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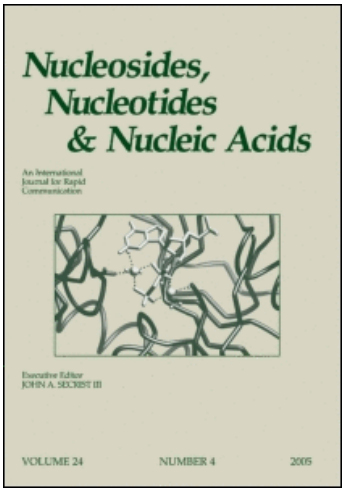

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Sequence-Dependent Structural Variations of DNA Revealed by Chemical Ligation

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SEQUENCE-DEPENDENT STRUCTURAL VARIATIONS OF DNA REVEALED BY CHEMICAL LIGATION

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ABSTRACT. The comparative study of nick-sealing efficiency under the action of BrCN or water-soluble carbodiimide was carried out for 14 dinucleotide combinations in double helix. The difference between the lowest (17%, GpG) and the highest (94%, CpT) coupling yields was found to be more than five fold, both condensing reagents showing a similar sequence-specific trend. A strong correlation observed between coupling yields at different dinucleotide combinations and ^{31}P NMR parameters supports the idea that variations in chemical ligation efficiency arise from sequence-specific modulations of the helix geometry and confirms close similarity of the intrinsic fine structure of intact and nicked DNAs.

INTRODUCTION

Chemical approaches for assembling extended double-stranded DNAs from synthetic oligonucleotides have been extensively studied in recent years as an alternative to the enzymatic method [1]. Different types of water-soluble carbodiimides [1,2] and cyanogen bromide (BrCN) [3,4] have been successfully used for condensation of oligonucleotides on complementary templates. The attractive features of non-enzymatic ligation are the high rate of BrCN-induced reaction (1-3 min); the possibility to obtain unusual molecules, for example, branched [5] or circular DNAs [6], to assemble a wide range of DNAs with preset modifications of the sugar-

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phosphate backbone; any scale of synthesis; good reproducibility of results and inexpensive condensing reagents. However, the efficiency of non-enzymatic assembling of several oligonucleotide blocks into longer DNA pieces is lower than that of the DNA ligase-catalyzed reaction, considering in both cases phosphodiester linkage formation. This may be due to the sensitivity of the chemical ligation method to the type of nucleotide residues to be joined. This effect was observed during 1-ethyl-3-(3'dimethylamino-propyl)carbodiimide, hydrochloride (EDC) - induced assembly of a 35 b.p. DNA duplex from six oligonucleotides [7] and nick-sealing in a 15-17 b.p. DNA [2], independently of whether the phosphate group was located at the 5'- or 3'-position of oligonucleotide.

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In the present communication a comparison study of chemical ligation efficiency was performed for most of the dinucleotide combinations in the double helix (14 out of 16). A series of three-component DNA duplexes I-XV (TABLE) were used as chemical ligase substrates. It should be noted that nucleotide sequences flanking the ligation junction were different. Both condensing reagents, EDC and BrCN, were used in parallel experiments to obtain more reliable results. Our results showed the strong dependence of chemical ligation efficiency on the type of the reacting nucleotide residues. Variations of coupling yields are closely related to the sequence-specific structure variations of phosphodiester linkages. The potentialities of chemical ligation for probing nucleic acid local conformations in solution are discussed.

EXPERIMENTAL

The following reagents were used: BrCN, EDC, 2-morpholinoethane sulfonate (MES) (Merck), [α-^{32}P]UTP (Isotop, Russia), terminal transferase ("Fermentas", Lithuania).

Buffer solutions: A (for BrCN-induced chemical ligation) - 0.25 M MES adjusted to pH 7.5 by $(C_2H_5)_3N$, 20 mM $MgCl_2$; B (for EDC-induced chemical ligation) - 50 mM MES, pH 6.0, 20 mM

$MgCl_2$; C (for terminal transferase) - 0.2 M K-cacodylate, pH 7.5, 1 mM $CoCl_2$, 0.5 mM dithiothreitol.

