A METHOD FOR DETERMINATION OF THE POSITION OF GUANOSINE RESIDUES IN OLIGODEOXYRIBONUCLEOTIDES INVOLVING MODIFICATION WITH GLYOXAL

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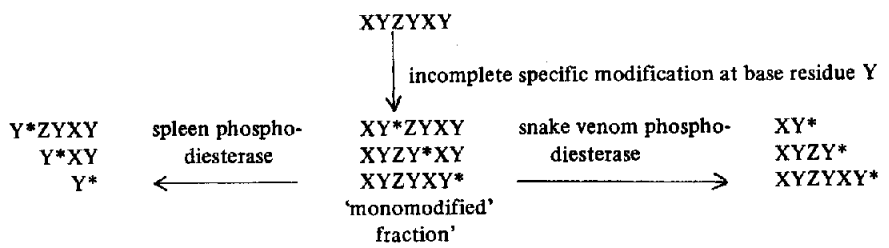
1. Introduction

Analysis of the primary structure of nucleic acids involves the use of various independent and complementary methods for determination of the base sequence within oligonucleotides [1–4]. We have previously suggested a method for determination of the positions of base residues in oligonucleotides based on arresting the attack of exonucleases in certain monomer units subjected to specific chemical modification [5,6].

The principle of the method is illustrated by scheme 1.

Scheme 1



The fraction of oligonucleotides that differ from each other by the position of the modified base ('monomodified fraction') is split by one of the exonucleases to give a combination of fragments of uniform terminal sequence with a modified base at the opposite terminus. The chain length of each of these fragments indicates the position of the given base in the starting oligonucleotide (detailed in [5–7]).

This principle is used as a basis for finding the positions of thymidine and cytidine residues in oligonucleotides modified with OsO_4 and HSO_3^- and NH_2OCH_3, respectively [5,6].

This paper is concerned with a similar method for determination of the positions of guanosine residues after modification of oligonucleotides with glyoxal. Each of these methods may be used independently or together with other ways of analysis of the primary structure of oligonucleotides.

2. Materials and methods

Oligodeoxyribonucleotides d(T–T–G), d(pT–A–G–T), d(T–C–G–T–G–G) were obtained by chemical synthesis [8].

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Glyoxal obtained from BDH was used as a fresh 1% solution in 0.1 M sodium phosphate (pH 8.5).

Snake venom phosphodiesterase (EC 3.1.4.1) (Worthington) was used as a solution (1 mg/ml) in 0.02 M Tris buffer (pH 8.5), 5×10^{-3} M $MgCl_2$. A minimum concentration of the enzyme was taken which ensures total degradation of 0.3 A_{260} unit of the non-modified oligonucleotide within 40 min at 37°C in 100 μl 0.05 M borate buffer (pH 8.6), 5×10^{-3} M $MgCl_2$.

Ion-exchange chromatography was carried out on microcolumns packed with DEAE-cellulose (Serva), using devices described in [9]. Oligonucleotides were desalted on Sephadex G-10 (Pharmacia, column diam. 2.8 mm, vol. 600 μl).

2.1. *Modification of oligonucleotides*

0.2–0.3 A_{260} unit of the desalted oligonucleotide evaporated in a hydrophobic glass tube was supplemented with 25 μl glyoxal solution. The mixture was incubated for 1 h at 40°C. The modified oligonucleotide was separated from glyoxal on Sephadex G-10 equilibrated with 0.05 M borate buffer (pH 8.6), 5×10^{-3} M $MgCl_2$.

2.2. *Hydrolysis of modified oligonucleotides*

Fractions containing the modified oligonucleotide (total vol. 60–75 μl) were supplemented with phosphodiesterase and with 1.5 μg bovine serum albumin. The mixture was incubated for 1–2 h at 37°C, then heated for 10 min at 100°C and diluted with bidistilled water to 300–400 μl.

the effect being especially noticeable when the modified base is charged [5]. The presence of a stable negative charge on the modified base made us use only the 'monomodified fraction' for further analysis, because the concomitant di- and polymodified oligonucleotides would have made the interpretation of the results difficult.

Glyoxal-modified guanine contains a vicinal diol group [10], which makes possible formation of a negatively-charged complex with borate. Such complexes are unstable and, when the excess of borate-ions is removed, they readily dissociate, the initial compounds being regenerated.

