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Base specific fractionation of double stranded DNA: affinity chromatography on a novel type of adsorbant

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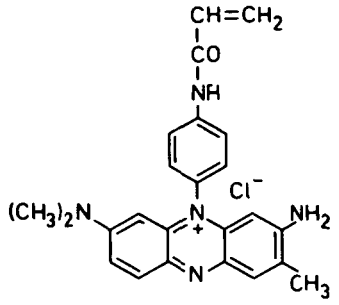
ABSTRACT

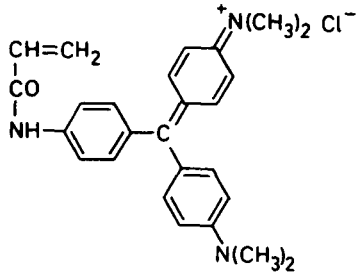
A material suitable for base-pair-specific 'affinity chromatography' of double stranded DNA is described. The synthesis of this material involves two successive polymerization reactions yielding solid particles of cross-linked bisacrylamide to which base-pair-specific dyes are covalently attached by spacers of polyacrylamide chains of different length. Materials with immobilized A·T-specific malachite green or G·C-specific phenyl neutral red were used for base-pair-specific fractionation of sheared DNA from bacterial sources, as well as of higher molecular weight Calf thymus DNA or defined fragments of coliphage lambda DNA. The excellent resolving power of this method of 'affinity chromatography' of nucleic acids is comparable only to DNA fractionation in buoyant density gradients in the presence of base-pair-specific ligands. A great advantage of this material is its simple handling and its chemical stability, which allows repeated re-use of the same column.

INTRODUCTION

The discovery and systematic development of dyes with remarkable base-pair-specificity when complexing double stranded DNA resulted in the development of several methods for DNA fractionation with improved resolving power. For example, derivatives of G·C-specific phenyl neutral red have been used for improvement of base-pair-specific fractionation of DNA on columns of hydroxyapatite (1). A·T-specific compounds such as methylgreen, "Hoechst 33258" (2) and antibiotics such as A·T-specific distamycin and netropsin (3) or G·C-specific actinomycin (4) have been applied to increase the resolving power of density gradients. Nevertheless, up to the present no material has been developed which takes advantage of immobilized base-pair-specific dyes for direct 'affinity chromatography' of nucleic acids. Hydroxyapatite, methylated kieselguhr and polyamine kieselguhr columns have been used successfully for base-pair-specific affinity chromatography of nucleic acids (5,6,7), but the resolving power of these materials is low and the reasons for their specificity are not really understood.



structure I



structure II

From our work on base-pair-specific dyes we obtained some ideas on the structure of their complexes with double stranded DNA (2,8,9). These investigations enabled us to synthesize defined acrylamide derivatives of G·C-specific phenyl neutral red (structure I) and of A·T-specific malachite green (structure II) without risking large changes in specificity and affinity of the parent compounds by the substituents.
Copolymerization of these acrylamide dyes with acrylamide itself yielded polyacrylamide chains containing dye residues with unchanged base pair specificity for their complex formation with DNA (10). On the basis of these results we developed the 'two step polymerization procedure' for total synthesis of a novel material for affinity chromatography of biopolymers (11). The general scheme of this procedure is shown in Fig. 1.
In the first polymerization step bisacrylamide is crosslinked to a solid material of macroreticular pore size structure. The second polymerization reaction takes advantage of the fact that these bisacrylamide particles contain about 1 mmol of unreacted acrylamide double bonds per gram of dried material. These unreacted double bonds are used for fixation of side chains containing "specific" subunits such as base-pair-specific DNA ligands. In the course of the second polymerization reaction an acrylamide derivative of the ligand is copolymerized with a large excess of acrylamide in a suspension of the bisacrylamide particles. Under these conditions polymer acrylamide chains with a single dye molecule per chain are formed and immobilized immediately by incorporation of the acrylamide residues on the bisacrylamide particles.

