Detection of non-homology-containing heteroduplex molecules

Michael J.Lichten and Maurice S.Fox

Massachusetts Institute of Technology, Department of Biology, 77 Massachusetts Avenue,
Cambridge, MA 02139, USA

Received 7 April 1983; Accepted 9 May 1983

ABSTRACT

Heteroduplex DNA molecules which contain both the wild-type and mutant sequences of a deletion nonhomology possess a characteristic electrophoretic mobility in agarose and can be readily separated from both the wild-type and deletion-containing parental homoduplex fragments. Because of the partial single stranded character of these deletion-containing heteroduplex molecules, they are selectively bound to nitrocellulose filters, and once bound, can be selectively detected by hybridization with radioactively labeled single-stranded DNA which is homologous to the sequences absent in the deletion mutation.

INTRODUCTION

Genetic evidence has been reported which suggests that, in a variety of organisms, both insertion and deletion mutations of substantial dimension are included in regions of heteroduplex DNA formed during the process of genetic recombination (1-8). This evidence, although suggestive, is indirect in nature, and a direct demonstration that nonhomology-containing heteroduplex structures are formed during recombination is still forthcoming.

In this paper, we describe the development of a methodology which allows detection of nonhomology-containing heteroduplex DNA molecules. When the nonhomology results from the presence of a deletion mutation in one of the strands, the molecules can be separated from both wild-type and deletion-containing homoduplex molecules by electrophoresis in agarose, and can be sensitively and quantitatively detected by a simple modification of the nitrocellulose-blotting technique of Southern (9). The application of this technique to the detection of deletion-containing heteroduplex molecules in vegetative DNA isolated from bacterio-

Table 1. Bacteriophage strains

<u>Strain</u>	<u>Genotype</u>
λ c60	<u>cI60</u>
λ gt7	<u>cIΔHindIII</u> <u>nin5</u> <u>b522</u> <u>ara6</u>
λ 31	<u>cI857</u> <u>Pam80</u> <u>plac5</u> <u>lacZΔW4680</u>
λ 44	<u>cI857</u> <u>Pam80</u> <u>plac5</u>
λ 333	<u>cIΔHindIII</u> <u>Pam80</u> <u>Sam7</u> <u>b522</u> <u>ara6</u>
λ 334	<u>cI857</u> <u>Pam80</u> <u>Sam7</u> <u>b522</u> <u>ara6</u>

Phage strains λ gt7 and λ 333 contain an unnamed 0.69 kB deletion, referred to in this paper as cI Δ HindIII, which deletes λ sequences between the Hind III sites at .760 and .774 on the λ map.

phage λ crosses will be described in a later publication (Lichten and Fox, in preparation).

MATERIALS AND METHODS

Bacteriophage strains

The bacteriophage λ strains which were used as a source of DNA for heteroduplex material are listed in Table 1. A physical map of λ , showing the positions of the relevant deletions and restriction sites, is shown in Figure 1.

Preparation of bacteriophage DNA

Phage stocks were grown in MJL266, a proC::Tn5 lac Δ U169 derivative of BNN45 (12) using the NZC broth method (13). Phage lysates were concentrated 50-fold by polyethylene glycol (PEG) precipitation (14) followed by resuspension of the PEG precipitate in SM buffer (15). Undissolved precipitate was removed by centrifugation at 10,000 rpm for 20 min. in a Sorvall SS34 rotor.

If the phage were to serve as a source of DNA, they were purified on CsCl step gradients as follows. Concentrated phage lysates were layered over a step gradient composed of three layers of CsCl dissolved in SM buffer to a density of 1.7, 1.5 and 1.3 respectively, and centrifuged at 24,000 rpm for 90 min. in a Beckman SW27 rotor. The phage were removed by side puncture, mixed with an equal volume of saturated CsCl, and layered under two CsCl layers of densities of 1.6 and 1.4 respectively, and

Figure 1. Physical map of bacteriophage λ .

