Dissociation of the AT-specific bifunctional intercalator [N-MeCys³,N-MeCys⁷]TANDEM from TpA sites in DNA

Michael C. FLETCHER,* Richard K. OLSEN† and Keith R. FOX*‡

*Department of Physiology and Pharmacology, Bassett Crescent East, University of Southampton, Southampton SO9 3TU, U.K., and †Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, U.S.A.

We have examined the dissociation of [N-MeCys³,N-MeCys⁷]TANDEM, an AT-selective bifunctional intercalator, from TpA sites in mixed-sequence DNAs by a modification of the footprinting technique. Dissociation of complexes between the ligand and radiolabelled DNA fragments was initiated by adding a vast excess of unlabelled calf thymus DNA. Portions of this mixture were subjected to DNase I footprinting at various times after adding the competitor DNA. Dissociation of the ligand from each site was seen by the time-dependent disappearance of the footprinting pattern. Within a natural DNA fragment (tyrT) the ligand dissociates from TTAT faster than

from ATAT. We found that the stability of complexes with isolated TpA steps decreases in the order ATAT > TTAA > TATA. Dissociation from each of these sites is much faster than from longer regions of (AT)_n. These results confirm the requirement for A and T base-pairs surrounding the TpA step and suggest that the interaction is strongest with regions of alternating AT, possibly as a result of its unusual structure. The ligand dissociates more slowly from the centre of (AT)_n tracts than from the edges, suggesting that variations in dissociation rate arise from sequence-dependent variations in local DNA structure.

INTRODUCTION

[N-MeCys³,N-MeCys⁷]TANDEM (Figure 1a) is a synthetic derivative of the quinoxaline group of antibiotics that includes echinomycin and triostin A [1]. These compounds contain an octadepsipeptide ring to which are attached two quinoxaline chromophores, and they bind to DNA by bifunctional intercalation [2,3]. The naturally occurring compounds, which are potent anti-tumour agents, contain four *N*-methyl substituents on the cysteine and valine residues, whereas the synthetic compounds, TANDEM and [N-MeCys³,N-MeCys⁷]TANDEM, lack four and two of these methyl groups respectively. Footprinting [4,5], n.m.r. [6,7] and X-ray crystallography [8–10] have shown that echinomycin and triostin bind selectively to the dinucleotide CpG; this has been explained by the formation of hydrogen bonds between the alanine NH of the ligand and guanine N-3, and between alanine CO and guanine 2-NH₂ [6–10]. In contrast, TANDEM and [N-MeCys³,N-MeCys⁷]TANDEM bind best to AT-rich DNAs [11]. Footprinting [12–15] and NMR [16–17] studies have shown that they are selective for the dinucleotide TpA. The affinity for TpA is much stronger when this dinucleotide is surrounded by AT base-pairs [12,13], and the binding to poly(dA-dT) is highly co-operative [11]. Although the molecular basis of this recognition has been less well characterized, it appears to involve hydrogen bonds between alanine NH on the ligand and adenine N-3 [16,17]; recognition of CpG is no longer possible, since the alanine CO groups are involved in intramolecular hydrogen bonds to the valine NH groups [18].

Kinetic experiments using SDS sequestration demonstrated that, while the dissociation of echinomycin and triostin from synthetic DNAs, such as poly(dG-dC), is described by a single exponential decay, dissociation from natural DNAs requires several exponential terms [19,20]. This complexity was explained by suggesting that it represented the parallel dissociation of the ligand from different classes of binding sites with individual

microscopic rate constants, the longest of which displayed a half-life of about 15 min. There have been few studies on the dissociation kinetics of the synthetic analogues. SDS sequestration revealed that the dissociation of TANDEM from natural DNAs was faster than echinomycin or triostin A and that only the slowest component could be resolved by simple mixing techniques [21]. In contrast, dissociation from poly(dA-dT), to which the ligand binds co-operatively, was much slower, confirming the preference for surrounding AT base-pairs. Although this dissociation was completely described by a single exponential decay, the time constant varied according to the starting ratio of ligand to DNA, becoming longer at higher levels of saturation [21]. This again reflects the co-operative nature of the interaction.