In this paper we describe the application of malachite green and phenyl neutral red substituted bisacrylamide particles for base-pair-specific fractionation of double stranded DNA up to molecular weights of 25×10^6. The high resolving power of this DNA chromatography is demonstrated by sep-

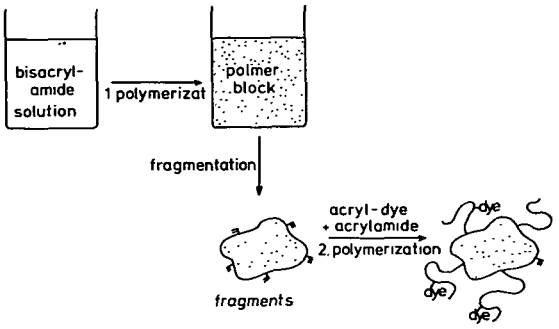


Figure 1: Scheme of the 'two step polymerization procedure' for synthesis of a novel material for affinity chromatography of nucleic acids.

aration of defined fragments of lambda DNA with not more than 2-3% difference in their (G+C) contents.

MATERIALS AND METHODS

Monomers for polymerization reactions.

Acrylamide and N,N'-methylenebisacrylamide were purchased from Serva (Heidelberg). The base pair specific dyes N-acryloyl-4'-amino-phenyl neutral red (APNR, structure I) and N-acryloyl-4-amino-malachite green (AMG, structure II) were synthesized as will be described elsewhere (12). Their concentrations were measured photometrically in aqueous solution by addition of concentrated sodium dodecylsulfate solution (25%, w/v) to a final concentration of 2.5% of the detergent. Molar extinction coefficients of 54,500 at 555nm for APNR and of 89,500 at 620nm for AMG were used. The base pair specificity parameter α and the affinity parameter σ were determined by the partition dialysis method published by Müller and Crothers (8). The α values of 2.04 for APNR and 0.29 for AMG were found to be similar to those of unsubstituted phenyl neutral red and malachite green. These values mean that both dyes show a strong preference for complex formation with two adjacent base pairs of the same base composition. APNR binds to two neighbouring G·C base pairs; in contrast, AMG prefers binding to two adjacent A·T-base pairs. The corresponding σ values of 8.3×10^3 and 1.7×10^4 respectively are in the same order of magnitude as those of their unsubstituted analogues (2,9).

Synthesis of crosslinked bisacrylamide particles.

The bisacrylamide material was synthesized by polymerization of a solution of 50g N,N'-methylenebisacrylamide in 300 ml of 33% (v/v) aqueous

methanol at 60°C. The polymerization was started by addition of 1 ml of N,N,N',N'-tetraethylenediamine (Fluka) and a solution of 0.2 g ammonium peroxidisulfate in 5 ml H_2O. Immediately after intensive stirring the magnetic stirrer was stopped. The solution solidified within one minute. The warm solid polymer block was rapidly reduced to smaller pieces which were suspended in methanol for further breakage by sieves with decreasing pore sizes. After final washings with several volumes of methanol and water the particles were stored in distilled water for the second polymerization reaction. (More details for preparation of the bisacrylamide material and its further characterization will be published in a separate paper (11).)

Coupling of base-pair-specific dyes to bisacrylamide particles.

In a typical batch 60 ml of settled bisacrylamide particles are suspended in a solution of 70 mmol acrylamide and 2.5×10^{-2} mmol dye hydrochloride in 20 ml H_2O. The copolymerization is started by addition of 50 µl 2-mercaptoethanol (Fluka) and 1 ml of a solution of 0.8 g sodium peroxide (Merck) in 100 ml 1 M sodium acetate buffer, pH 5.5. Nitrogen is bubbled through the suspension for 5 minutes before the bottle is closed and kept at room temperature for about 2 hours. The viscous suspension is transferred onto a buchner funnel and the aqueous supernatant is sucked off. The red or green colored particles are intensively washed with aqueous buffer, boiled in 1 M sodium perchlorate and again washed until no further release of colored material can be observed. The particles are normally stored in the dark at 4°C. The capacity for DNA was nearly the same for the malachite green and the phenyl neutral red substituted material used in this work. Both materials absorbed about 0.11 mg of sheared Calf thymus DNA per ml of settled polymer in 10 mM sodium phosphate buffer, pH 6.0.

General procedure for chromatography of DNA on dye-substituted bisacrylamide.