Preparation of synthetic oligomers

Oligonucleotides 7-12, 14-16 (SCHEME) with terminal phosphate groups were synthesized by the hydrophosphoryl method in semiautomatic regime as in [8]. Oligonucleotides 1-6, 13, 17, 18 were obtained by the phosphoramidite method on a Victoriya 4M (Russia) DNA synthesizer [9]. 3'-Phosphorylated oligomers were obtained from precursors containing 3'-terminal uridine by periodate oxidation of the 2',3'-cis-hydroxyl system followed by ß-elimination [10]. The synthesized oligomers were analyzed and finally purified by reverse-phase HPLC [11]. The synthesis of oligonucleotides a-h was carried out by phosphoramidite chemistry on a "Cyclon" (Biosearch) DNA synthesizer. After deprotection, the products were desalted on Biogel P-2 and purified by denaturing polyacrylamide gel electrophoresis (PAGE). For 3'-end-labelling we used [α-^{32}P]UTP and terminal transferase [10]. The primary structure of the oligomers was confirmed by a modified Maxam-Gilbert procedure [12].

Chemical ligation of oligonucleotides

A mixture of oligonucleotides with 0.1 mM final concentration in buffer A or B was cooled from 85 to 0°C in 2-3 hours. The ratio of oligonucleotides (3'-^{32}P-labelled oligomer : the oligomer which is phosphate donor : template) was 1:1.2:1.5.

a) The BrCN-induced reaction. A 10 M solution of BrCN in absolute acetonitrile was added (1/10 of the total volume) to the mixture of oligomers in buffer A. The reaction was carried out for 1-3 min at 0°C.

b) The EDC-induced reaction. EDC in buffer B was added to the mixture of oligomers in the same buffer to give a final concentration of 0.15 M. The reaction mixtures were incubated at 0°C in the dark. Aliquots of the reaction mixtures were collected every 24 hours. The oligonucleotide fractions were precipitated with ethanol as usual and analysed by electrophoresis in denaturing 8% polyacrylamide gel. After

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autoradiography, the bands containing the reaction products and starting materials were cut off and the radioactivity was measured on a "Delta-300" (Tracor) counter. The coupling yield was calculated as the ratio of the reaction product radioactivity to the total radioactivity.

RESULTS AND DISCUSSION

The real gene containing 183 b.p. [13] was subdivided into the 18(1-18) or 8(a-h) fragments (SCHEME) in such a manner that at the ligation junctions different combinations of nucleotide residues were brought together. Besides, the duplex components were designed to minimize alternative structure formation and to provide overlapping by 7 or more nucleotide residues. This type of subdivision allowed the examination of fourteen out of the sixteen dinucleotide combinations in the double helix. Diagrams of duplex constructions from oligomers 1-18 and a-h are shown in the TABLE. Some combinations were examined twice using nick-containing duplexes which only differed in their template length (compare systems V and V' in the TABLE). All the duplexes contained 3'-phosphate and 5'-hydroxyl groups at the ligation sites.

The results of EDC- and BrCN-induced chemical ligation are summarized in the TABLE and FIG. 1. The PAGE analysis of some reaction mixtures is shown in FIG. 2. One can see that the coupling yields varied from 17 to 94% depending on the nature of nucleotides to be joined. In several cases the variation in the template length influenced the yield of ligation products (compare, for example, duplexes X and X' in the TABLE). This phenomenon calls for additional studies. It is essential that both condensing reagents showed a similar sequence specific trend despite the difference in the reaction rates (3 min and several days). Such a correlation between the actions of BrCN and EDC has been noted earlier for the family of DNA duplexes containing various sugar-phosphate backbone modifications at the ligation site [4].