We have recently developed the footprinting technique for examining the kinetics of dissociation of sequence specific ligands from individual DNA-binding sites [22]. In this technique a complex between the ligand and a radiolabelled DNA fragment is dissociated by adding a vast excess of unlabelled calf thymus DNA. Aliquots are removed from the dissociation mixture at various times after adding the competitor DNA and are subjected to DNase I footprinting. The dissociation is revealed by the rate of disappearance of the footprinting pattern. In the present study we have used the modified footprinting technique to examine the dissociation of [N-MeCys³,N-MeCys⁷]TANDEM from TpA sites in both natural and synthetic DNA fragments.

MATERIALS AND METHODS

Chemicals and enzymes

[N-MeCys³,N-MeCys⁷]TANDEM was prepared as previously described [13]. Since this drug is only sparingly soluble in aqueous systems, it was stored as a 2 mM stock solution in dimethyl sulphoxide and diluted to working concentrations in 10 mM Tris/HCl, pH 8.0, containing 10 mM NaCl immediately before

‡ To whom correspondence should be addressed.

Figure 1 (a) Structures of TANDEM, [N-MeCys³,N-MeCys⁷]TANDEM and triostin A, and (b) sequence of the *tyrT* DNA fragment

(a) TANDEM, R₁ = R₂ = H; [N-MeCys³,N-MeCys⁷]TANDEM, R₁ = CH₃, R₂ = H; triostin A, R₁ = R₂ = CH₃. (b) The numbering scheme is as previously described [4]. The sequence is written from 3'–5' so that lower numbers are located towards the bottom of the gel. The underlined bases correspond to positions bearing the radioactive phosphates.

use. The final concentration of dimethyl sulphoxide was always less than 5% (v/v) and had no effect on DNase I digestion. DNase I was purchased from Sigma, and stored as previously described [4].

DNA fragments

The *tyrT* DNA fragment (Figure 1b) was prepared from plasmid pKMA-98 as previously described [4,12], by digesting with *Eco*RI and *Ava*I and labelling at the 3'-end of the *Eco*RI site with [α -³²P]dATP using reverse transcriptase. The preparation of plasmids containing the synthetic DNA inserts has been previously described [23,24]. In each case the inserts were cloned into the *Sma*I site of pUC19. Radiolabelled fragments containing the inserts were obtained by digesting with *Hind*III, labelling at the 3'-end with [α -³²P]dATP using reverse transcriptase, and cutting again with *Eco*RI. The radiolabelled DNA fragments of interest were separated from the remainder of the plasmid using 6 (*tyrT*) or 8% (fragments containing synthetic inserts) polyacrylamide gels.

DNase I footprinting

Samples for footprinting were prepared by mixing 9 μ l of radiolabelled DNA (about 50 pmol of base-pairs) with 9 μ l of [N-MeCys³,N-MeCys⁷]TANDEM, at concentration of 100 μ M in 10 mM Tris/HCl, pH 8.0, containing 10 mM NaCl. This mixture was left for at least 30 min to ensure equilibration of the drug and DNA. Dissociation of the antibiotic from the DNA was initiated by adding 10 μ l of calf thymus DNA (6 mM base-pairs). Aliquots (4 μ l each) were removed from this mixture at various times and digested with 2 μ l of DNase I (150 units/ml dissolved in 2 mM MgCl₂/2 mM MnCl₂/20 mM NaCl). The digestion reaction was stopped after 12 s by the addition of 4 μ l of formamide containing 10 mM EDTA and 0.1% (w/v) Bromophenol Blue. The footprinting pattern of the mixture at equilibrium was determined by mixing the radiolabelled DNA with calf thymus DNA before addition of the antibiotic and digesting

in the same manner. Digestion patterns of the native DNA and the complex before dissociation were obtained in the usual manner by mixing 1.5 μ l of radiolabelled DNA with 2 μ l of drug and digesting for 1 min with 2 μ l of DNase I (0.03 unit/ml). In each case the digestion was such that only about 10% of the material was cleaved by the enzyme, thereby ensuring 'single-hit kinetics'. As a result of the much higher enzyme concentration required in the presence of the unlabelled DNA, it is not always possible to obtain the same extent of digestion in each lane. It is therefore important to compare the relative cleavage patterns of bands between different lanes, rather than merely examining the intensity of individual bands.