The deletions used in this study are indicated by open rectangles. LacZ sequences contained in λ lac5 are indicated by crosshatched areas. Sequences contained in the 0.56 kb HindIII-HindIII restriction fragment which was used to make radioactive probe are indicated by a solid rectangle. Restriction enzyme recognition sites are as follows: ●--Bgl I, ○--Bgl II, □--Pvu I. Only restriction enzyme recognition sites relevant to this study are indicated. Positions of the Bgl I sites in lacZ were determined by D. Moir (personal communication). The position and extent of lacZΔW4680 is from Malamy et. al. (10). Positions of all other deletions and restriction sites are from Davis et. al. (11).

centrifuged as before. The phage band was removed by side puncture, mixed with CsCl in SM buffer to a final density of 1.5, and centrifuged at 35,000 rpm for 24 hrs. in a Beckman SW50.1 rotor. The phage were removed by side puncture and dialysed against SM buffer without gelatin. DNA was isolated from these phage by extraction with an equal volume of phenol (equilibrated with 50 mM TRIS-HCl, 250 mM NaCl, 1 mM EDTA, pH 8.5), followed by ethanol precipitation and resuspension in TE buffer (10 mM TRIS-HCl, 1 mM EDTA, pH 8.0).

Artificial heteroduplex DNA molecules

Phage DNA heteroduplexes were prepared by a slight modification of the procedure described by Davis et. al. (11). Equal amounts of both parental phage DNAs were mixed at a final concentration of 30 to 50 μ gm/ml. NaOH was added to a final concentration of 0.1 M, and the mixture was incubated at room temperature for 30 min. TRIS-HCl (pH 7.2) was added to a final concentration of 0.2 M, formamide was added to a final concentration of 50%

(vol./vol.), and the mixture was incubated for 3 hrs. at 26°-30°C. The heteroduplex mixture was then ethanol precipitated, and resuspended in TE buffer at a final concentration of 300-500 µgm/ml. Sham heteroduplexes were prepared by the same procedure, but the NaOH and TRIS-HCl were added simultaneously.

Restriction enzyme digestion

All restriction enzymes were obtained from New England Biolabs, and were used according to the supplier's recommendations, though at a 2 to 4-fold higher concentration. Restriction enzyme digestions were terminated prior to electrophoresis by adding a 1/5 volume of Ficol-EDTA sample buffer (10% Ficol (w./vol), 100 mM Na₃EDTA (pH 8.5) and 0.25% bromphenol blue). Most digestion mixtures were subsequently incubated at 65°C. for 5 min. and quenched in ice water prior to electrophoresis. Restriction enzyme digestions for preparation of DNA for end labeling were terminated by phenol extraction.

Agarose gel electrophoresis

Samples were subjected to electrophoresis in horizontal agarose gels at a voltage gradient of 1.25 volts/cm. For most purposes, TRIS-borate-EDTA buffer (11) was used. For gels used to purify restriction fragments, TRIS-acetate-EDTA buffer (11) was used.

Transfer of DNA to nitrocellulose

DNA was transferred from agarose gels to nitrocellulose (Millipore or Schleicher and Schuell) by two different methods. The method referred to as "denaturing transfer" in the text is the method described by Southern (9) with modifications described by Davis et. al (11). In the method referred to as "native transfer" in the text, gels were soaked for 1 hr. in 1.5 M NaCl, 0.5 M TRIS-HCl (pH 7.4) and then transferred directly to nitrocellulose.

Preparation of radioactive probe

Plasmid pKB280 (16) was used as a source of the cI HindIII fragment. Purified plasmid DNA was digested with Hind III and displayed on an agarose gel. The band corresponding to the 564 base pair cI HindIII fragment was cut out of the gel and purified by the glass powder method (17). Radioactive probes for hybridization to DNA bound to nitrocellulose filters were prepared by a

modification of the nick-translation method of Rigby et. al. (18). 0.25 μ gm of DNA in 10 μ l of NT buffer (50 mM TRIS-HCl, 5 mM MgCl₂, 10 mM dithiothreitol, pH 8.0) was prenicked at 12°C. with sufficient DNase I to yield about 1 nick per duplex molecule (precalibrated DNase I was a gift of A. Wyman). The nicking reaction was terminated by incubation at 65°C for 5 min. 50 μ Ci of α^{32} P dCTP (Amersham Searle, 3000 Ci/mmol) was added, as were cold dGTP, dATP, and dTTP to a final concentration of 30 μ M. 10 units of DNA polymerase I (New England Biolabs) were added, and the mixture incubated at 12°C for 90 min. The reaction was terminated by ethanol precipitation.