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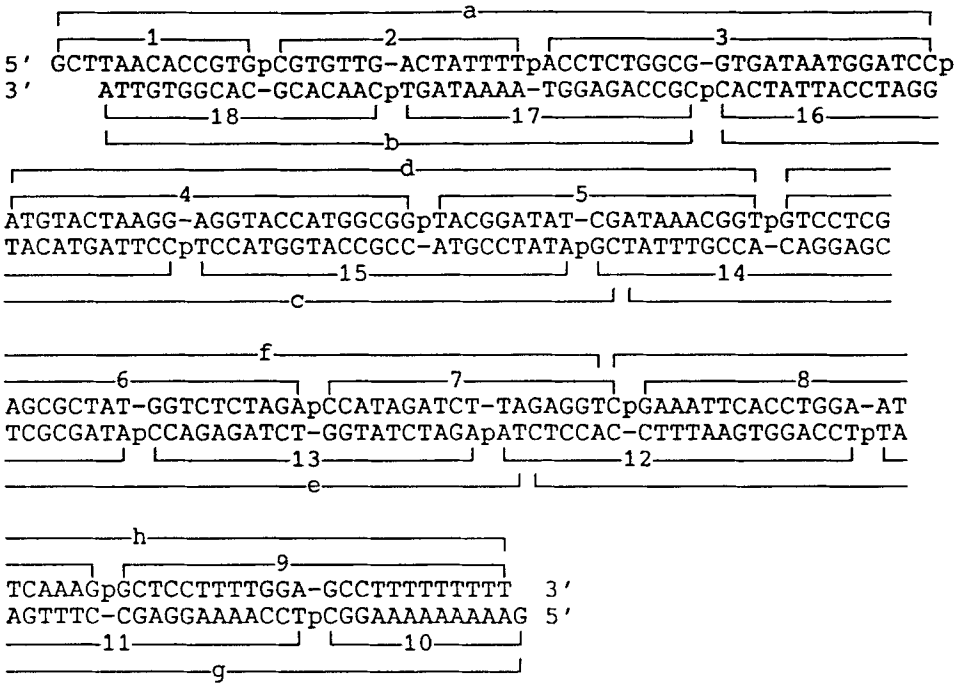

SCHEME. Symbol d is omitted here and throughout the paper; p marks the ligation site; horizontal lines indicate oligonucleotides.

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Our experimental data and results from the literature suggest that oscillation of the coupling yields may be due to different orientation and proximity of phosphate and hydroxyl groups in the ligation site caused by sequence-dependent modulations of the DNA local structure.

Dickerson and Drew [14] described in detail the helix parameter variations along the B-helical dodecamer d(CGCGAATTCGCG) (single-crystal X-ray analysis). The reasons for this conformational polymorphism are not clearly understood yet. One model was proposed by Calladine [15], who stated that the steric clash between purine on the opposite helix strands at adjacent base pairs may be responsible for the geometric irregularities in B-DNA which are observed mainly in mixed PyPu sequences. Purine-purine clashes may be

TABLE. The results of chemical nick-sealing in DNA duplexes containing different residues at the reaction site

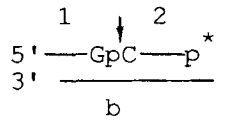
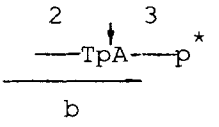
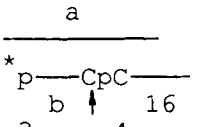
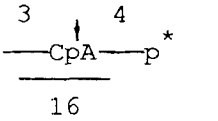
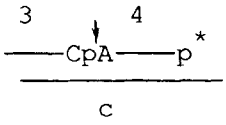
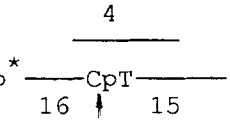
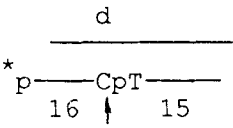
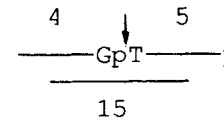
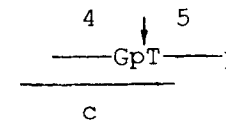
Duplex number	Diagrammic representation of duplexes studied*	Nucleotides facing the nick	Yield of chemical ligation product, % (±2)**			
			BrCN	EDC		
			3 min	2 days	4 days	6 days
I		GpC	87	80	88	90
II		TpA	51	50	65	75
III		CpC	42	19	37	38
IV		CpA	33	10	12	19
IV'		CpA	19	13	18	20
V		TpC	79	59	60	67
V'		TpC	76	75	78	83
VI		GpT	83	68	72	83
VI'		GpT	88	75	77	88