Gel electrophoresis

All samples were heated to 95 °C for 3 min prior to electrophoresis. The products of digestion were resolved on 8% (w/v), 40 cm-long polyacrylamide gels containing 8 M urea. Gels were run at 1500 V for about 2 h. These were then fixed in 10% acetic acid, transferred to Whatmann 3MM paper, dried under vacuum at 80 °C and subjected to autoradiography at –70 °C with an intensifying screen. Bands in the digests were assigned by comparison with Maxam–Gilbert dimethyl sulphate/piperidine markers specific for guanine or guanine and adenine, or by comparison with previously published digestion patterns.

RESULTS

tyrT DNA

Figure 2 presents the time course of dissociation of [N-MeCys³,N-MeCys⁷]TANDEM from *tyrT* DNA, measured at three different temperatures. Previous studies have shown that both TANDEM [12] and [N-MeCys³,N-MeCys⁷]TANDEM [13] each produce two clear footprints on this DNA fragment, located around positions 88 (ATAT) and 110 (ATAA). These are evident in the

Figure 2 Dissociation of [*N*-MeCys³,*N*-MeCys⁷]TANDEM from *tyrT* DNA

Aliquots were removed from the mixture at various times after adding the unlabelled calf thymus DNA and subjected to short (12 s) digestion by DNase I. The time after adding the competitor DNA (in min) is indicated at the top of each lane. 'con' indicates digestion of the DNA alone in the absence of any ligand; '0' corresponds to digestion of a complex with 50 μ M [*N*-MeCys³,*N*-MeCys⁷]TANDEM before adding the unlabelled DNA. In the track labelled 'Eq' the labelled and unlabelled DNA were mixed before adding the ligand and represents the true equilibrium distribution of the ligand in the dissociating mixture. The temperature of the dissociation is indicated at the top of each panel. The black boxes show the position of the footprints and correspond to the sequences ATAT (lower) and TTAT (upper). Residue numbers refer to the numbering scheme for *tyrT* DNA shown in Figure 1(b).

zero-time lanes in Figure 2 as clear gaps in the pattern of DNase I products. Other TpA steps at positions 33 (CTAA), 60 (TTAC), and 68 (GTAA) do not produce footprints, as previously reported, though the precise DNase I cleavage pattern between positions 53 and 60 is modified in the presence of the ligand (at 20 °C), and may correspond to weaker interaction with the sequence GTAA. The last lane in each panel shows the cleavage pattern obtained when the radiolabelled *tyrT* DNA is mixed with unlabelled calf thymus DNA before adding the ligand. This pattern corresponds to the distribution of the ligand at equilibrium and is essentially the same as that seen in the control lanes. This confirms that, at equilibrium, insufficient [*N*-MeCys³,*N*-MeCys⁷]TANDEM is bound to *tyrT* DNA to produce a footprint. Minor differences between the tracks labelled 'con' and 'Eq' arise from variations in the extent of digestion; tracks 'con' and '0' do not contain any unlabelled DNA, in contrast with all the other lanes, and so require lower concentrations of DNase I. The remaining lanes show the time course of the dissociation, after adding the competitor DNA, and reveal time-dependent changes in the footprinting pattern, which eventually

becomes like that in the control. This reflects dissociation of the ligand from the labelled DNA.

