Minor-groove-modified oligonucleotides: synthesis of decadeoxynucleotides containing hypoxanthine, N²-methylguanine and 3-deazaadenine, and their interactions with restriction endonucleases *Bgl* II, *Sau* 3AI, and *Mbo* I (Nucleosides and Nucleotides Part 75)

Akira Ono and Tohru Ueda*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

Received February 26, 1987; Accepted March 6, 1987

Abstract

Decadeoxynucleotides containing hypoxanthine, N²-methylguanine, 3-deazaadenine in the recognition sequences of restriction endonucleases *Bgl* II, *Sau* 3AI, and *Mbo* I were synthesized. These decanucleotides modified in the base moieties facing in to the minor groove were strongly resistant to hydrolysis by *Bgl* II and partially resistant to that of *Sau* 3AI and *Mbo* I. The decadeoxynucleotide containing 3-deazaadenine in place of adenine was bound to *Bgl* II strongly, whereas the nucleotides containing hypoxanthine and N²-methylguanine were bound less tightly.

Introduction

Sequence-specific DNA-binding proteins such as restriction endonucleases have been reported to bind to the major groove of the DNA helix.² All of the DNA methylations related to the restriction-modification systems so far found occur in the major groove.³ An X-ray diffraction study of a co-crystalline complex between *Eco* RI and an oligonucleotides containing the recognition sequence showed that *Eco* RI was bound to the major groove.⁴ However, other studies using modified DNA's and oligonucleotides have suggested a role for the minor groove in the recognition of restriction endonucleases.⁵ For instance, the modified recognition sequence containing 3-methyladenine in place of adenine was resistant to *Eco* RI digestion.^{5b}

In this paper we report the synthesis of decadeoxynucleotides containing hypoxanthine, N²-methylguanine, 3-deazaadenine (Fig. 1) in the recognition sequences of restriction endonucleases *Bgl* II⁶ (AGATCT), *Sau* 3AI⁷ (GATC), and *Mbo* I⁸ (GATC), and their interaction with these enzymes.

Fig.1

Materials and Methods

Enzymes and Nucleosides

T₄ Polynucleotide kinase (*E. coli* B), Bgl II, and Sau 3AI were purchased from Takara Shuzo Co. Ltd. Mbo I was purchased from Pharmacia P-L Biochemical Inc. The 2'-deoxynucleosides were purchased from Yamasa Shoyu Co. Ltd.

Synthesis of modified nucleotides

Melting points were determined on a Yanaco MP-3 micromelting point apparatus and are uncorrected. UV spectra were taken with a Shimadzu UV-260 spectrophotometer. ¹H-NMR spectra were recorded on a JEOL JNM-FX 270FT spectrometer with tetramethylsilane as an internal standard. Chemical shifts are expressed in ppm (δ), and signals are described as s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Field desorption mass spectra (FD-MS) were taken on a JEOL JMS-01SG-2 mass spectrometer.

Triethylammonium 5'-O-dimethoxytrityl-2'-deoxyinosine 3'-O-(o-chlorophenyl phosphate) (1)

A solution of 2'-deoxyinosine (2.0g, 7.9mmol) and dimethoxytrityl chloride (DMTrCl, 3.2g, 1.2eq) in pyridine (20 ml) was stirred at room temperature overnight. Methanol (5ml) was added to the solution and the solvent was removed *in vacuo*. The residue was taken up in CHCl₃ and washed with 5% NaHCO₃ solution. The organic layer was separated, passed through a Whatman 1PS filter paper and the filtrate was concentrated. The separated crystals were collected to give 3.2g (5.8mmol, 73%) of 5'-O-dimethoxytrityl-2'-deoxyinosine (2). An aliquot was recrystallised from ethanol for analysis. UV (in MeOH) λ_{max} 236nm, 274nm (shoulder), 282nm (shoulder). ¹H-NMR (DMSO-d₆): 12.36 (1H, s, HN-1), 8.18 (1H, s, H-8), 7.98 (1H, s, H-2), 7.34-6.78 (13H, m, dimethoxytrityl), 6.34 (1H, t, H-1'), 5.37 (1H, d, HO-3'), 4.42 (1H, m, H-3'), 3.98 (1H, m, H-4'), 3.72 (6H, s, methoxy groups of di-

Fig. 2

methoxytrityl), 3.15 (2H, m, H-5'), 2.79-2.34 (2H, m, H-2'). FD-MS m/z : 554 (M^+).