A chromatographic column of 1.5 x 25 cm (Whatman) equipped with two adaptors was packed to about 10-20 cm height with dye-substituted bisacrylamide suspended in 10 mM sodium phosphate buffer, pH 6.0, 1 mM EDTA. The column was eluted with 1-2 volumes of the same buffer to remove dust particles which may have formed by breakage of particles during the packing procedure. Flow rates from 2 ml/hr to 100 ml/hr were used without any problems. Shrinkage of the material was not observed even in 5 M sodium perchlorate solution. The DNA (about 1 mg or less) was loaded onto the column from the top and elution with a sodium perchlorate gradient was started. The shape of the gradient was formed by use of two peristaltic pumps with different flow rates and was controlled continuously by con-

ductivity measurements of the effluent at the lower end of the column. For continuous registration of optical density at 258 nm a PM 2 A spectrophotometer (Zeiss) equipped with a quartz flow cell of 1 cm light path (Hellma) was used. Conductivity and optical density were simultaneously recorded on a two channel recorder.

DNAs.

Bacterial DNAs from Micrococcus luteus, Escherichia coli and Clostridium acidiurici were prepared from frozen (wet) cells (Merck, Darmstadt) by a variation of the method described by Marmur (13,14,8,9). For preparation of DNA from Halobacteria a wildtype strain was grown in a medium which contained in 1000 ml: NaCl, 250.0 g; $MgSO_4 \cdot 7\ H_2O$, 20 g; trisodium citrate· $2\ H_2O$, 3.0 g and Casamino Acids (Difco), 5 g. The cells were cultured in 10 l polyethylene bottles in daylight at room temperature and were aerated under vigorous stirring with about 50 l of air per hour. When the optical density at 570 nm reached 0.600 the cells were harvested at 15,000 rpm in a Sorvall RC 2B centrifuge, equipped with the KBS rotor. A clear red lysate was obtained when the pellets were suspended in 2 M NaCl. For precipitation of nucleic acids and membrane fragments the viscous solution was mixed with a concentrated aqueous solution (50%, w/v) of polyethyleneglycol 6000 (Serva) to a final concentration of 10% of polyethyleneglycol. When this solution was kept at 4°C for about 2 hours and centrifuged at 10,000 rpm, red pellets were obtained. For isolation of DNA the pellets were treated in the same way as the frozen cells of other bacteria as described above. All bacterial DNAs were obtained in a molecular weight range from 4-8 x 10^6 as determined from sedimentation velocity (15). High molecular weight Calf thymus DNA (M_r 25 x 10^6) was purchased from Sigma (type I) or from Boehringer (Mannheim). Sheared Calf thymus DNA from Sigma and bacterial DNAs (M_r 7.5 x 10^5) were obtained by shearing high molecular weight DNA in a Virtis 45 homogenizer at 20,000 rpm at 0.5°C for 4 hours, followed by purification as described in a previous paper (1). Samples of DNA from bacteriophage lambda, digested by endo R·EcoR I or endo R·Hind III, were generous gifts of Boehringer (Mannheim).

Agarose gel electrophoresis.

Agarose gel electrophoresis was performed on 0.8% agarose (Seakem) slab gels in a vertical electrophoresis apparatus using 36 mM Tris·HCl, 30 mM sodium phosphate, 10 mM EDTA, pH 7.5 and 1 μg/ml ethidium bromide. The DNA fragments on the gel were visualized in an Original Hanau UV-Fluotest

and photographed with a Polaroid 195 land camera equipped with close up kit and red-orange filter.

Melting curves for DNA fractions.

The fractions of the columns were dialyzed extensively against 10 mM sodium phosphate buffer, pH 7.1, before melting in a Gilford spectrophotometer 260 equipped with a thermoprogrammer 2527. A heating rate of 1°C/min was normally employed.

RESULTS

Fractionation of sheared DNA.

For evaluation of elution conditions with optimal resolving power we started our experiments with a mixture of 3 bacterial DNAs of different base composition which were sheared to an average molecular weight of 7.5×10^5. This standard "DNA triple mix" contained DNAs of Micrococcus luteus (72% G+C), Escherichia coli (52% G+C) and Clostridium acidiurici (34% G+C) in an O.D. ratio of 1/1/1 at 258nm. This mixture was dialyzed against 10 mM sodium phosphate buffer, pH 6.0, 1 mM EDTA and stored at 4°C. For separation runs all columns were equilibrated with this buffer, before they were loaded with DNA samples.

The low pH value is necessary since the malachite green derivative has a pK value of 7.2 for its conversion into the carbinol base form (Reinholz, unpublished results), which shows no affinity for DNA, causing the capacity and resolving power of the malachite green column to decrease substantially with increasing pH values. The phenyl neutral red material showed no dependence of its separation parameters on the pH value within the range of pH 5 to pH 9. For reasons of simplicity the phenyl neutral red polymer columns were used in the same buffer as the malchite green polymer.