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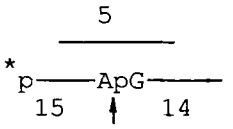
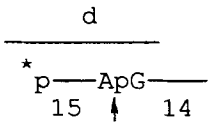
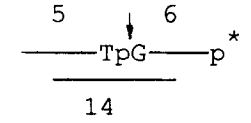
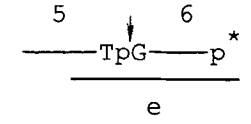
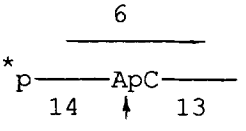
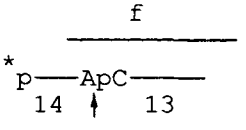
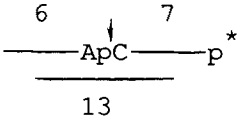
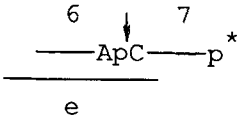
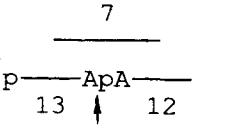
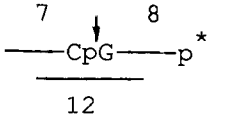
TABLE. Continued.

VII		GpA	33	-	-	-
VII'		GpA	46	51	54	57
VIII		TpG	-	29	33	39
VIII'		TpG	39	34	43	58
IX		CpA	25	19	26	39
IX'		CpA	53	30	39	57
X		ApC	47	48	52	59
X'		ApC	72	38	41	69
XI		ApA	62	28	34	52
XII		CpG	30	30	33	38

(continued)

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TABLE. Continued.

XIII	h —————— p*——TpT—— 12 ↑ 11	TpT	81	66	72	85
XIV	8 ↓ 9 ——GpG——p* g	GpG	50	13	15	17
XV	9 —————— *p——TpC—— 11 ↑ 10	CpT	89	82	89	94

For sequence and explanations see the SCHEME; nucleotide residues and phosphate groups facing the nick are indicated; vertical arrows mark the site of the oligonucleotide junction; p marks 3'-terminal ^{32}P-labelled phosphate group; duplexes having the same number differ only in the template length; the strand polarity is as in duplex I.

**For reaction conditions see Experimental.

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relieved by flattening the propeller twist, by rolling the base pair into the major groove, by sliding the base pair and by decreasing the twist. Arising helical irregularities can be accomodated by small changes in torsion angles along the sugar-phosphate backbone and about the glycosyl bond. According to another point of view the sequence-dependent variations in the DNA helix are a direct consequence of the intrinsic stacking interactions between neighboring base pairs. In other words, charge patterns in the base pairs and their interactions are responsible for local conformational variations [16].

It seems to be likely that disruption of the covalent backbone of DNA (nicked DNA) will result in strong perturbations of fine structural modulations. However, from recent X-ray diffraction analysis it follows that the