Compound 2 (500mg, 0.9mmol) was dried by distillation with anhydrous pyridine twice and the residue was dissolved in anhydrous dioxane (10ml). A 0.2 M solution of *o*-chlorophenylphosphorotriazolid⁹ (1.5eq) was added to the solution under cooling in an ice-bath and the whole was kept at room temperature for 1 hr, then 50% pyridine-H₂O (1ml) was added, and the solvent was removed in vacuo. The residue was taken up in CHCl₃, washed with 0.1 M triethylammonium bicarbonate, and the organic layer was separated, passed through a Whatman 1PS filter paper, and the filtrate was concentrated in vacuo. The residue was triturated in ether-hexane to give the title compound 1 (820mg, 0.89mmol, 99%) (Fig. 2). UV (in MeOH) λ_{max} 236nm, 275nm (shoulder), 283nm (shoulder). ¹H-NMR (CDCl₃): 13.00-12.50 (1H, br, onium proton of triethylammonium), 11.61 (1H, br, HN-1), 8.09 (1H, s, H-2), 7.88 (1H, s, H-8), 7.68-6.73 (17H, m, dimethoxytrityl and *o*-chlorophenyl), 6.43 (1H, t, H-1'), 5.24 (1H, m, H-3'), 4.45 (1H, m, H-4'), 3.90-3.70 (8H, m, H-5' and methoxy groups of dimethoxytrityl), 3.09 (6H, q, methylene groups of triethylamine), 2.90-2.70 (2H, m, H-2'), 1.29 (9H, t, methyl groups of triethylamine) FD-MS m/z : 846(M^+), 948(M^+ +102).

Triethylammonium 5'-O-dimethoxytrityl-2'-deoxy-N²-acetyl-N²-methylguanosine 3'-(*o*-chlorophenyl phosphate) (3).

Acetic anhydride (0.48mmol, 10eq) and 4-dimethylaminopyridine (30mg, 0.5eq) were added to a suspension of 2'-deoxy-N²-methylguanosine¹⁰ (150mg, 0.53mmol) in pyridine (5ml) and the whole was refluxed for 2 hr. The solvent was concentrated and the residue was dissolved in MeOH (2ml). 1N NaOH (in 50%MeOH, 2ml) was added to the solution and, after 5 min, the solution was

neutralised by addition of 1N HCl (in 50%MeOH, 2ml). The solution was concentrated and the separated crystals were collected to give 2'-deoxy-N²-acetyl-N²-methylguanosine (4, 59mg). The filtrate was concentrated and additional crystals (31mg) were obtained (total yield, 52%). An aliquot was recrystallized from ethanol for analysis. mp 180-181°. Anal. Calcd for C₁₃H₁₇N₅O₅ (323.31): C, 48.30; H, 5.30; N, 21.66. Found: C, 47.82; H, 5.28; N, 21.19. UV (in MeOH) λ_{max} 277nm, 254nm. ¹H-NMR (DMSO-d₆): 12.80-12.40 (1H, br, HN-2), 8.28 (1H, s, H-8), 6.26 (1H, t, H-1'), 5.32 (1H, d, HO-3'), 4.92 (1H, t, HO-5'), 4.37 (1H, m, H-3'), 3.87-3.82 (1H, m, H-4'), 3.60-3.46 (2H, m, H-5'), 3.30 (3H, s, MeN-2), 2.67-2.57 (1H, m, H-2'a), 2.33-2.20 (1H, m, H-2'b), 2.22 (3H, s, Ac).