For direct comparison of the resolving power of both materials, A·T-specific malachite green-substituted and G·C-specific phenyl neutral red-substituted bisacrylamides of similar capacities for sheared DNA were used. Both materials were prepared from bisacrylamide particles taken from the same polymerization batch. The corresponding columns with similar dimensions were run with similar flow rates and recorded with the same recorder settings. The slopes of gradients were adjusted in such a way that the elution profiles for columns of both materials became comparable as shown in Fig. 2a and 2b.

Both elution profiles show 3 more or less pronounced peaks. The se-

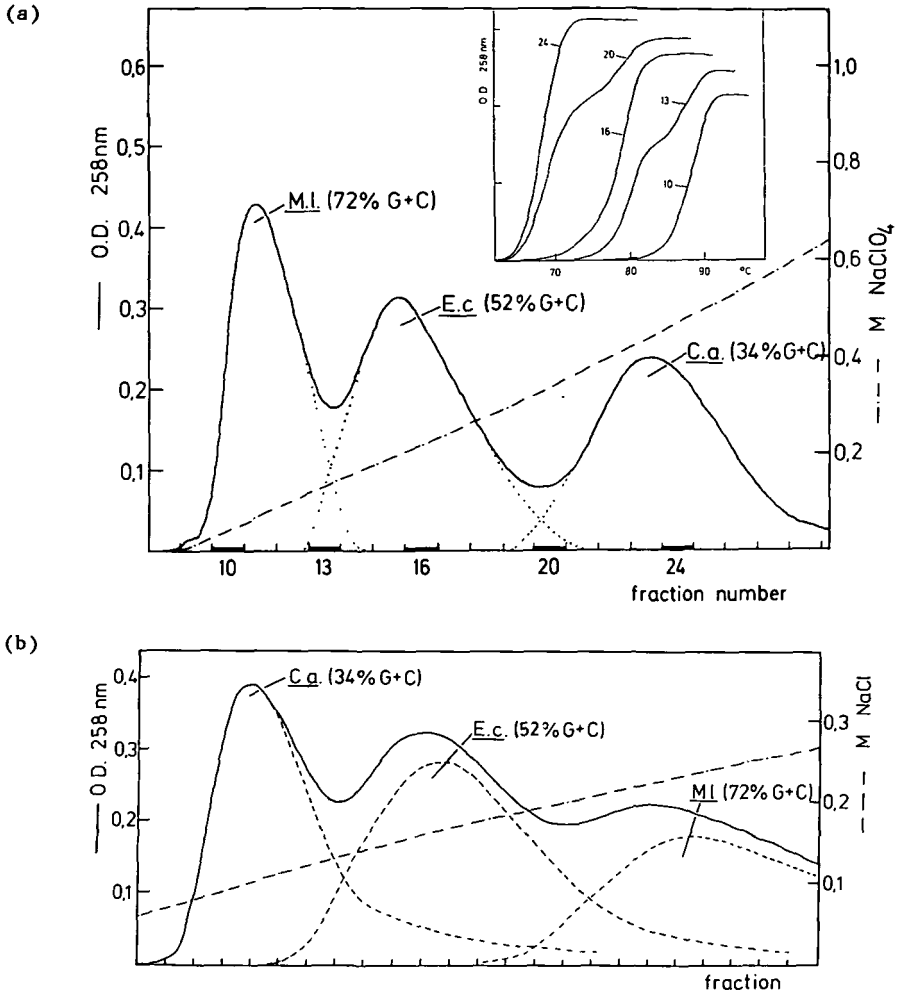


Figure 2: Elution of about 1.0 mg of a 'triple mix' of sheared bacterial DNAs (molecular weight about 7.5 x 10^5) from dye substituted bisacrylamide columns. (*a*) Malachite green column: 1.5 x 16.0 cm, 5.8 ml/hr flow rate at room temperature. (b) Phenyl neutral red column: 1.5 x 15.8 cm, 5.8 ml/hr flow rate at room temperature. Melting curves for several fractions are shown in the insert to (*a*).

quence of elution for the 3 different DNA-species is reversed for the two materials, according to their opposite base pair specificity. Nevertheless, the peaks in the elution profile of A·T-specific material in Fig. 2*a* are obviously sharper than those in the corresponding elution profile of G·C-specific material in Fig. 2b. To get further information about the real distribution of the 3 DNA-species we measured the melting curves for frac-

tions of both runs (insert of Fig. 2a). From this analysis it can be seen that the peaks for single DNAs show pronounced tailing in the case of phenyl neutral red, which is absent in the run of malachite green material (dotted lines in the diagrams). Possible reasons for this remarkable difference between two dyes with similar specificity and affinity parameters (see materials and methods) are discussed later.