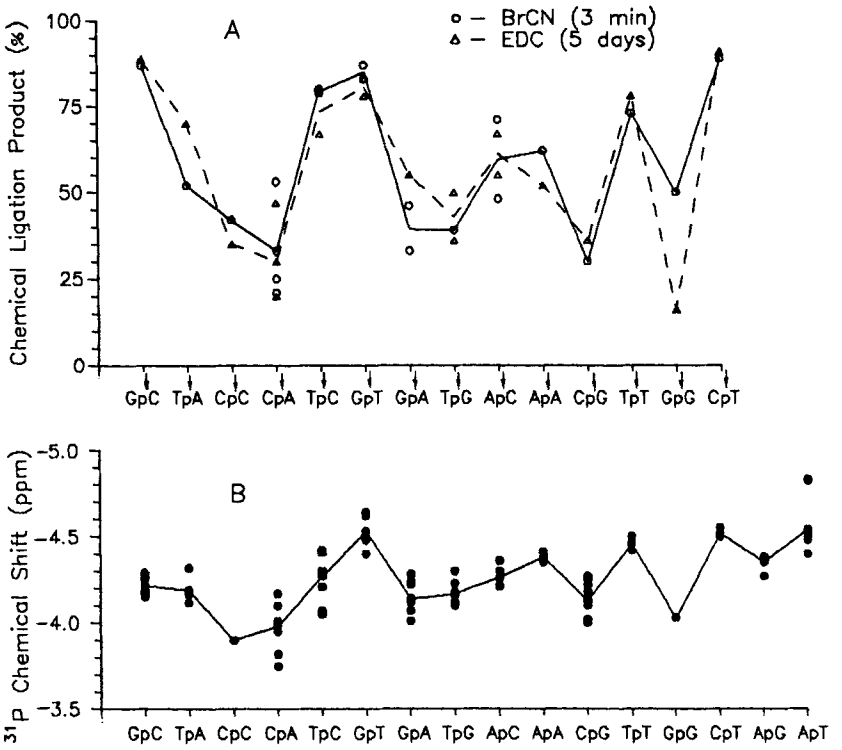

FIGURE 1. A. Plots of variations in the coupling yields as a function of the dinucleotide combinations at the nicked site. Nucleotide residues facing the nick are indicated on the abscissa axis; the polarity of the nicked strand of the duplex is 5'-3'. The averaged yields of chemical ligation products for different duplexes with a similar nicked site are connected by solid (BrCN) or dotted (EDC) lines.
B. A sequence dependence of ^{31}P chemical shifts of phosphate groups in different positions of DNA duplexes (data for six nonmodified duplex oligonucleotides were taken from [20]). The averaged values for identical N_1pN_2 are connected by a solid line. Nucleotide residues surrounding the phosphate group are indicated; the strand polarity is 5'-3'.

intrinsic sequence-dependent properties of the double-stranded DNA can be maintained even if one strand is severed through the loss of a phosphate group [17]. An NMR study also has confirmed that only small local distortions occur when a nick is introduced into the DNA duplex [18]. Consequently, the cohesive internal forces stabilizing the DNA double

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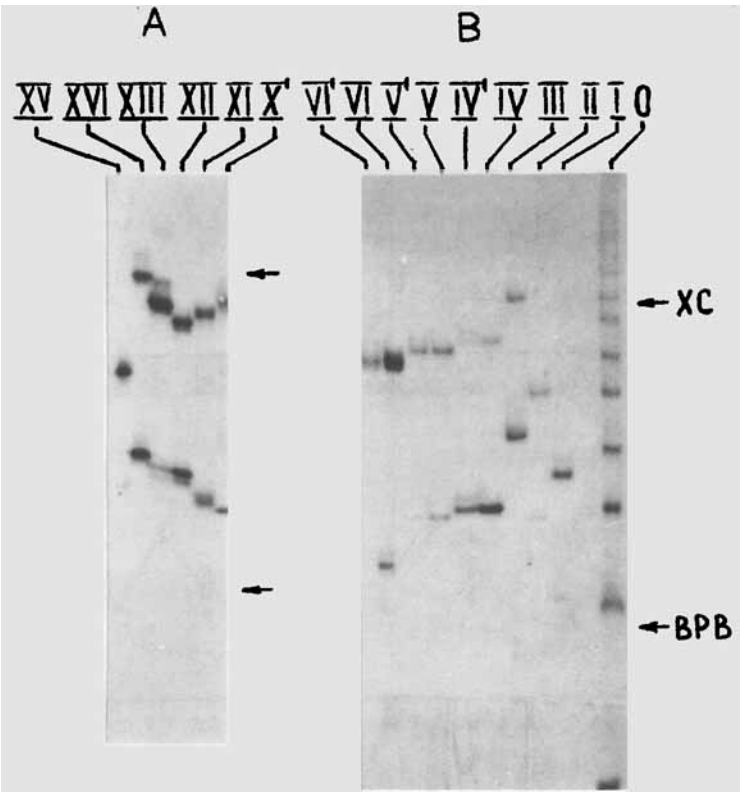

FIGURE 2. Electrophoretic analysis (8% denaturing PAG) of the reaction mixture containing duplexes I-VI' and X'-XV. BrCN-induced nick ligation; 3 min reaction (A). EDC-induced nick ligation; 6 days reaction (B). For duplex structure see the SCHEME and the TABLE; for conditions see Experimental. Line numbers correspond to duplex numbers (TABLE). XC and BPB, xylene cyanol and bromphenol blue markers, respectively. Length markers differing in 10 nucleotides are in line 0.

helix, largely base stacking and hydrogen bonding, are great enough to overcome the loss of connectivity associated with disruption of the covalent backbone of DNA. Therefore, we can use the local parameters of intact DNA duplexes suggesting minor distortions due to the nick in order to test the interrelationship between chemical ligation data and sequence-dependent variations of the DNA structure.

