deeper at lower ($< 40°$) temperatures than unmodified tRNA, on the other hand, at $> 40°$ there was practically no difference in melting behaviour. This effect was connected with a modification of the looped G's in tRNA molecules followed by disturbance of stacking interactions in the loops. This weakening of the structure caused quick disordering of the packed conformation under heating. The double-helical regions resistant to modification melted as in control tRNA since their structure was not affected by kethoxalation.

The $tRNA_2^*$ melted non-cooperatively since the whole three-dimensional structure was profoundly affected by exhaustive modification.

The analysis made in this work demonstrated clearly that chemical modification (at least kethoxalation) of nucleic acids could not be considered as a local event having no influence outside the modified base. The degree of conformational disorders produced by modification depended seriously on the chemical nature of nearest neighbours of the modified base.

In particular, some results found by Litt and Greenspan [15] in functional study of kethoxalated tRNA's may be connected with the fact that in the case of kethoxalation of G_{20} in diHU-loop of the

$tRNA^{Phe}_{yeast}$ and $tRNA^{Val}_{E.\ coli}$ this base is surrounded by purines and in the light of our data the disturbance of the stacking interactions may be insignificant. On the contrary, when G's were modified in the anticodons of the $tRNA^{Phe}_{E.\ coli}$ and $tRNA^{Phe}_{yeast}$, the 5' halves of the anticodon loops consisting of pyrimidines may loose ordered conformation.

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