Influence of molecular weight on elution conditions.

In the course of our tests for base pair specificity of dye-substituted polyacrylamides we regularly observed preferential precipitation of the high molecular weight DNA if solutions of copolymers were added to mixtures of DNAs of different molecular weights but the same average base composition. Since our dye-substituted bisacrylamide materials contain similar copolymers, we expected an analogous influence of molecular weight of a DNA species on its elution conditions.

In order to test this prediction a mixture of two ^{3}H-labelled and sheared DNA species of different base composition, 34% and 72% G+C respectively, were separated on a malachite green column in presence of similar amounts of their unlabelled high molecular weight analogues (Fig. 3).

Both sheared DNAs were eluted at lower salt concentrations than their high molecular weight analogues. The difference in the salt concentrations, which are necessary for elution of the analogue DNAs with different mole-

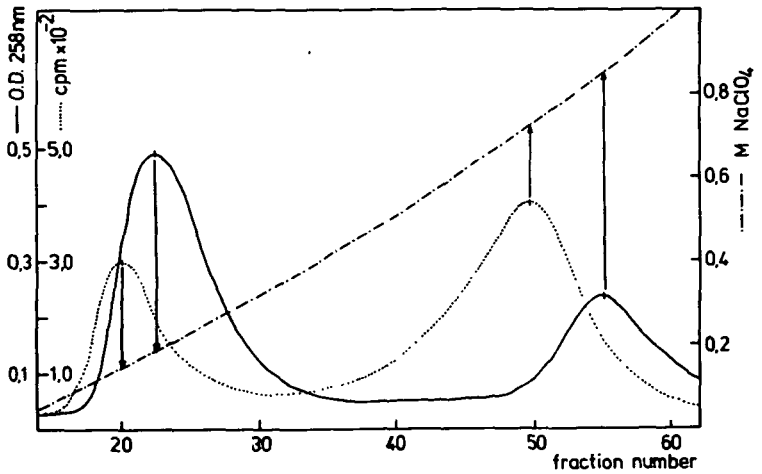


Figure 3: Elution of a DNA mixture of two bacterial DNAs of different base composition (Cl. acidiurici, 34% G+C and M. luteus, 72% G+C) from a malachite green column: 1.5 cm x 16 cm. Both DNA species consist of analogues of ^{3}H-labelled sheared DNA (M_r about 7.5 x 10^5) and unlabelled high molecular weight DNA (M_r about 8.0 x 10^6).

cular weights, increases with increasing (A+T)-content of the DNA-species, e.g. from 0.146 to 0.186 M $NaClO_4$ for M. luteus DNA and from 0.720 M to 0.846 M $NaClO_4$ for Cl. acidiurici DNA.

Fractionation of high molecular weight DNA.

Since the malachite green substituted material gave better resolution in the case of sheared DNAs, only this material was tested for separations of high molecular weight DNA. Calf thymus DNA is well known to contain several highly repetitive DNA fractions (satellites) with considerably higher (G+C) contents than the 'main' DNA (e.g. 16). The separation and purification of these satellites for sequence studies could only be achieved by two or more ultracentrifuge runs using buoyant density gradients in the presence of base pair specific ligands (16,17,18).

Figure 4 shows the elution profile for a separation run of 0.5 mg of Calf thymus DNA (high molecular weight, Boehringer) on a malachite green column. Some of the satellites are clearly visible as separate peaks. Melting curve analysis shows that DNAs eluted in the first and second peak are the satellites II and I with about 68% and 59% G+C respectively (normally characterized by their buoyant densities in neutral CsCl-density gradient as components with densities of 1.723 g/cm^3 and 1.715 g/cm^3 respectively).