Looking first at the data obtained at 20 °C, footprints (indicated by the black boxes) are evident around positions 110 and 90. The lower footprint, around position 90 (ATAT), is still evident 5 min after adding the unlabelled DNA; by 30 min the pattern in this region resembles that in the control. By contrast, bands in the footprint around position 110 (TTAT) begin to appear much earlier, and the pattern has returned to that in the control by 10 min. In addition, the modified cleavage pattern around position 60 is very similar to that in the control after 2 min, and has completely reverted to the control pattern by 5 min. It therefore appears that dissociation from ATAT is slower than from TTAT and that the weaker interaction with TTAC is faster still.

A similar effect can be seen at 37 °C, except that the pattern changes more quickly. Bands within the footprint at position 90 begin to reappear about 2 min after adding the competitor DNA, whereas the footprint at position 110 has already vanished by this time. Indeed, the latter footprint is less well defined at this higher temperature, and weak bands within the binding site are visible, even in the zero-time ('0') lane. The pattern around position 60 is not modified by the ligand at this higher temperature. It again appears that dissociation from ATAT is slower than from TTAT. At 55 °C dissociation from the upper site (TTAT) is complete by the first time point (0.5 min). The footprint at the lower site (ATAT) is still evident at 0.5 min, though the pattern has returned to that in the control by the 1 min time point. At each temperature we consistently observe that dissociation from TTAT is faster than ATAT.

Synthetic Inserts

Since the studies with *tyrT* DNA indicate that [*N*-MeCys³,*N*-MeCys⁷]TANDEM dissociates from each of its TpA-binding sites at different rates, we investigated the dissociation from fragments containing isolated TpA steps within synthetic DNA inserts. Figure 3 shows the dissociation of [*N*-MeCys³,*N*-MeCys⁷]TANDEM from fragments containing variations of the 'Dickerson dodecamer' (CGCGAATTCGCG), with the central sequences ATAT, TATA and TTAA. Previous footprinting studies have shown that they each contain TANDEM-binding sites and suggested that the binding preference was TATA > ATAT > TTAA. It can be seen that the footprint with ATAT persists for about 2–5 min after adding the unlabelled DNA. In contrast the footprint with TATA has disappeared by the first time point (30 s). Bands in the footprint with TTAA (which is less clear in the time 0 lane, as previously indicated [15]) reappear after 1–2 min. It therefore appears that the dissociation times decrease in order ATAT > TTAA > TATA. On lowering the temperature to 4 °C (not shown) the dissociation from TATA and TTAA is about 5 and 10 min respectively. Dissociation from all three of these fragments is faster than from similar sites within the *tyrT* sequence, confirming that the dissociation from each TpA site is influenced by the nature of the surrounding sequences. In each instance the slowest dissociation is from the tetranucleotide sequence ATAT, consistent with previous observations that the ligand binds exceptionally well to poly(dA-dT) [11]. We have therefore examined the dissociation from sequences containing longer stretches of (AT)_n.

The dissociation of [*N*-MeCys³,*N*-MeCys⁷]TANDEM from fragments containing the inserts (AT)₅GC(AT)₅ and (AT)₁₀CCCG(AT)₁₀ is presented in Figure 4. With (AT)₅GC(AT)₅ the ligand produces a clear footprint throughout the entire insert; a few bands around the central GpC are less

Figure 3 Dissociation of [*N*-MeCys³,*N*-MeCys⁷]TANDEM from fragments containing the inserts CGCGATATCGCG, CGCGTATACGCG and CGCGTTAACGCG

The time (in min) after adding the unlabelled DNA is indicated at the top of each lane. 'con' indicates digestion of the DNA alone in the absence of any ligand; '0' corresponds to digestion of a complex with 50 μ M [*N*-MeCys³,*N*-MeCys⁷]TANDEM before adding the unlabelled DNA. In the track labelled 'Eq' the labelled and unlabelled DNA were mixed before adding the ligand, and represents the true equilibrium distribution of the ligand in the dissociating mixture. The tracks labelled 'GA' are Maxam–Gilbert formic acid/piperidine markers specific for guanine and adenine. The square brackets indicate the position of the dodecamer insert; the black boxes show the position of central four AT base-pairs.