Compound 4 (59mg, 0.18mmol) was dried by distillation twice with anhydrous pyridine and the residue was dissolved in pyridine (3ml). DMTrCl (1.2eq) was added and the whole was kept at room temperature for 2 hr. MeOH (1ml) was added and the solvent was concentrated in vacuo. The residue was taken up in CHCl₃ and washed with 5% NaHCO₃ solution. The organic layer was separated through a Whatman 1PS filter paper, then the solvent was removed in vacuo. The residue was applied to a column of silica gel (Kieselgel, 10g). The product was eluted with CHCl₃-MeOH (20:1) and concentrated to a small volume which was added dropwise to a mixture of ether-n-hexane (1:1). The precipitate was collected by centrifugation to give 5'-O-dimethoxytrityl-2'-deoxy-N²-acetyl-N²-methylguanosine (5, 95mg, 0.15mmol, 85%). UV (in MeOH) λ_{max} 275nm, 236nm, 282nm (shoulder). ¹H-NMR (CDCl₃): 12.91 (1H, s, HN-2), 7.78 (1H, s, H-8), 7.41-6.78 (13H, m, dimethoxytrityl), 6.33 (1H, t, H-1'), 4.64-4.60 (1H, m, H-3'), 4.14-4.10 (1H, m, H-4'), 3.78 (6H, s, methoxy groups of dimethoxytrityl), 3.47 (3H, s, MeN-2), 3.50-3.41 (1H, m, H-5'a), 3.31-3.26 (1H, m, H-5'b), 2.72-2.62 (1H, m, H-2'a), 2.54-2.43 (1H, m, H-2'b), 2.42 (3H, s, acetyl). FD-MS m/z: 625 (M⁺).

Compound 5 (79.2mg, 0.13mmol) was dried by distillation twice with anhydrous pyridine and the residue was dissolved in dioxane (1ml). 0.2 M solution of o-chlorophenyl phosphoroditriazolidide (1.5eq) was added and the solution was kept at room temperature for 1 hr. 50% pyridine-water (1ml) was added to the

solution under cooling in an ice-bath and the solvent was removed in vacuo. The residue was taken up in CHCl_3 , washed with 0.1 M triethylammonium bicarbonate, and the organic layer was separated through a Whatman 1PS filter paper, then the solvent was removed in vacuo. The residue was triturated in ether-n-hexane (1:1) to give title compound (3, 103mg, 0.11mmol, 85%) (Fig. 2). UV (in MeOH) λ_{max} 276nm, 263nm, 236nm, 283nm (shoulder). FD-MS m/z : 917 (M^+), 1019 ($\text{M}^+ + 102$). $^1\text{H-NMR}$ (CDCl_3): 7.77 (1H, s, H-8), 7.66-6.73 (17H, m, dimethoxytrityl and o-chlorophenyl), 6.37 (1H, t, H-1'), 5.18 (1H, m, H-3'), 4.44 (1H, m, H-4'), 3.76 (6H, s, methoxy groups of dimethoxytrityl), 3.44 (3H, s, MeN-2), 3.33-3.29 (2H, m, H-5'), 2.97 (6H, q, methylene groups of triethylamine), 2.76-2.74 (2H, m, H-2'), 2.41 (3H, s, acetyl), 1.25 (9H, t, methyl groups of triethylamine).

Triethylammonium 5'-O-dimethoxytrityl-2'-deoxy-3-deazaadenosine 3'-(o-chlorophenyl phosphate) (6)

2'-Deoxy-3-deazaadenosine¹¹ was protected by the similar procedure as reported.¹² A solution of 2'-deoxy-3-deazaadenosine (36mg, 0.14mmol) and dimethylformamide dimethylacetal (0.2ml) in dimethylformamide (1ml) was kept at room temperature for 2 days, then the solvent was evaporated in vacuo. The residue was dissolved in pyridine (1ml), DMTrCl (2eq) was added, and the whole was kept at room temperature for 1 day. MeOH (0.5ml) was added and the solvent was removed in vacuo. The residue was taken up in CHCl_3 , washed with 5% NaHCO_3 solution, and the organic layer was separated through a Whatman 1PS filter paper. The filtrate was concentrated to a small volume which was added dropwise to a mixture of ether-n-hexane (1:1). The precipitate was collected by centrifugation to give the 5'-O-dimethoxytrityl-2'-deoxy-3-deazaadenosine (7, 46mg, 0.08mmol, 57%). FD-MS m/z : 607 (M^+). $^1\text{H-NMR}$ (CDCl_3): 8.70 (1H, s, dimethylaminomethylene), 7.97 (1H, s, H-8), 7.96 (1H, d, H-2), 7.04 (1H, d, H-3), 7.41-6.78 (13H, m, dimethoxytrityl), 6.24 (1H, t, H-1'), 4.63-4.61 (1H, m, H-3'), 4.12 (1H, m, H-4'), 3.78 (6H, s, methoxy groups of dimethoxytrityl), 3.41-3.36 (2H, m, H-5'), 3.21 (3H, s, N-Me a), 3.14 (3H, s, N-Me b), 2.70-2.40 (2H, m, H-2').