Without any further interpretation of other details of this elution diagram, we can estimate from these two peaks that the resolving power of our malachite green material is sufficient for extensive separation of two DNA species which differ by about 10% in their average (G+C) content, if the DNA species possess the low complexity which is typical for many

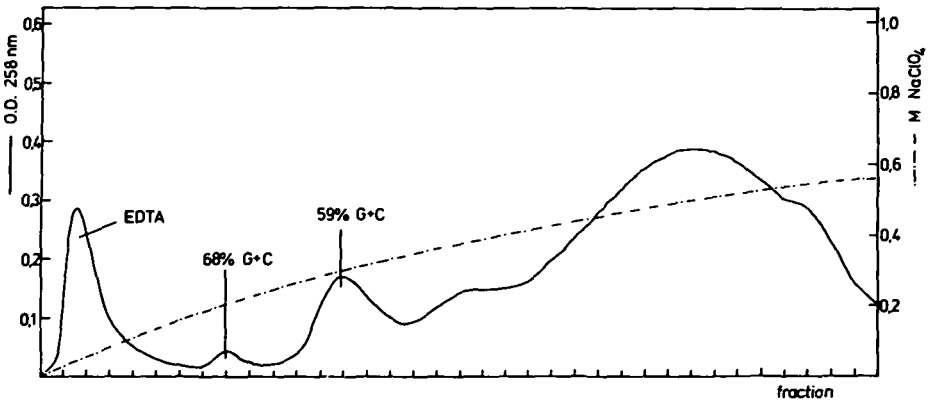


Figure 4: Elution of 0.5 mg of high molecular weight DNA of Calf thymus (M_r about 25 x 10^6) from a malachite green column: 1.5 x 18 cm, 1.9 ml/hr flow rate at room temperature.

satellite DNAs. If a DNA pair with a similar difference in the average (G+C) content of its two components but with a higher complexity is used, such as DNA from Halobacteria (19), the separation is less complete, due to higher complexity of the genome of bacteria (Fig. 5).

For the same reasons the separation for the 'triple-mix' of sheared DNAs of bacteria is not as complete as one would expect from the considerable differences in their average (G+C) contents.

Fractionation of DNA fragments of defined molecular weight and defined base composition.

As shown in the preceeding chapters, a precise definition of the resolving power of our columns is drastically complicated by the influences of polydispersity and complexity encountered with all DNA preparations. These influences may be avoided by the use of DNA digests obtained by the action of restriction nucleases on plasmids or phage DNAs. For a first test we applied an endo R·EcoR I digest of DNA of the hybrid plasmid pGM 214 to the malachite green column (Fig. 6). The plasmid had been constructed previously from the Col E1 - Ap vector (pSF 2124) and the 1400 base pair repeat unit of Calf thymus satellite I (20). The separation of the small satellite fragment (M_r: 0.93×10^6) in fraction 22-25 from an excess of the larger vector fragment (M_r: 7.35×10^6) and incompletely digested hybrid plasmid DNA in fraction 29-34 is absolutely complete as can be seen from electrophoresis pattern in the insert of Figure 6. The difference in the base composition between vector DNA (50% G+C) and satellite DNA (59% G+C) is similar to that of those mixtures cited in the earlier sections.

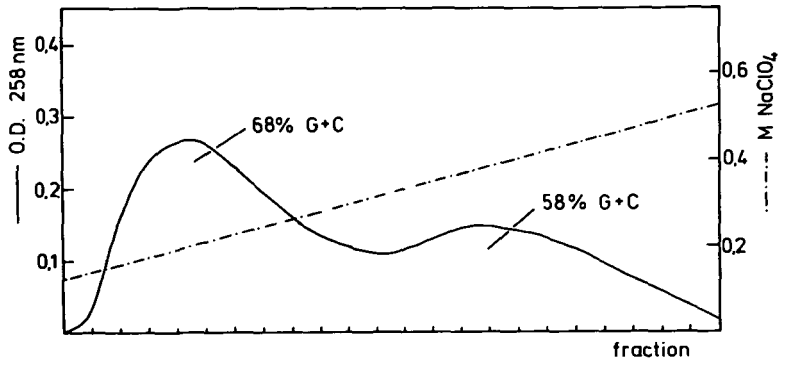


Figure 5: Elution of 0.5 mg of high molecular weight DNA of Halobacterium (M_r about 10×10^6) from a malachite green column: 1.5 x 16.0, 5.8 ml/hr flow rate at room temperature.

