Identification of lysine residues at the binding site of bacteriophage-Pf1 DNA-binding protein

Akira TSUGITA and G. Geoff KNEALE*

European Molecular Biology Laboratory D-6900 Heidelberg, Federal Republic of Germany

(Received 23 November 1984/23 January 1985; accepted 31 January 1985)

The accessibility of NH₂ groups in the DNA-binding protein of Pf1 bacteriophage has been investigated by differential chemical modification with the reagent ethyl acetimidate. The DNA-binding surface was mapped by identification of NH₂ groups protected from modification when the protein is bound to bacteriophage-Pf1 DNA in the native nucleoprotein complex and when bound to the synthetic oligonucleotide d(GCGTTGCG). The ability of the modified protein to bind to DNA was monitored by fluorescence spectroscopy. Modification of the NH₂ groups in the native nucleoprotein complex showed that seven out of the eight lysine residues present, and the *N*-terminus, were accessible to the reagent, and were not protected by DNA or by adjacent protein subunits. Modification of these residues did not inhibit the ability of the protein to bind DNA. Lysine-25 was identified by peptide mapping as being the major protected residue. Modification of this residue does abolish DNA-binding activity. Chemical modification of the accessible NH₂ groups in the complex formed with the octanucleotide effectively abolishes binding to DNA. Peptide mapping established that, in this case, lysine-17 was the major protected residue. The differences observed in protection from acetimidation, and in the ability of the modified protein to bind DNA, indicate that the oligonucleotide mode of binding is not identical with that found in the native nucleoprotein complex with bacteriophage-Pf1 DNA.

The Pf1 DNA-binding protein is one of the major proteins synthesized in bacterial cells after infection with bacteriophage Pf1 (Kneale & Marvin, 1982). The role of this protein in phage replication and assembly is thought to be similar to that of the gene 5 product of bacteriophage fd, namely the conversion of replicative-form (double-stranded) DNA into viral (single-stranded) DNA, and the prepackaging of the circular viral DNA into an elongated form before its incorporation into the virion (for a review, see Denhardt *et al.*, 1978). The intracellular complex between the Pf1 DNA-binding protein and viral DNA appears as a flexible helical structure under the electron microscope (Gray *et al.*, 1982). X-ray fibre diffraction of the complex shows that each turn of the helix consists of six protein dimers in a pitch of 4.5 nm (45 Å), and each subunit of the dimer covers four

nucleotides of the two opposing DNA strands (Kneale *et al.*, 1982).

The Pf1 DNA-binding protein consists of a single polypeptide chain of *M_r* 15400; its amino acid sequence has recently been determined and shows no sequence homology with the fd gene 5 protein, in spite of many functional similarities (Maeda *et al.*, 1982). It is found in free solution as a dimer, but in the presence of octanucleotides it aggregates to form tetramers (Kneale, 1983). Binding of the protein to DNA is accompanied by a pronounced shift from 340 to 330 nm in the fluorescence emission spectrum (arising from the single tryptophan residue), and this property can be used to monitor DNA-binding activity (G. G. Kneale & R. W. Wijnaendts; unpublished work).

In order to map the binding site of the Pf1 DNA-binding protein, we have performed chemical modification with ethyl acetimidate, a reagent that is specific for α - and ϵ -NH₂ groups (reviewed by Inman *et al.*, 1983). This reagent can be used under mild conditions, and does not affect the

* To whom correspondence and reprint requests should be sent at the following present address: University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

ionic character of the protein. The approach we have used consists of modification of accessible NH_2 groups in the nucleoprotein complex, followed by removal of the DNA. Modification of those residues previously protected by DNA was then performed with ^{14}C -labelled reagent, allowing identification of lysine residues at the binding site by peptide mapping. The DNA-binding activity of the protein was monitored at both stages. The same method was also used to study the protein-oligonucleotide interaction, with the unexpected result that the protected lysine residues in the two types of complex were not identical.