Compound 7 (22mg, 0.036mmol) was converted to the title compound 6 (Fig. 2) (26mg, 0.029mmol, 80%) by the procedure

Table 1.

		Tm(°C)
I	GGAGATCTCC	42
II	GGAIATCTCC	32
III	GGAG ^{Me} ATCTCC	46
IV	GGAGAT ^{3C} CTCC	36

Melting temperatures of deca-nucleotide duplexes. T_m 's were measured in a buffer of 0.01M sodium cacodylate containing 0.1M NaCl at pH 7.5.

described previously. UV (in MeOH) λ_{max} 324nm, 281nm, 275nm, 231nm. $^1\text{H-NMR}$ (CDCl_3): 9.18 (1H, s, dimethylaminomethylene), 8.12 (1H, s, H-8), 7.90 (1H, d, H-2), 7.75-6.73 (18H, m, H-3, dimethoxytrityl, o-chlorophenyl), 6.25 (1H, dd, H-1'), 5.32 (1H, m, H-3'), 4.50 (1H, m, H-4'), 3.75 (6H, s, methoxy groups of dimethoxytrityl), 3.37 (2H, m, H-5'), 3.23 (3H, s, N-Me a), 3.19 (3H, s, N-Me b), 3.10-3.05 (7H, m, H-2'a, methylene groups of triethylamine), 2.70 (1H, m, H-2'b), 1.32 (9H, t, methyl groups of triethylamine).

Fig.3 Mobility shift analysis of decanucleotides II-IV.

Synthesis of decadeoxynucleotides

The decadeoxynucleotides listed in Table 1 were prepared by the solid phase phosphotriester method.¹³ The protected oligomers on the polymer support were released from the resin and deprotected by treatment with tetramethylguanidinium pyridine-2-aldoximate¹⁴ followed by ammonia, then with 80% AcOH. All the decamers were purified by anion exchange column chromatography (DEAE-Toyopearl 650s) followed by reversed phase HPLC (Gilson Microsorb C-18) to a purity of over 99%. The sequences of the purified decamer were confirmed by 5'-phosphate labelling and successive partial digestion with snake venom phosphodiesterase¹⁵, followed by mobility shift analysis¹⁶ of the partial hydrolyzates. The autoradiograms are shown in Fig 3.

Melting temperature determination

Melting temperature was measured by a Shimadzu UV-260 spectrophotometer equipped with a temperature controller (140/SPR-5, Shimadzu) and a thermometer (Model 2542, Yokokawa electric Co.), and the results are summarized in Table 1.

CD spectra measurements

The CD spectra of decamers were measured on a JASCO J-40 spectropolarimeter in 0.1M NaCl containing 0.01M sodium cacodylate (pH 7.5) at 17°, and are shown in Fig. 4.

5'-Labelling of decadeoxynucleotides

The oligonucleotide (1 nmol) was incubated with γ -³²P-ATP and polynucleotide kinase (1 unit) in 50 mM Tris-HCl (pH 9.6), 10 mM DTT, 2 mM spermine, and 100 mM KCl in a total volume of 15 μ l at 37°C for 30 min. The product was adsorbed onto Whatman DE 50 (bicarbonate form) and washed with 0.4 M triethylammonium bicarbonate, then eluted with 2.0 M triethylammonium bicarbonate. The eluate was concentrated in vacuo to leave the product.