strongly protected. It can be seen that the dissociation is very slow, and that, by 30 min, it has not returned to that in the control. The pattern in the track labelled 'Eq' is very similar to that in the control, confirming that, at equilibrium, insufficient ligand is bound to the labelled DNA to generate a footprint. It may be significant that the various bands within this footprint do not reappear at the same rate. For example the band corresponding to the first ApT step above the central GC is evident only 5 min after adding the competitor DNA, whereas the next two ApT steps are barely visible even after 30 min. This is consistent with the suggestion that dissociation from the centre of an (AT)_n block is slower than towards the ends. A similar result is evident for the dissociation from (AT)₁₀CCCG(AT)₁₀, for which the footprint extends throughout the entire insert. This experiment was performed at 37 °C and reveals that, even at this elevated temperature, the pattern slowly returns to that in the control and is incomplete by the longest time point. It may be significant that bands towards the centre of the insert, around the CCCG, reappear earlier than towards the centre of each AT block. This is especially noticeable for the three clear bands above the central G (ApTpCpC) and the first ApT step below. These returned to the same intensity as the control by 5 min, whereas cleavage of the other ApT steps is still attenuated. Bands in the centre of the lower (AT)₁₀ tract are hardly visible, even at the longest time point.

DISCUSSION

Sequence selectivity

The results presented here show that the dissociation of [*N*-

Figure 4 Dissociation of [*N*-MeCys³,*N*-MeCys⁷]TANDEM from fragments containing the inserts (AT)₅GC(AT)₅ and (AT)₁₀CCCG(AT)₁₀

The time (in min) after adding the unlabelled DNA is indicated at the top of each lane. 'con' indicates digestion of the DNA alone in the absence of any ligand; '0' corresponds to digestion of a complex with 50 μ M [*N*-MeCys³,*N*-MeCys⁷]TANDEM before adding the unlabelled DNA. In the track labelled 'Eq' the labelled and unlabelled DNA were mixed before adding the ligand, and represents the true equilibrium distribution of the ligand in the dissociating mixture. The dissociation from (AT)₅GC(AT)₅ was performed at 20 °C, whereas that from (AT)₁₀CCCG(AT)₁₀ was performed at 37 °C. The track labelled 'G' is a Maxam–Gilbert dimethyl sulphate/piperidine marker specific for guanine. The square brackets indicate the position of the inserts. The central guanine in the sequence (AT)₅GC(AT)₅ is indicated by an arrow.

MeCys³,*N*-MeCys⁷]TANDEM from TpA sites in mixed sequence DNA fragments is slow and that the individual rate constants vary according to the nature of the flanking sequences. The slowest dissociation is observed with regions containing alternating A and T residues, an effect which is evident even at the tetranucleotide level, since ATAT yields a much slower dissociation than TTAA or TATA. Even though TATA consists of alternating purines and pyrimidines, each TpA step is located in the sequence GTAT (ATAC). The faster dissociation from this site, compared with ATAT, confirms the preference for flanking A and T bases.

The data for *tyrT* DNA show that dissociation from TTAT is faster than from ATAT. It therefore appears that flanking AT bases are not only required for tight binding, but their precise arrangement influences the kinetic parameters. This requirement is unusual, since the ligand does not make any hydrogen-bonded interactions with the surrounding bases, though there are van der Waals interactions between the sugar protons and the alanine and valine side chains. The sequence specificity of TANDEM itself is puzzling, since although the formation of internal hydrogen bonds between the alanine CO groups and valine NH

groups prevents interaction with the 2-amino group of guanine, this does not explain the pronounced selectivity for binding to TpA as opposed to CpG. Indeed the hydrogen bonds between the ligand and N-3 of adenine, proposed from n.m.r. studies of the complex with GATATC [17], should be equally feasible with N-3 of guanine. It appears that hydrogen-bonding patterns alone can not explain its unusual sequence binding preferences and that local DNA structure may play an important role. There is clearly something special about regions of alternating AT which generates unusually good TANDEM-binding sites.