End labeled lambda DNA Bgl II fragments were prepared in a similar manner, but the prenickling step was omitted, 10 μ Ci α^{32} P dGTP was used in place of α^{32} P dCTP, the large subtilisin fragment of DNA polymerase I (New England Biolabs) was substituted for DNA polymerase I, and the reaction incubated for 30 min. at room temperature.

Hybridization conditions

DNA fragments immobilized on nitrocellulose filters were hybridized with radioactive probe in 6xSPE (1xSPE = 0.18 M NaCl, 0.001 M EDTA, 0.01 M sodium phosphate pH7.0), 0.5% (w/vol) SDS, 15% (vol/vol) formamide, 100 μ gm/ml heat denatured sonicated chicken blood DNA (Sigma Chemical Co.). Hybridization reactions contained 4 ml of the above buffer for every 100 cm² of filter and 3-30 μ Ci of heat denatured radioactive probe in a heat-sealed plastic bag. Reaction mixtures were incubated with agitation at 55°C for 12-18 hrs. Filters were then rinsed in 2xSPE, washed in 500-1000 ml. 2xSPE, 0.5% (w/vol) SDS for 5-12 hrs at 55°C, rinsed in 2xSPE, and air dried.

Autoradiography

Radioactive filters were exposed to preflashed Kodak AR X-ray film (19) at -70°C, using Dupont Cronex Lightning Plus intensifying screens. Film density was determined by scanning autoradiograms with a Joyce-Lobel scanning densitometer, and the area under peaks on the density traces was determined using a Numonics Corp. electronic graphics calculator.

Figure 2. Electrophoretic mobility of deletion-containing heteroduplex DNA fragments in agarose.

DNA samples were digested with Bgl II and displayed on a 1% agarose gel. DNA in the gel was visualized by ethidium bromide fluorescence. Lane a-- λ 333 (cI Δ HindIII). Lane b-- λ 334 (cI857). Lane c--mixture of λ 333 and λ 334 DNA which was annealed without prior denaturation (cI857 + cI Δ HindIII sham heteroduplex). Lane d--mixture λ 333 and λ 334 DNA which was annealed with prior denaturation (cI857 + cI Δ HindIII heteroduplex). Arrows indicate the positions of the cI857/cI857 homoduplex, the cI857/cI Δ HindIII heteroduplex, and the cI Δ HindIII/cI Δ HindIII homoduplex Bgl II fragments.

RESULTS

Electrophoretic behavior of deletion-containing heteroduplex molecules in agarose

In order to determine the electrophoretic mobility in agarose of DNA molecules which were hybrid for the wild-type and mutant alleles of a deletion, artificial heteroduplex molecules were constructed. Initial experiments examined the electrophoretic behavior of heteroduplex molecules which contained both the mutant and wild-type sequences of cI Δ HindIII. DNA extracted from cI857 and cI Δ HindIII-carrying phage (λ 334 and λ 333) was mixed, denatured, and annealed. As a control, a portion of the same DNA mixture was carried through the annealing procedure without prior denaturation. The heteroduplex and control DNA mixtures were digested with the restriction endonuclease Bgl II, which cuts in sequences flanking the cI Δ HindIII deletion. The resulting fragments were displayed on an agarose gel. A photograph of a typical gel is shown in Figure 2.

A novel DNA species is present in the Bgl II digestion of the denatured and reannealed mixture which is not present in Bgl II digestions of either the sham heteroduplex mixture or in the two individual parent DNA samples. This fragment, the presumptive cI857/cI Δ HindIII heteroduplex fragment, migrates at a position intermediate between the 2.5 kb. cI857 Bgl II fragment and the

Table 2. Mobility in agarose of various deletion containing heteroduplex DNA fragments.

deletion	restriction fragment	fragment size (kb)		apparent fragment size (kb)	
		+/+	Δ/Δ	+/Δ	
<u>cI</u> ΔHindIII	Bgl II	2.45	1.77	2.01 +/- 0.03	
<u>lacZ</u> ΔW4680	Bgl I	2.1	0.8	1.33 +/- 0.06	
<u>b522</u>	Pvu I	9.5	6.1	7.1	(0.35%)
				7.7	(0.5 %)
<u>nin5</u>	Bgl II	9.7	6.7	7.7	(0.35%)
				8.1	(0.5 %)

Molecular weights of homoduplex restriction fragments were obtained from previous determinations (11, D. Moir, personal communication). Apparent molecular weights of the +/Δ heteroduplex fragments were determined by measuring gels. Apparent molecular weight of the cI+/cIΔHindIII heteroduplex fragment presented is the average of eight independent determinations, performed using 0.35%, 0.5%, 0.75%, and 1.0% agarose gels. Apparent molecular weight of the lacZ+/lacZΔW4680 heteroduplex fragment presented is the average of three independent determinations, using 0.75% and 1.0% agarose gels. All other apparent molecular weights are the result of single determinations. Agarose concentration in gels is indicated in parentheses.