Materials and methods

Preparation of the Pfl nucleoprotein complex

Bacteriophage-Pfl-infected cell extracts of *Pseudomonas aeruginosa* were prepared and depleted of free DNA (Kneale & Marvin, 1982). The nucleoprotein complex was purified by sucrose-gradient centrifugation and precipitation with poly(ethylene glycol) (Kneale, 1983). The purity of the complex was established by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, agarose-gel electrophoresis and u.v.-absorption spectroscopy.

Preparation of the Pfl DNA-binding protein

The Pfl DNA-binding protein was isolated from crude extracts of the Pfl nucleoprotein complex (Kneale, 1983). Briefly, the partially purified complex was dissociated in 1 M-MgCl₂ and the viral DNA removed by high-speed centrifugation. Purification of the protein to homogeneity was obtained by chromatofocusing on Pharmacia PBE 74. The protein peak eluted at pH 5.9 was pooled, precipitated with $(\text{NH}_4)_2\text{SO}_4$ and dialysed against 50 mM-NaHCO₃.

Labelling the Pfl nucleoprotein complex

Acetimidation of accessible NH_2 groups in the complex was accomplished by the addition of 10 mg of ethyl acetimidate to 1.5 mg of the Pfl nucleoprotein complex in 50 mM-NaHCO₃ buffer, pH 8.4. The reaction was allowed to proceed for 2 h at 20°C, then quenched by the addition of NH_4HCO_3 . After dialysis against 50 mM-NaHCO₃, pH 8.4, the viral DNA was removed by centrifugation at 200 000 g for 2 h in the presence of 1 M-MgCl₂, and the supernatant dialysed back into 50 mM-NaHCO₃, pH 8.4. Remaining NH_2 groups in the protein were then labelled by the addition of 0.8 mg of [^{14}C]ethyl acetimidate (370 μCi ; Amersham International) for 1 h at room temperature, followed by reaction with excess unlabelled reagent (10 mg, Sigma) for 1 h. The sample was dialysed against 50 mM-NaHCO₃, pH 8.4, then

against 100 mM-pyridine acetate buffer, pH 7.0, before chymotryptic digestion.

The experiment was also performed in the reverse order, i.e. the radioactive reagent was added to the complex and the unlabelled reagent added after removal of DNA.

Labelling the octanucleotide-protein complex

A 210 μg portion of the deoxyoligonucleotide d(GCGTTGCG) was added to 1.5 mg of the Pfl DNA-binding protein (approximately twice the stoichiometric binding ratio) in 50 mM-NaHCO₃. At this protein concentration (6 μM -dimer) the protein is essentially fully bound to the octanucleotide (G. G. Kneale & R. W. Wijnaendts, unpublished work). A 10 mg portion of unlabelled ethyl acetimidate was then added and the reaction allowed to continue at 20°C for 2 h. The protein was precipitated with an equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$ and dialysed against 50 mM-NaHCO₃, pH 8.4. U.v. spectroscopy was used to check that the oligonucleotides had been removed by this procedure. The protein was then allowed to react with the ^{14}C -labelled reagent as described above.

Peptide mapping

Chymotrypsin digestion was used since this has been found to give a reasonable cleavage pattern (Maeda *et al.*, 1982). The imidated protein was incubated with 4% (w/w) chymotrypsin in 0.1 M-pyridine/collidine/acetate buffer, pH 8.2, for 16 h at 37°C. The digest was chromatographed on a strong cationic resin, SP- (sulphopropyl)-Sephadex C-25, using pyridine/acetate buffers (the experimental conditions are noted in the legend to Fig. 1) as described earlier (Maeda *et al.*, 1982). Radioactivity and amino acids after hydrolysis of the eluents were analysed, and radioactive peaks were further purified by gel filtration on a Bio-Gel P4 column (0.9 cm $\times$ 100 cm) with 70% (v/v) formic acid and SP-Sephadex chromatography with a linear pyridine/acetate buffer gradient [0.25% (pH 3.9)/0.75% (pH 4.25)]. The radioactive peak was characterized by amino acid composition (Tsugita & Scheffler, 1982), manual Edman degradation to determine the *N*-terminal residues (Maeda *et al.*, 1982), as well as carboxypeptidase A (or A plus B) digestion, to determine the C-terminal sequences (Tsugita *et al.*, 1979).