Comparison with SDS sequestration

Although the present studies should be compared with results obtained using the technique of SDS sequestration [13], the results are not directly comparable, since we have used [*N*-MeCys³,*N*-MeCys⁷]TANDEM, whereas TANDEM was used in the previous work. Nonetheless the binding properties of these two ligands are very similar and, on the basis of the concentration required to generate clear DNase I footprints, the derivative bearing two *N*-methyl groups binds more tightly. Comparison of the dissociation times for echinomycin and quinomycin C or triostins A and C consistently show that the more hydrophobic compounds dissociate more slowly from DNA [20]. We therefore expected that the dissociation times for [*N*-MeCys³,*N*-MeCys⁷]TANDEM should be slower than TANDEM. SDS-sequestration experiments with TANDEM demonstrated that most of the dissociation from mixed sequence DNA was too fast to measure, though 14% dissociated with a time constant of 18 s [13]. This is faster than the dissociation from the two good footprinting sites in *tyrT* DNA, but this difference may be due to differences in the ligands. We presume that the greater portion of the dissociation from natural DNA, which is too fast to measure, corresponds to interaction at sites which do not yield footprints. It is known that DNAase I footprinting only detects the strongest binding sites and there are other examples of interactions which do not produce clear footprints [25].

In contrast, dissociation from poly(dA-dT) was completely described by a single exponential, though the time constant varied according to starting ratio of ligand to DNA, becoming slower at higher levels of saturation. This extrapolated to time constants of about 50 s at very low occupancy, compared with 600 s at saturation. The dissociation that we observe from blocks of (AT)_n (greater than 30 min) is comparable with the dissociation from poly(dA-dT) at high levels of occupancy, while the dissociation from isolated ATAT sites is more closely related to the dissociation rate measured at low levels of occupancy. It should be noted that the DNA concentration in the present experiments is vanishingly small (nanomolar), so that the interaction is limited by the binding constant, whereas the previous work used much higher DNA concentrations (500 μM) so that binding was limited by stoichiometry. TANDEM binds to poly(dA-dT) with an association constant of about $1 \times 10^7 \text{ M}^{-1}$ [11,13]; as a result, the (AT)_n sites should be fully saturated under the starting conditions in the footprinting experiments.

Another problem with SDS sequestration, and one which is obviated in the present experiments, is that the detergent makes a significant contribution to the ionic strength (2% SDS $\equiv$ 10.08). Both the binding strength and co-operativity in the interaction of TANDEM with poly(dA-dT) decrease at elevated ionic strength [13]. Although the present study, using footprinting methods to observe the dissociation, provides a less rigorous estimate of the rate constants, it has two clear advantages. Firstly, it does not

suffer from any artefacts that might be caused by addition of the solvent. Secondly it can be used to examine the dissociation from individual binding sites within mixed sequence DNAs which contain several binding sites within different sequence environments.

Co-operativity

The result with fragments containing blocks of (AT)_n suggest that [*N*-MeCys³,*N*-MeCys⁷]TANDEM dissociates more slowly from the centre of these regions than the edges. This cannot be explained on the basis of different tetranucleotide sequences, since every TpA step in (AT)₅GC(AT)₅ and (AT)₁₀CCCG(AT)₁₀ is in the context ATAT. In addition, dissociation from the isolated ATAT sites in the *tyrT* DNA fragment and the dodecamer insert is similar to that from the edges of the (AT)_n tracts. The stronger binding to the centre of (AT)_n tracts could have several explanations. First, co-operativity might arise from interactions between ligands bound to adjacent sites. Secondly, drug binding may perturb the DNA helix in such a way that the binding of further ligands is facilitated. Thirdly the structure of (AT)_n, which is known to be unusual [26], may vary along its length, even in the absence of added ligand, and the drug binding simply reflects and exaggerates these differences.