1.8 kb. cIΔHindII Bgl II fragment. This position corresponds to an apparent duplex length of 2.0 kb. Similar results were obtained when DNA molecules hybrid for the wild-type and mutant alleles of either lacZΔW4680, nin5, or b522 were examined. These results are summarized in Table 2. In all cases, denaturation and reannealing of mixtures of wild-type and deletion-containing phage DNA created a novel restriction fragment which migrated at a position characteristic of a duplex molecule of slightly smaller than the average of the molecular weights of the parental duplex molecules.

Selective retention of deletion-containing heteroduplex molecules by nitrocellulose

Since DNA molecules which are hybrid for the wild-type and mutant alleles of a deletion contain a region of single-strand DNA, it was reasoned that such molecules should be bound to nitrocellulose without prior denaturation. In order to test this prediction, heteroduplex and sham heteroduplex DNA mixes were prepared with DNA from cI857 and cIΔHindIII-carrying phage, cut

Figure 3. Transfer of end-labeled DNA fragments to nitrocellulose.

DNA samples were digested with Bgl II, end labeled, and displayed on a 1% agarose gel. Lanes a and b were transferred to nitrocellulose with prior denaturation; lanes c and d were transferred to nitrocellulose without prior denaturation. Lanes a,c--cI857 + cIΔHindIII sham heteroduplex. Lanes b,d--cI857 + cIΔHindIII heteroduplex. Arrows indicate the positions of the cI857/cI857 homoduplex, the cI857/cIΔHindIII heteroduplex, and the cIΔHindIII/cIΔHindIII homoduplex Bgl II fragments.

with Bgl II, and the resulting fragments were end-labeled by filling in the single-strand termini created by the restriction enzyme with radioactive nucleotide triphosphates. The radioactive fragments were displayed on an agarose gel, and the DNA in the gel was transferred to nitrocellulose by two different methods. In the first method, the standard denaturing transfer method of Southern (9), DNA in the gel was denatured by soaking the gel in 0.5 M NaOH, 1.5 M NaCl for 30 minutes, followed by a 30 minute neutralizing soak in 0.5 M TRIS-HCl (pH 7.4), 1.5 M NaCl. In the second method the gel was soaked for 60 minutes in 0.5 M TRIS-HCl (pH 7.4), 1.5 M NaCl, thus allowing the DNA in the gel to remain in its native state. The DNA in both gels was then transferred to a nitrocellulose filter by blotting. An autoradiogram of this filter is shown in Figure 3. Whereas all the DNA species present in the gel were retained by the nitrocellulose filter if the DNA had been denatured prior to transfer, only the cI857/cIΔHindIII heteroduplex fragment was retained efficiently upon transfer without prior denaturation.

An estimate of the relative efficiencies of retention of the cI857/cIΔHindIII heteroduplex fragment and of duplex DNA fragments upon transfer with or without prior denaturation was made by densitometer tracing of the autoradiogram shown in Figure 3. 70% of the heteroduplex fragment retained upon transfer with

Figure 4. Hybridization of radioactive probe to DNA fragments transferred to nitrocellulose. DNA samples were digested with Bgl II and displayed on a 1% agarose gel. Lanes a and b were transferred with prior denaturation; lanes c and d were transferred without prior denaturation. The filter was then hybridized with radioactive cI probe and exposed to film. Lanes a,c--cI857 + cIΔHindIII sham heteroduplex. Lanes b,d--cI857 + cIΔHindIII heteroduplex. Arrows indicate the positions of the cI857/cI857 homoduplex, the cI857/cIΔHindIII heteroduplex, and the cIΔHindIII/cIΔHindIII homoduplex Bgl II fragments.

prior denaturation was retained upon transfer without prior denaturation; in contrast, 14-20% of the homoduplex fragments retained upon transfer with prior denaturation were retained upon transfer without prior denaturation.