DNA-binding measurements

The ability of the modified protein to bind to single-stranded DNA was monitored by fluorescence spectroscopy. We have previously used fluorescence spectroscopy to determine the equilibrium-binding parameters of the native Pfl DNA-binding protein (G. G. Kneale & R. W. Wijnaendts, unpublished work). That study re-

vealed that binding to polynucleotides is essentially independent of DNA sequence, and that titration with poly(dT) is a convenient assay. Fluorescence-emission spectra of the protein (5 μ M-dimer concn.) before and after equilibrium with poly(dT) (60 μ M-nucleotide concn.) were reported as described elsewhere (Kneale, 1983). Co-operative binding of the native protein results in a shift in the emission spectrum from 340 to 330 nm, and the peak shape can be analysed to give the fraction (Θ) of protein bound to DNA. Apparent binding constants were determined by the method of Kelly *et al.* (1976), with the equation:

$$K_{app.} = \frac{\Theta}{(1 - \Theta) \cdot (N_0 - \Theta P_0)}$$

Where N_0 and P_0 are the total concentrations of nucleotide-binding sites and of protein dimers respectively. The binding-site size was taken as 4 nucleotides per dimer.

Results

Chemical modification of the Pf1 nucleoprotein complex

In order to determine which, if any, of the lysine residues are protected from acetimidation when the Pf1 DNA-binding protein is bound to viral DNA, we performed the following experiment. The native Pf1 nucleoprotein complex was incubated with the reagent ethyl acetimidate to block all accessible NH_2 groups (see the Materials and methods section). The complex was then dissociated in 1 M-MgCl₂ and the DNA removed. The modified protein was tested for its ability to bind DNA by monitoring the shift in the fluorescence emission spectrum on binding poly(dT). The results showed that the protein was essentially fully bound to the polynucleotide. NH_2 groups that had been protected in the nucleoprotein complex were then labelled with [1-¹⁴C]ethyl acetimidate. The derivative had a stoichiometry of 0.79 mol of ¹⁴C/mol of protein. The labelled protein was again tested for DNA-binding activity. The results shown in Table 1 show that less than 5% of the protein was bound [corresponding to a decrease in the apparent binding constant ($K_{app.}$) of at least a factor of 10³].

The ¹⁴C-labelled protein was then digested with α -chymotrypsin and the peptides separated by cation-exchange chromatography. The resulting elution profile revealed the presence of essentially a single narrow peak of radioactivity (Fig. 1b). The peak fractions were pooled and further purification on Bio-Gel P4 indicated that the labelled peptide was ²²Thr-Ser-Thr-Lys-Thr-Gly-Glu-Ile-Tyr³⁰ (stoichiometry 0.73 mol of ¹⁴C/mol of pep-

Table 1. DNA-binding parameters of the chemically modified Pf1 DNA-binding protein

Apparent binding constants ($K_{app.}$) were estimated from the fraction of protein bound (Θ), which was determined from fluorescence spectra as described in the Materials and methods section.

Lysine residues not modified	Θ	$K_{app.}$ (M ⁻¹)
All (native protein)	0.98	5×10^6
Lys-25*	0.95	2×10^6
Lys-17†	<0.06	$<4 \times 10^3$
None (fully modified)	<0.03	$<2 \times 10^3$

* Protein isolated after modification in the complex with Pf1 DNA.

† Protein isolated after modification in the complex with d(GCGTTGCG).

tide), containing the single lysine residue, Lys²⁵ (Table 2).