This work was supported by a grants from the Science and Engineering Research Council and The Royal Society. K. R. F. is a Lister Institute Research Fellow.

REFERENCES

- 1 Waring, M. J. and Fox, K. R. (1983) in *Molecular aspects of Anti-cancer Drug Action* (Neidle, S. and Waring, M. J., eds.), pp. 127–156, Macmillan, London
- 2 Wakelin, L. P. G. and Waring, M. J. (1974) *Nature (London)* **252**, 653–657
- 3 Wakelin, L. P. G. and Waring, M. J. (1976) *Biochem. J.* **157**, 721–740
- 4 Low, C. M. L., Drew, H. R. and Waring, M. J. (1984) *Nucleic Acids Res.* **12**, 4865–4879
- 5 Van Dyke, M. W. and Dervan, P. B. (1984) *Science* **225**, 1122–1127
- 6 Gao, X. and Patel, D. J. (1988) *Biochemistry* **27**, 1744–1751
- 7 Gilbert, D. A. and Feigon, J. (1991) *Biochemistry* **30**, 2483–2494
- 8 Wang, A. H.-J., Ughetto, G., Quigley, G. J. and Rich, A. (1986) *J. Biomol. Struct. Dyn.* **4**, 319–342
- 9 Ughetto, G., Wang, A. H. J., Quigley, G. J., van der Marel, G., van Boom, J. H. and Rich, A. (1985) *Nucleic Acids Res.* **13**, 2305–2323
- 10 Wang, A. H.-J., Ughetto, G., Quigley, G. J., Hakoshima, T., van der Marel, G. A., van Boom, J. H. and Rich, A. (1984) *Science* **225**, 1115–1121
- 11 Lee, J. S. and Waring, M. J. (1978) *Biochem. J.* **173**, 128–144
- 12 Low, C. M. L., Olsen, R. K. and Waring, M. J. (1984) *FEBS Lett.* **176**, 414–419
- 13 Low, C. M. L., Fox, K. R., Olsen, R. K. and Waring, M. J. (1986) *Nucleic Acids Res.* **14**, 2015–2033
- 14 Waterloh, K., Olsen, R. K. and Fox, K. R. (1992) *Biochemistry* **31**, 6246–6253
- 15 Lavesa, M., Olsen, R. K. and Fox, K. R. (1993) *Biochem. J.* **289**, 605–608
- 16 Address, K. J., Gilbert, D. E., Olsen, R. K. and Feigon, K. J. (1992) *Biochemistry* **31**, 339–350
- 17 Address, K. J., Sinsheimer, J. S. and Feigon, J. (1993) *Biochemistry* **32**, 2498–2508
- 18 Viswamitra, M. A., Kennard, O., Cruse, W. B. T., Egert, E., Sheldrick, G. M., Jones, P. G., Waring, M. J., Wakelin, M. J. and Olsen, R. K. (1981) *Nature (London)* **289**, 817–819
- 19 Fox, K. R., Wakelin, L. P. G. and Waring, M. J. (1981) *Biochemistry* **20**, 5768–5779.
- 20 Fox, K. R. and Waring, M. J. (1981) *Biochim. Biophys. Acta* **654**, 279–286
- 21 Fox, K. R., Olsen, R. K. and Waring, M. J. (1982) *Biochim. Biophys. Acta* **696**, 315–322
- 22 Fletcher, M. C. and Fox, K. R. (1993) *Nucleic Acids Res.* **21**, 1339–1344
- 23 Waterloh, K. and Fox, K. R. (1991) *J. Biol. Chem.* **266**, 6381–6388
- 24 Carpenter, M. L., Marks, J. N. and Fox, K. R. (1993) *Eur. J. Biochem.* **215**, 561–566
- 25 Fox, K. R., Marks, J. N. and Waterloh, K. (1991) *Nucleic Acids Res.* **19**, 6725–6730
- 26 Klug, A., Jack, A., Kennard, O., Shakked, Z. and Steitz, T. (1979) *J. Mol. Biol.* **131**, 669–680