Hybridization of radioactive probe to deletion-containing heteroduplex fragments bound to nitrocellulose

In order to determine whether deletion-containing heteroduplex DNA molecules which were bound to nitrocellulose without prior denaturation could be detected by hybridization to radioactive probe, Bgl II-digested non-radioactive heteroduplex and sham heteroduplex DNA mixes isolated from cI857 and cIΔHindIII-carrying phage were displayed on an agarose gel similar to the one illustrated in Figure 2. One half of the gel was transferred to nitrocellulose with prior denaturation, and the other half was transferred to the same filter without prior denaturation. The nitrocellulose filter was then hybridized to a radioactive probe made from the 0.56 kb Hind III fragment illustrated in Figure 1. Since the sequences contained within this fragment are absent in molecules carrying the cIΔHindIII deletion, this fragment would be expected to hybridize only to DNA molecules which contained cI857 sequence. An autoradiogram of this filter is shown in Figure 4. A strong signal is present at the cI857/cIΔHindIII

Figure 5. Detection of $cI857/cI\Delta HindIII$ heteroduplex molecules. Various amounts of the $cI857/cI\Delta HindIII$ artificial heteroduplex mixture were mixed with 0.5 $\mu\text{gm.}$ of *E. coli* DNA, digested with Bgl II, and displayed on a 1% agarose gel. DNA in the gel was transferred without prior denaturation. The filter was then hybridized with radioactive cI probe and exposed to film.

Lane a-- 1.1×10^{-4} $\mu\text{gm.}$ of the artificial heteroduplex mixture.
 Lane b-- 3.5×10^{-5} $\mu\text{gm.}$ of the artificial heteroduplex mixture.
 Lane c-- 1.1×10^{-5} $\mu\text{gm.}$ of the artificial heteroduplex mixture.
 Arrows indicate the positions of the $cI857/cI857$ homoduplex, the $cI857/cI\Delta HindIII$ heteroduplex, and the $cI\Delta HindIII/cI\Delta HindIII$ homoduplex Bgl II fragments.

position in both the denatured and native portions of the filter; in contrast, a signal is present at the $cI857/cI857$ position only in the portion of the filter where material was denatured prior to transfer. Quantitation of film density at the $cI857/cI\Delta HindIII$ position revealed that the native-transferred heteroduplex fragment bound radioactive probe about 80% as efficiently as did the heteroduplex material which was denatured prior to transfer. No binding of radioactive probe by duplex fragments transferred to nitrocellulose without prior denaturation was detected. In addition to being highly selective, this method was also quite sensitive. As little as one picogram of the $cI857/cI\Delta HindIII$ heteroduplex Bgl II fragment could be readily detected (Figure 5).

DISCUSSION

We have described the development of a methodology which allow sensitive and quantitative detection of DNA molecules which

contain both the mutant and wild-type sequences of a deletion nonhomology in a region of heteroduplex DNA. Advantage was taken of several properties of these heteroduplex molecules. They exhibit a characteristic electrophoretic mobility in agarose, and are selectively retained when transferred to nitrocellulose in the absence of prior denaturation. In addition, these heteroduplex molecules, when bound to nitrocellulose in the native state, can be selectively detected by hybridization with single-stranded DNA homologous to the single-strand region present in the region of heteroduplex.

The most important of these properties is the electrophoretic mobility in agarose of deletion-containing heteroduplex molecules. All of the deletion-containing heteroduplex DNA fragments examined in this study migrated to a position intermediate between those assumed by the corresponding wild-type and deletion-containing parental homoduplex fragments, and which differed only slightly from that expected of a homoduplex DNA fragment of the corresponding molecular weight. This property holds when the single-stranded loop constitutes either a major or minor fraction of the total mass of the heteroduplex molecule, and appears to be fairly independent of the total size of the heteroduplex molecule in question.

The physical parameters which determine the mobility of DNA in agarose are not well understood, but it has been suggested that, in the molecular weight range where the mobility of a DNA fragment is inversely proportional to the logarithm of its molecular weight, the fragment assumes a globular conformation in the gel (20, 21). Linear single-strand DNA molecules migrate more rapidly in agarose than do duplex molecules of the same length, presumably because the single-strand molecules can assume a more compact globular structure (22, 23). The observation that heteroduplex fragments which contain an internal single-strand loop migrate to very nearly the same position anticipated for a completely duplex molecule of the same mass suggests that the single strand loop present in deletion-containing heteroduplex molecules causes these molecules to present the gel medium with a configuration of very nearly the same effective cross-section as would a duplex molecule of the same molecular weight.