^{31}P NMR spectroscopy is known to provide a powerful probe of nucleic acid structural variations in solution and, in

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particular, of local conformational heterogeneity in the sugar-phosphate backbone [19 and references there]. Thus, Gorenstein et al [20,19] have shown that variations in ^{31}P chemical shifts of individual phosphates in duplex oligonucleotides may be attributed to torsional angle changes of the phosphodiester groups. Using the $J_{H3'-P}$ coupling constants measured and the Karplus relation, they calculated the Zeta torsional angles (C3'-03'-P-05') which are the most variable in the B form of the double helix and obtained a strong correlation between Zeta and ^{31}P chemical shifts. Additionally, the ^{31}P chemical shifts of duplex B-DNA phosphates correlate reasonably well with the calculated helix twist sum, helix roll angle and other local helical parameters.

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We made a comparison between these ^{31}P NMR spectroscopy data and our chemical ligation results for all the 14 dinucleotide combinations in the double helix (FIG. 1). As one can see, average ^{31}P chemical shifts and oscillations of the coupling yields show a similar sequence-specific trend: the downfield shift of the ^{31}P NMR resonance and the lowering of the coupling yield for the 5'-Pyr Pu-3' sequence (note that this sequence produces the largest local perturbations, see above); the upfield shift of the ^{31}P signal and the maximal coupling yield for GpT, TpT and CpT dinucleotide combinations. The plot of ^{31}P chemical shifts *vs* phosphate position along the 5'-3' strand for duplexes (Fig. 1B) contains data for ApT and ApG dinucleotides which are absent in Fig. 1A (the sequence dependence of chemical ligation efficiency). Taking into account the NMR data, the coupling yields can be predicted as ~80-90% for the ApT and ~40-50% for the ApG nucleotide combinations. Additional experiments show a good coincidence with this prediction [21].

A direct comparison of the coupling yields at different nucleotide combinations with the experimental (X-ray study) and computed sequence dependence of the local helix twist in dodecamer d(CGCGAATTCGCG) [16] shows parallel variations in these parameters as well (FIG. 3).

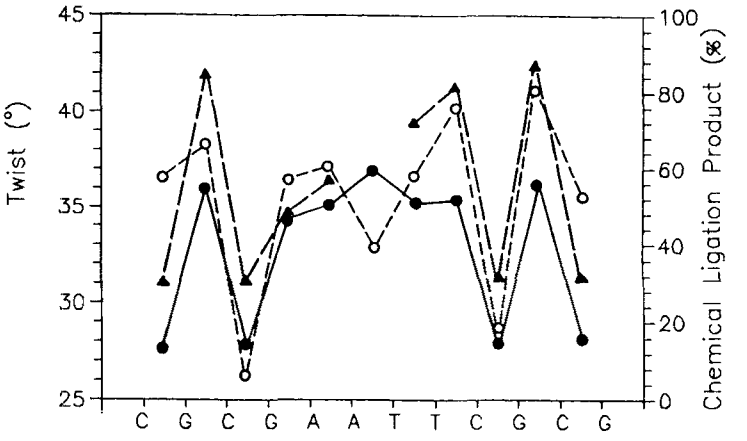

FIGURE 3. A sequence dependence of the local helical twist in dodecamer d(CGCGAATTCGCG). The calculated data for planar base pairs without a propeller twist (closed circles), experimental (X-ray analysis) results (open circles). Data were taken from [16]. For comparison, a sequence dependence of nick-sealing efficiency (BrCN-activation) is presented for the same dinucleotide combinations (closed triangles). The ApT combination is absent in our study.

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CONCLUSION

The similarities revealed between the coupling yields (the reactions induced by chemical reagents) at different dinucleotide combinations and the ^{31}P resonances of phosphate groups within the corresponding dinucleotides in the double helix strongly support the hypothesis that variations in chemical ligation efficiency arise from sequence-specific modulations in the helix geometry. The present research allowed us to show the direct correlation between the reaction ability and the secondary structure-directed arrangement of the reactive groups. It is worth noting that the obtained correlations do not depend on the nature of the condensing reagent.

To a first approximation, the coupling ability of two nucleotide residues in the double helix might be predicted on the basis of the nearest neighbor model which describes the helix parameters in terms of the dinucleotide structure

neglecting the distant effects. In fact, the different sequences flanking the dinucleotide combinations being studied, hardly affect the obtained regularities. Our results favour the view that local structure, dictated by the intrinsic properties of double-stranded DNA, is somewhat "frozen" near the nick.

And finally, the knowledge of the coupling efficiency range depending on the nature of reacting nucleotide residues is very important for optimal subdivision of the DNA duplexes to be chemically assembled.

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