As a control, the reverse experiment was also performed: the accessible lysine residues in the nucleoprotein complex were ¹⁴C-labelled, followed by removal of the DNA and incubation with the unlabelled reagent. In this case the derivative had a stoichiometry of 6.7 mol of ¹⁴C/mol of protein. Separation of peptides by cation-exchange chromatography gave the elution pattern in Fig. 1(a). Further purification of these fractions showed that all NH_2 groups, with the exception of that of Lys²⁵, had been extensively labelled (Table 2).

This confirmed that the N-terminal and the seven remaining lysine residues are indeed accessible in the nucleoprotein complex and that Lys²⁵ is essentially protected (Fig. 1a and Table 2).

Modification of the protein bound to d(GCGTTGCG)

The Pf1 DNA-binding protein binds to single-stranded DNA regardless of sequence. We have chosen to use the octanucleotide d(GCGTTGCG), since its binding to the protein has previously been characterized (Kneale, 1983). In order to determine if the lysine residues involved in DNA binding were identical with those involved in binding the octanucleotide, a similar experiment to that described above was performed. The experiment was also designed to reveal any additional protection arising from protein-protein interactions in the native nucleoprotein complex, as these are substantially decreased in the complex with octanucleotide.

The Pf1 DNA-binding protein was incubated with the octanucleotide d(GCGTTGCG) under conditions where protein is fully bound. Accessible NH_2 groups were then modified with ethyl acetimidate. After disruption of the complex and removal of the octanucleotide, the protein was

Table 2. Characterization of the labelled peptides

(a), (b), and (c) refer to corresponding experiments in Fig. 1. Peptides I and II in expts. (b) and (c) are obtained from the peaks noted in Figs. 1(b) and 1(c) after further purification. Chymotryptic peptides: C113-144, for example, indicates a peptide containing residues 113-144 of the complete sequence. Amino acid composition was analysed with an amino acid analyser (Durrum D500) after hydrolysis with HCl and trifluoroacetic acid (Tsugita & Scheffler, 1982). The values are ratios of amino acids observed, normalized to the theoretical value for a given amino acid (usually leucine, alanine or glutamic acid), and the values in parentheses are theoretical numbers of amino acids in the peptides (N.D. = not determined). *N*-Termini were determined by manual Edman degradation (Maeda *et al.*, 1982). C-Terminal sequences were determined by carboxypeptidase digestion (Tsugita *et al.*, 1979). Stoichiometry is expressed as mol of ^{14}C /mol of peptide. Peptide concentrations were determined by amino acid analysis after hydrolysis. The amino acid sequence is shown below the Table with lysine residues (K) highlighted by boxing. Observed chymotryptic digestion points are arrowed.