Conditions for the digestion with restriction endonucleases

Method A) 5'-³²P-oligonucleotide (5000 to 10000 cpm) was incubated with Bgl II (12 units) in 10 mM Tris-HCl (pH 7.5), 7 mM MgCl₂, 100 mM NaCl, and 7 mM 2-mercaptoethanol at 15°C for 4 hr. Method B) 5'-³²P-labelled GGAGATCTCC (5000 to 10000 cpm) and cold GGAGATCTCC (final concentrations of 1.25×10^{-6} M as duplex) were incubated with Bgl II (12 units) at 15°C, in the presence of a modified decamer of appropriate concentrations, in the same buf-

fer as described in method A. An aliquot of the reaction mixture was removed and adsorbed on to a DEAE cellulose plate (Polygram Cel 300 Macherey-Nagel) at 1, 2, 3, 4, and 5 min after incubation to determine the initial velocities.

Method C) 5'- ^{32}P -labelled GGAGATCTCC (5000 to 10000 cpm) and cold GGAGATCTCC (final concentrations of 0.75×10^{-6} , 1.25×10^{-6} , 2.25×10^{-6} , 3.25×10^{-6} , $4.25 \times 10^{-6}\text{M}$) were incubated with Bgl II (12 units) in the presence of the decamer containing 3-deazaadenine in concentrations of 0.5×10^{-6} , $1.0 \times 10^{-6}\text{M}$, or in the presence of the decamer containing 7-deazaadenine ¹⁷ in concentrations of 0.1×10^{-6} , 0.25×10^{-6} or $0.5 \times 10^{-6}\text{M}$ in the same conditions as described in method A. The initial velocities were determined by the same procedure described in method B.

Method D) 5'- ^{32}P -labelled decamer (5000 to 10000 cpm) together with the unlabelled decamer in appropriate concentrations were incubated with Mbo I (40 units in 100 mM Tris-HCl pH 8.0, 20 mM MgCl_2 , and 100mM NaCl at 15°C), or Sau 3AI (30 units in 10 mM Tris-HCl pH 7.5, 7 mM MgCl_2 , and 100 mM NaCl at 15°C). The

Fig.4 The CD spectra of decamer duplexes in 0.1M NaCl, 0.01M sodium cacodylate pH 7.5 at 18°C .

initial velocities were determined by the same procedure as described in method B.

All digests in the above methods were developed by homo-chromatography and the radioactive spots were excised and counted with a toluene based scintillator on a Beckman LS-230 liquid scintillation counter.

Results and Discussions

Melting temperatures (T_m) and CD spectra of decadeoxynucleotides

The T_m 's of modified oligonucleotides are shown in Table 1, and the CD spectra in Fig. 4. The decanucleotides containing N²-methylguanine showed higher T_m than the natural decamer. The CD spectra of these natural and modified oligonucleotides exhibited similar patterns of B-type DNA. Thus it can be stated that the N²-methyl group on the guanine moiety stabilized the B-DNA like conformation, possibly N²-methyl group increase the stacking ability of guanine ring system. The duplex containing hypoxanthine showed a lower T_m when compared with the parent decamer as might be expected from the lack of a hydrogen bond present in the G:C pair. The CD patterns are apparently very different. This

Fig.5 The inhibition experiment in the Bgl II digestion of GGAGATCTCC in the presence of minor groove modified decamers II-IV. The conditions are described in Method B.

is probably due primarily to the considerable difference in the UV spectra of hypoxanthine and guanine moieties, and there seems to be no essential difference in the duplex formation.

As one would expect, the difference in CD spectra between the decanucleotides containing 3-deazaadenine or adenine is very small, as one is only replacing the methine function with azomethine. The T_m of the decamer containing 3-deazaadenine was lower than that of the parent decamer, probably due to the lower stacking ability of the imidazopyridine system than that of the purine system.