The other property of deletion-containing heteroduplex molecules that has been taken advantage of is their retention by nitrocellulose in their native state, and their selective hybridization with probe which contains sequences homologous to the single strand loop present in such molecules. While deletion-containing heteroduplex molecules are efficiently bound to nitrocellulose in their native state, homoduplex molecules are retained to a lesser extent. The retention of duplex fragments observed might be the result of the presence of small, stable single-strand regions in a subfraction of the homoduplex molecules examined; alternatively, it could be the result of transitory single strand regions formed by "breathing" of the DNA duplex during the transfer process. These nitrocellulose-bound homoduplex molecules hybridize to radioactive probe very inefficiently, if at all, while nitrocellulose-bound deletion-containing heteroduplex molecules efficiently hybridize to a radioactive probe homologous to sequences present in the single-strand loop. As little as one picogram of the cI857/cIΔHindIII heteroduplex Bgl II fragment could be detected using this method. This amount corresponds to about one heteroduplex molecule per 200 *E. coli* genomes; alternatively, it would correspond to about one heteroduplex molecule per 10^4 lambda genomes. Therefore, although this methodology has proven useful in examining the products of phage λ recombination for the presence of deletion-containing heteroduplex molecules, additional increases in sensitivity may be necessary before it can be applied to the study of recombination in organisms with more complex genomes.

ACKNOWLEDGEMENT

This work was supported by grant number AI05388 from the National Institute of Health (to M.S.F.); M.L. was supported by training grant number GM07287 from the National Institute of Health and a Johnson and Johnson Foundation Graduate Fellowship.

REFERENCES

1. Doermann, A. H. and Parma, D. H. (1967) J. Cell. Physiol. 70(Sup. 1), 147-164
2. Berger, H. and Warren, A. J. (1969) Genetics 63, 1-5

3. Makin, V., Szybalski W. and Blattner, F. R. (1982) Genetics 102, 299-317
4. Lichten, M. and Fox, M. S. (1983) Genetics 103: 5-22
5. Lataste, H., Calverys J. P. and Sicard, A. M. (1980) J. Bacteriol. 144, 422-424
6. Claverys, J. P., Roger, M. and Sicard, A. M. (1980) Molec. gen. Genet. 178, 191-201
7. Fink, G. R. and Styles, C. A. (1974) Genetics 77, 231-244
8. Lawrence, C. W., Sherman, F., Jackson M. and Gilmore, C. A. (1975) Genetics 81, 615-629
9. Southern, E. (1975) J. Mol. Biol. 98, 503-517
10. Malamy, M. H., Fiandt M. and Szybalski, W. (1972) Molec. gen. Genet. 119, 207-222
11. Davis, R.W., Botstein, D. and Roth, J. R. (1980) Advanced Bacterial Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. St. John, T. and Davis, R. (1981) J. Mol. Biol. 152, 285-315
13. Blattner, F., Williams, G., Bulechl, A., Thompson, K., Faber, H., Furlong, L., Grumwal, D., Kiefer, D., Moore, D., Schumm, D., Sheldon, E. and Smithies, O. (1977) Science 196, 161-169
14. Yamamoto, K. R. and Alberts, B. M. (1970) Virology 40, 734-744
15. White, R. L. and Fox, M. S. (1974) Proc. Nat. Acad. Sci. USA 71, 1544-1548
16. Backman, K. and Ptashne, M. (1978) Cell 13, 65-71
17. Vogelstein, B. and Gillespie, D. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 615-619
18. Rigby, P. W. J., Dieckmann, M., Rhodes C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251
19. Laskey, R. A. and Mills, A. D. (1975) Eur. J. Biochem. 56, 335-341
20. Fisher, M. P. and Dingman, C. W. (1971) Biochemistry 10, 1895-1899
21. McDonell, M. W., Simon M. N. and Studier, F. W. (1977) J. Mol. Biol. 110, 119-146
22. Aaij, C. and Borst, P. (1972) Biochim. Biophys. Acta 269, 192-200
23. Hayward, G. S. and Smith, M. G. (1972) J. Mol. Biol. 63, 383-395