Amino acid composition															
Expt. in Fig. 1...				(a)					(b)					(c)	
Amino acid	Pep- tide...	CI-14	C15-21	C22-30	C35-55	C68-76	C97-112	C113-144	C121-144	C15-21	C22-30	I	C15-21	I	II
Asp		1.0 (1)	1.0 (1)		1.0 (1)	0.3	1.1 (1)	3.0 (3)	3.0 (3)	1.0 (1)	0.2	1.0 (1)	0.4	1.0 (1)	3.0 (3)
Thr		1.1 (1)	2.7 (3)	2.9 (3)			0.9 (1)			2.6 (3)	2.8 (3)	3.2 (3)	3.1 (3)		
Ser		0.3	0.3	0.8 (1)	2.3 (2)		0.2	1.2 (1)	1.3 (1)	0.6	0.7 (1)	0.3	0.9 (1)	1.5 (1)	
Glu		2.2 (2)		1.0 (1)	3.1 (3)	2.0 (2)	0.9 (1)	5.0 (5)	6.4 (6)	0.3	1.0 (1)	0.2	0.9 (1)	4.8 (5)	
Pro					2.1 (2)	0.8 (1)	2.0 (2)	3.0 (3)	4.3 (4)					2.0 (2)	
Gly		2.0 (2)	0.2	1.3 (1)	3.4 (3)	2.4 (2)	2.5 (2)	1.3 (1)	1.3 (1)	0.4	1.2 (1)	0.2	1.3 (1)	1.4 (1)	
Ala		0.9 (1)	0.3		3.0 (3)	0.2	2.9 (3)	8.2 (8)	5.0 (5)	0.1		0.2	0.4	8.0 (8)	
Cys						0.9 (1)									
Val		0.9 (1)	0.9 (1)			0.8 (1)	1.6 (2)			0.9 (1)		1.0 (1)	0.1		
Met		1.2 (2)					0.7 (1)								
Ile				0.7 (1)	2.0 (3)	0.2		0.2			0.6 (1)		0.5 (1)		
Leu		1.0 (1)			0.2	1.0 (1)	2.0 (2)	0.3				0.1			
Tyr			0.8 (1)	0.7 (1)	0.6 (1)					0.7 (1)	0.6 (1)	0.9 (1)	0.8 (1)	0.2	
Phe		0.7 (1)			0.7 (1)	1.0 (1)									
His															
Lys		0.2 (0)	1.2 (1)	0.5 (1)	0.7 (1)	0.5 (1)	0.5 (1)	1.8 (3)	2.0 (3)	1.2 (1)	0.5 (1)	0.6 (1)	1.0 (1)	2.6 (3)	
Arg					1.1 (1)	0.1		1.9 (2)	0.7 (1)					2.0 (2)	
Trp		N.D. (14)													
Stoichiometry ...	0.92	0.78	0.08	0.70	0.70	0.68	0.81	2.79	2.07	0.03	0.73	0.62	0.06	0.14	
N-terminus ...	Met	Val	—	—	—	Glu	Val	Ala	Ala	—	Thr	Val	—	—	
C-terminus ...	Leu-Trp	Thr-Tyr	Ile-Tyr	Glu-Tyr	Glu-Tyr	Phe	Glu-Ala	Lys-Ala	Ala-Lys-Ala	Thr-Tyr	Glu-Ile-Tyr	Thr-Tyr	Ile-Tyr	Lys-Ala	
Labelled															
amino acid ...	Met ¹	Lys ¹⁷	Lys ²⁵	Lys ⁴⁸	Lys ⁷⁵	Lys ¹⁰⁵	Lys ¹³⁵	Lys ¹³⁵	Lys ¹³⁵	Lys ¹⁷	Lys ²⁵	Lys ¹⁷	Lys ²⁵	Lys ¹³⁵	
							Lys ¹³⁸	Lys ¹³⁸	Lys ¹³⁸					Lys ¹³⁸	
							Lys ¹⁴³	Lys ¹⁴³	Lys ¹⁴³					Lys ¹⁴³	
↓ ↓

Fig. 1. Chromatography of chymotryptic peptides of the modified DNA-binding protein of phage Pf1 on SP-Sephadex C-25

The digest of 100 nmol of the modified protein was applied to a column (0.6 cm $\times$ 8 cm) and eluted with a gradient of pyridine/formate/acetate buffer. The gradient was made with 20 ml of each buffer as follows: chamber 1, 0.1% pyridine/formate (pH 2.6); chamber 2, 0.1% pyridine/acetate (pH 3.8); chamber 3, 0.25% (pH 3.9); chamber 4, 0.75% (pH 4.25); chamber 5, 1.5% (pH 4.5); chamber 6, 2.5% (pH 4.7); chamber 7, 5.0% (pH 4.9); chamber 8, 7.5% (pH 5.0); chamber 9, 20% (pH 5.18) (chambers 2-9 contain pyridine/acetate). The elution was carried out at 37°C and 2 ml fractions were collected. Further elutions were carried out with a second gradient of 20% pyridine/acetate (pH 5.2) to 30% (pH 6.0) at 50°C. The profile was obtained by counting the radioactivity of 50 μ l of eluate from every second fraction.

tested for DNA-binding activity. In this case, binding was almost completely abolished under standard experimental conditions (Table 1). Further reaction of the sample with [14 C]ethyl acetimidate was then performed. The stoichiometry of the derivative was 0.68 mol of 14 C/mol of protein. Table 2 gives the results of peptide mapping of the labelled protein, indicating the major labelled peptide to be 15 Val-Thr-Lys-Thr-Asp-Thr-Tyr 21 (stoichiometry 0.62 mol of 14 C/mol of peptide), i.e. not the peptide labelled in the previous experiment, but the adjacent peptide. The second largest peak arises from the C-terminal fragment, 113-144-peptide, which contains three lysine residues. Thus the stoichiometry per lysine residue here is less than 8% of that of the major peptide (Table 2).