Interaction of Bgl II with decanucleotides

We have previously reported that major groove modified decanucleotides containing 7-deazaadenine, N^6 -methyladenine, 5-methylcytosine, and N^4 -methylcytosine in the recognition sequence of Bgl II were not cleaved but were strongly bound to Bgl II.^{17, 1} Now it is shown that the minor groove modified decanucleotides (II-IV) are also resistant to hydrolysis by Bgl II (in the method A conditions, data not shown). It is conceivable that the minor groove alteration of the base moiety in the recognition sequence may cause either a loss (or decrease) of the binding to the enzyme, or the inability of the phosphodiester cleavage due to some alteration of the binding mode. To clarify this point an inhibition experiment was carried out on the cleavage of GGA-GATCTCC by Bgl II in the presence of minor groove modified decamers. The result is summarized in Fig. 5. As is apparent, the decamer containing 3-deazaadenine was bound to the enzyme, while the other two, containing hypoxanthine or N^2 -methylguanine, were bound less tightly. Kinetic analysis disclosed that both the decamers containing 3-deaza- or 7-deaza-adenine inhibited the hydrolysis of the natural decamer competitively, the K_i values being $1.7 \times 10^{-6} M$ and $0.9 \times 10^{-6} M$, respectively (Fig. 6), which were smaller than the K_m value ($3.3 \times 10^{-6} M$) for the natural decamer. It can be stated that the decamer containing 3-deazaadenine does bind to the recognition site of the enzyme but the binding mode may be somewhat different due to a loss of a proton-accepting function related to the azomethine group of adenine thus resulting in the resistance to hydrolysis. The 2-amino group of the

Fig.6 The inhibition experiment in the Bgl II digestion of GGAGATCTCC. The conditions are described in Method C.

guanine moiety in the recognition sequence seems to be crucial for the binding of Bgl II.

It has been generally recognized that sequence-specific DNA-binding proteins bind to the major groove of B-DNA by sequence-specific hydrogen bonds.² In fact, all of the naturally-occurring DNA methylations which result in resistance to the hydrolysis by restriction enzymes are found in the major groove.³ However the present result and some previous findings⁵ show that minor groove alterations also affect the function of restriction enzymes. The role of the minor groove can be tentatively suggested as the

Table 2.

		Km(M)	rel.Vmax
I	GGAGATCTCC	2.2×10^{-7}	100
II	GGAIATCTCC	8.0×10^{-6}	100
III	^{Me} GGAGATCTCC	1.3×10^{-7}	74
IV	^{3C} GGAGATCTCC	5.5×10^{-7}	60

Effect of the base modifications upon
Mbo I activity.
The conditions are described in
Method D.

Table 3.

		rel.V
I	GGAGATCTCC	100
II	GGAIATCTCC	80
III	GGAG ^{Me} ATCTCC	0
IV	GGAGAT ^{3C} CTCC	60

Effect of the base modifications upon *Sau* 3AI activity. The conditions are described in Method D.

following; the major groove of B-DNA is broad and deep enough (11.7 Å and 8.5 Å) to accommodate an α -helix of proteins (10.7 Å)¹⁸ as the recognition site (for *Bgl* II in the present case), and once the site is filled, the catalytic site of *Bgl* II would be made available by hydrogen bond-interaction with sugar-phosphate backbone and minor groove bases. The presence of the N²-amino group of guanine may be essential for binding (and cleavage) and the 3-azomethine function of the adenine moiety may be essential for cleavage.

Interaction of *Mbo* I with decanucleotides

Mbo I hydrolysed all modified decamers although the rates of cleavage were somewhat slower than that of parent decamer. The *K_m* values and relative *V_{max}* values are summarised in Table 2.

Interaction of *Sau* 3AI with decanucleotides

Sau 3AI exhibits a larger *K_m* value (2×10^{-5} M) with the natural substrate when compared with other restriction enzymes as described above. Unfortunately we were unable to determine the *K_m* and *V_{max}* values of the decamers with this enzyme due to shortage of the decamers, therefore, only the relative velocities are shown in Table 3. It is noteworthy that the decamer containing N²-methylguanine was totally resistant to hydrolysis.