The reported method for determination of the position of guanine residues in oligonucleotides is based on this property of the borate complexes. The method is illustrated by determination of guanine residues in synthetic oligonucleotides d(pT–A–G–T), d(T–T–G–T–T) and d(T–C–G–T–G–G) (see scheme 2).

An oligonucleotide is modified with glyoxal and split with exonuclease in borate buffer. As degradation in the presence of the modified residues having additional negative charge is slower, a number of fragments are formed with modified guanine residues at the 3′-ends. After the removal of borate, these fragments lose the additional negative charges. Therefore fragments of the same length but of different degree

Scheme 2

TCG*TGG		TCG⁻*		TCG*
TCGTG*G		TCGTG⁻*		TCGTG*
TCGTGG*	snake venom phospho-	TCGTGG⁻*	removal	TCGTGG*
TCG*TG*G	diesterase in	TCG⁻*TG⁻*	of borate	TCG*TG*
TCG*TGG*	borate buffer	TCG⁻*TGG⁻*	buffer	TCG*TGG*
TCGTG*G*	⟶	TCGTG⁻*G⁻*	⟶	TCGTG*G*
TCG*TG*G*		TCG⁻*TG⁻*G⁻*		TCG*TG*G*

G*, glyoxal-modified guanosine residue

G⁻*, negatively charged complex of G* with borate

3. Results and discussion

Unusual or modified bases, if present in nucleic acids, slow down their hydrolysis by exonucleases, of modification have now negative charges determined only by the number of phosphate groups in them. In ion-exchange chromatography these fragments give separate peaks, the number of which is

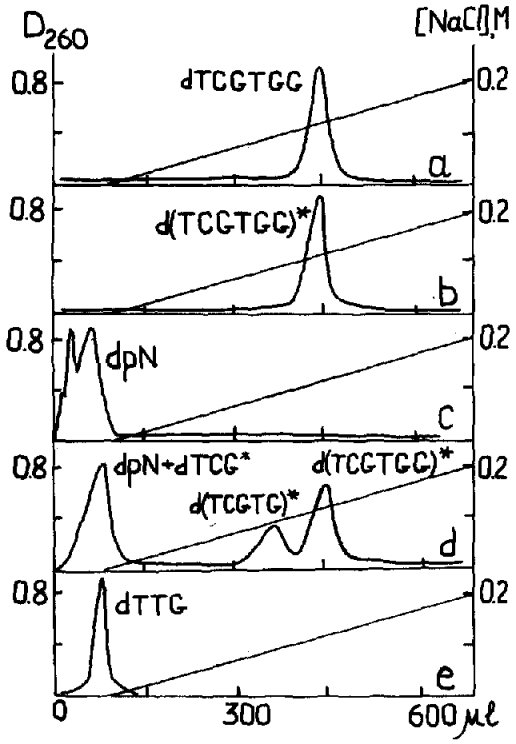

Fig.1. Chromatography of d(T–C–G–T–G–G) (a), of the product of its modification with glyoxal (b) and of their snake venom phosphodiesterase hydrolysates (c and d, respectively). Chromatography of d(T–T–G) (e). DEAE-cellulose column (50 µl). NaCl linear gradient in 0.05 M sodium acetate (pH 5.5) containing 7 M urea, 20°C. Elution rate 300 µl/h.

equal to that of guanine residues (without taking into consideration the peak of mononucleotides). The position of the peaks indicates that of the guanine residues in the starting oligonucleotide. In case of the hexanucleosidepentaphosphate d(T–C–G–T–G–G), the modified and the starting oligonucleotides are eluted at the same concentration of NaCl (fig.1a,b). This result demonstrates that glyoxal modification does not cause considerable degradation of oligonucleotides and does not affect their chromatographic behaviour.

The products of hydrolysis of modified d(T–C–G–T–G–G) with snake venom phosphodiesterase are found to contain the expected (scheme 2) hexa- and pentanucleotide derivatives (fig.1d). dTCG* is presumably eluted together with mononucleotides, as suggested by the result of chromatography of d(T–T–G) (fig.1e).

Results which are in line with the expected ones were also obtained with d(T–T–G–T–T) and d(pT–A–G–T).

It should be noted that, although the modified base considerably retards hydrolysis with phosphodiesterase, it does not stop it completely. Therefore on longer hydrolysis, the content of oligonucleotides in the mixture decreases, although qualitatively nothing seems to change.

The method is simple, requires no costly reagents and can be used to find the positions of guanine residues in natural and synthetic oligonucleotides. The resolution of the method depends exclusively on that of the techniques used for separation of the hydrolysis products.

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