Discussion

We have demonstrated that, of the nine NH_2 groups in the Pf1 DNA-binding protein, only one is appreciably protected from acetimidation when the protein is bound to Pf1 viral DNA (in the native nucleoprotein complex) or to the synthetic oligonucleotide d(GCGTTGCG). However, the residue protected is different in the two types of complex; Lys 25 is protected in the native complex, but Lys 17 is protected when the protein is bound to the octanucleotide. A further important difference is the observation that acetimidation of the seven accessible lysine residues and the N-terminus in the native complex has little or no effect on the ability of the protein to bind DNA (as judged by fluorescence spectroscopy), whereas acetimidation of the seven accessible NH_2 groups in the octanucleotide complex effectively abolishes DNA binding. In the latter case the accessible lysine residues, according to our results, would include Lys 25 , and modification of this residue might be expected to inhibit binding to polynucleotides. It should be noted that the binding experiments alone could be explained in another way, namely that modification of surface NH_2 groups in the protein-octanucleotide complex affect the ability of the protein subunits to bind adjacent to one another, thereby decreasing the co-operative component of the binding (as opposed to the intrinsic

(a) The DNA-protein complex was modified with radioactive ethyl acetimidate. After removal of DNA, the protein was further modified with non-radioactive reagent. (b) The DNA-protein complex was modified with non-radioactive reagent and after removal of DNA, the protein was modified with radioactive reagent. (c) The protein-octanucleotide complex was modified with non-radioactive reagent and after removal of nucleotide, the protein was modified radioactively.

binding constant). However, in this case one would expect the peptide-mapping experiment to reveal additional labelled lysine residues, corresponding to those initially protected by adjacent protein subunits (in the complex with viral DNA), and this is not observed.

The results imply two modes of binding: the polynucleotide mode and the oligonucleotide mode. Two such binding modes were recently suggested for the gene 5 protein of fd bacteriophage (Alma *et al.*, 1982, 1983) and for the gene 32 protein of T4 bacteriophage (Kowalczykowski *et al.*, 1981), both on the basis of spectroscopic binding studies. The two modes could be characterized by a decreased salt-dependence of binding to oligonucleotides, as well as differences in stoichiometry of binding. Alma *et al.* (1982, 1983) have pointed out an important difference between the oligonucleotide-binding modes of the two proteins; only one gene 32 protein subunit but at least five fd gene 5 protein subunits can bind in the oligonucleotide mode. Since our experiments involved binding to an octanucleotide, which binds four subunits of the Pfl DNA-binding protein (i.e. two dimers), this phenomenon resembles that of the fd gene 5 protein. However, our results are based on a comparison of chemical-modification data which reveal directly certain residues protected in each of the two binding modes. They cannot be compared in detail with the other single-stranded-DNA-binding proteins referred to above, for which no comparable data exist.

In the present study there are a number of interesting features in the vicinity of the two lysine residues implicated in the alternative binding modes. The two labelled peptides are adjacent to each other in the sequence and both have a high threonine content. In fact the sequence from residue 16 to residue 26 is very unusual in that every alternate residue is threonine (Fig. 2). A possible function for this might be to provide a hydrogen-bonding network for interaction with the sugar-phosphate backbone of the DNA. A tyrosine residue is centrally placed in the sequence, four residues removed from both Lys¹⁷ and Lys²⁵. Thus, apart from the replacement of serine by aspartate, there is a symmetrical arrangement of

Fig. 2. Sequence of the Pfl DNA binding in the vicinity of the labelled lysine residues, showing the possible modes of binding

amino acid side chains centred at Tyr²¹. From this it is tempting to speculate that, in the oligonucleotide mode of binding, the sugar-phosphate backbone may be running in an opposite (antiparallel) sense to that in the polynucleotide mode. Possibly the latter interaction is governed by the structural constraints imposed on binding the polynucleotide chain into a helical structure.