Conclusion

Of three restriction endonucleases tested, *Bgl* II was shown to interact with the minor groove of substrate decamers in addition to the interaction with the major groove as previously reported^{17,1}. The N²-amino group of guanine and the 3-azomethine group of adenine seem to be essential for the binding and/or

cleavage. In the case of Mbo I the recognition of the minor groove base moieties is not essential. Although the precise similarity or dissimilarity between Sau 3AI and Mbo I (recognizing the same sequence GATC) is not clear, it is noteworthy that the Km value of Sau 3AI with a natural decanucleotide is one hundred times larger than that of Mbo I.

* To whom correspondence should be addressed

References

1. Ono, A., Ueda, T., (1987) *Nucleic Acids Res.*, **15**, 219-232
2. Seeman, N.C., Rosenberg, J.M. Rich, A., (1976) *Proc. Nat. Acad. Sci. USA* **73**, 804-808
3. a) Ehrlich, M., Wang, R.Y.-H., (1981) *Science*, **212**, 1350-1357
 b) Ehrlich, M., Gama-Sosa, M.A., Carreira, L.H., Ljungdahl, L.G., Kuo, K.C., Gehrke, C.W., (1985) *Nucleic Acids Res.*, **13**, 1399-1412
 c) Butkus, V., Klimasauskas, S., Kersulyte, D., Vaitkevicius, D., Lebionka, A., Janulaitis, A., (1985) *Nucleic Acids Res.*, **13**, 5727-5746
4. Frederick, C.A., Grable, J., Melia, M., Samudzi, C., Jen-Jacobson, L., Wang, B.-C., Greene, P., Boyer, H.W., Rosenberg, J.M., (1984) *Nature*, **309**, 327-331
5. a) Modrich, P., Rubin, R.A., (1977) *J. Biol. Chem.*, **252**, 7273-7278
 b) Lu, A.-L., Jack, W.E., Modrich, P., (1981) *J. Biol. Chem.*, **256**, 1320-13206
 c) Dwyer-Hallquist, P., Kezdy, F.J., Agarwal, K.L., (1982) *Biochemistry*, **21**, 4693-4700
 d) Wolfes, H., Fliess, A., Pingoud, A., (1985) *Eur. J. Biochem.*, **150**, 105-110
6. a) Firrotta, V., (1976) *Nucleic Acids Res.*, **3**, 1747-1760
 b) Duncan, C.H., Wilson, G.A., Young, F.E., (1978) *J. Bacteriol.*, **134**, 338-344
7. a) Sussenbach, J.S., Monfoort, C.H., Schiphof, R., Stobberingh, E.E., (1976) *Nucleic Acids Res.*, **3**, 3193-3202
 b) Streek, R.E., (1980) *Gene*, **12**, 267-275
8. Gelinas, R.E., Myer, P.A., Roberts, R.J., (1977) *J. Mol. Biol.*, **114**, 169-179
9. Broka, C., Hozumi, T., Arentzen, R., Itakura, K., (1980) *Nucleic Acids Res.*, **8**, 5461-5471
10. Boryski, J., Ueda, T., (1985) *Nucleosides and Nucleotides*, **4**, 595-606
11. Itoh, T., Yamaguchi, T., Mizuno, Y., (1981) *J. Carbohydr. Nucleosides Nucleotides*, **8**, 119
12. Mizuno, Y., Kitano, S., Nomura, A., (1975) *Nucleic Acids Res.*, **2**, 2193-2207
13. Miyoshi, K., Arentzen, R., Huang, T., Itakura, K., (1980) *Nucleic Acids Res.*, **8**, 5507-5517
14. Reese, C.B., Yan, L., (1978) *Tetrahedron Letters*, 4443-4446

Nucleic Acids Research

15. Silberklang, M., Gillum, A.M., RajBhandary, U.L., (1977) Nucleic Acids Res., 4, 4091-4108
16. Jay, E., Bambara, R., Padmanabhan, R., Wu, R., (1974) Nucleic Acids Res., 1, 331-353
17. a) Ono, A., Sato, M., Ohtani, Y., Ueda, T., (1984) Nucleic Acids Res., 12, 8939-8949
b) Ueda, T., Sato, M., Ono, A., (1986) Chemica Scripta, 26, 199-203
18. Wang, J.-H., (1986) Nature 319, 183-184