It is well known that tyrosine residues in model peptides can bind to single-stranded DNA through stacking interactions and that the binding is greatly enhanced by electrostatic interactions with nearby lysine side chains (Helene & Lancelot, 1982). Tyrosine residues have also been implicated in the interaction of the fd gene 5 protein with DNA (Day, 1973). In view of the lysine-modification experiments and the amino acid sequence in the vicinity of lysine residues involved, we predict that Tyr²¹ will be involved in the binding of both oligo- and poly-nucleotides to the Pfl DNA-binding protein, together with one of the two lysine residues, Lys¹⁷ or Lys²⁵, depending on the mode of binding.

The chemical protection data in Table 2 also show the high accessibility of the amino groups not involved in binding. All eight lysine residues, as well as the *N*-terminal methionine residue, must be situated at the surface of the native nucleoprotein complex. This may be contrasted with results obtained for the fd virion, in which only one of the six amino groups in the coat protein could readily be modified (Armstrong, *et al.*, 1983). This provides evidence for an open subunit structure of the nucleoprotein precursor complex, compared with the close packed arrangement of subunits found in the virion.

We thank Mr. F. Vilbois for excellent technical assistance.

References

- Alma, N. C. M., Harmsen, B. J. M., van Boom, J. H., van der Marel, G. & Hilbers, C. W. (1982) *Eur. J. Biochem.* **122**, 319–326
- Alma, N. C. M., Harmsen, B. J. M., de Jong, E. A. M., Ven, J. V. D. & Hilbers, C. W. (1983) *J. Mol. Biol.* **163**, 47–62
- Armstrong, J., Hewitt, J. A. & Perham, R. N. (1983) *EMBO J.* **2**, 1641–1646
- Day, L. A. (1973) *Biochemistry* **12**, 5329–5339
- Denhardt, D. T., Dressler, D. & Ray, D. S. (eds.) (1978) *The Single-Stranded DNA Phages*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Gray, C. W., Kneale, G. G., Leonard, K. R., Siegrist, H. & Marvin, D. A. (1982) *Virology* **116**, 40–52
- Helene, C. & Lancelot, G. (1982) *Prog. Biophys. Mol. Biol.* **39**, 1–68
- Inman, J. K., Perham, R. N., Du Bois, G. C. & Appella, E. (1983) *Methods Enzymol.* **91**, 559–569

- Kelly, R. C., Jensen, D. E. & von Hippel, P. D. (1976) *J. Biol. Chem.* **251**, 7240–7250
- Kneale, G. G. (1983) *Biochim. Biophys. Acta* **739**, 216–224
- Kneale, G. G. & Marvin, D. A. (1982) *Virology* **116**, 53–60
- Kneale, G. G., Freeman, R. & Marvin, D. A. (1982) *J. Mol. Biol.* **156**, 279–292
- Kowalczykowski, S. C., Lonberg, N., Newport, J. W. & von Hippel, P. H. (1981) *J. Mol. Biol.* **145**, 75–104
- Maeda, K., Kneale, G. G., Tsugita, A., Short, N. J., Perham, R. N., Hill, D. F. & Peterson, G. B. (1982) *EMBO J.* **1**, 255–261
- Tsugita, A. & Scheffler, J. J. (1982) *Eur. J. Biochem.* **124**, 585–588
- Tsugita, A., Gregore, I., Kubota, I. & van den Broek, R. (1979) in *Cytochrome Oxidase*, (King, T. E., Oriei, Y., Chance, B. & Okunuki, K., eds.), pp. 67–77, Elsevier/North-Holland Biomedical Press, Amsterdam