From the relatively large distance between the two peaks in Figure 6 we concluded that DNA fragments with even smaller differences in their (G+C) content should be separable on malachite green substituted columns. Endo R·EcoR I digest of coliphage lambda DNA proved to be a suitable test mixture for this assumption. It is well known that lambda DNA consists of two halves which are different in their average base composition (21). By digestion with endo R·EcoR I, six fragments of molecular weights from 2.13 to 13.7 x 10^6 are obtained, which are easily separated by electrophoresis on agarose gels and should contain substantial differences in their (A+T) contents (21,22). For a rough estimation of these differences we investigated an endo R·EcoR I digest of lambda DNA for its behaviour in neutral CsCl density gradient centrifugation. The resolution of the UV-scan after 48 hours at 30,000 rpm was poor due to the relative low molecular weights of the fragments. Only two peaks were distinguishable. From their buoyant densities, which were calculated from marker DNA (Cl. acidiurici, ρ = 1.6955 g/cm^3) (G+C) contents of 53.2% and 45.5% respectively were determined (23). For undigested lambda DNA 50.2% G+C was calculated under identical conditions. From these data the difference in the (G+C) content between the six different DNA fragments could be estimated not to exceed 4%.

The elution profile for a separation of 0.5 mg of lambda DNA digested by endo R·EcoR I on a malachite green-substituted column is shown in Figure 7. Five distinct peaks are obtained. The inserted electrophoresis tracks in Fig. 7 prove that each of the peaks represents mainly one fragment of the complete digest in track 1, with the exception of the peak from fraction 30-34, which contains the fragments F and D. The fragments E and

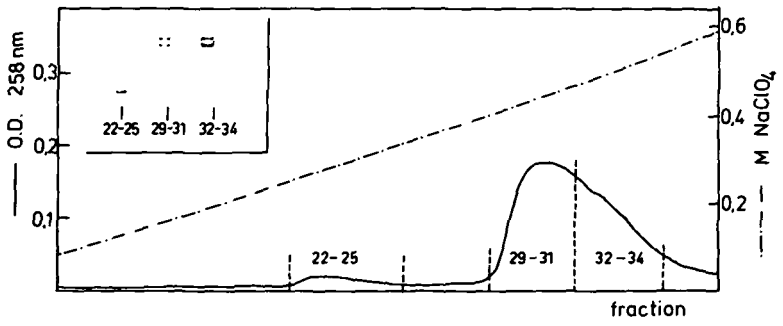


Figure 6: Elution of 0.3 mg of an endo R·EcoR I digest of plasmid pGM 214 (20) DNA from a malachite green column: 1.5 x 16.0 cm, 5.8 ml/hr flow rate at room temperature.

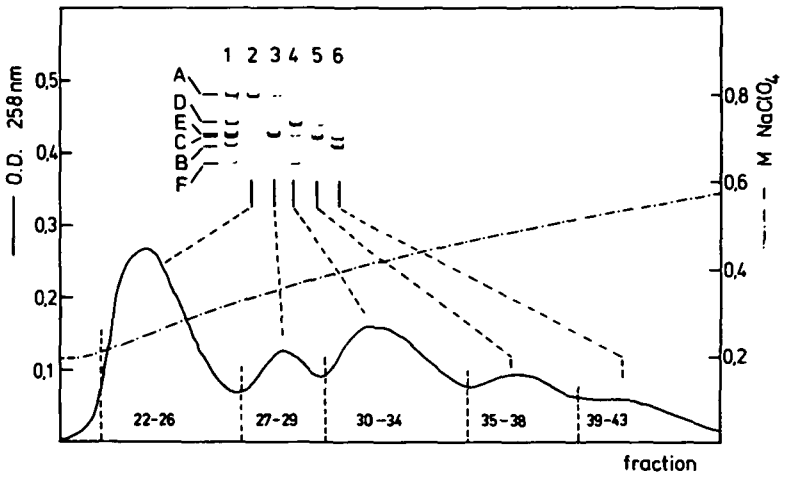


Figure 7: Elution of 0.45 mg of lambda DNA digested by endo R·EcoR I from a malachite green column: 1.5 x 16 cm, 5.8 ml/hr flow rate at room temperature.

C with similar molecular weights of 3.37 and 3.48 x 10^6 respectively, which are not separated by gel electrophoresis, are easily separated on the malachite green-substituted column due to their different base compositions (track 3 and 5 in Fig. 7).

From detailed work on the digestion of lambda DNA by endo R·EcoR I (22) and endo R·Hind III (24) respectively, the distribution of the six or seven DNA fragments in the genetic map of lambda is well known. If DNA fragments in the digests of both enzymes are designated by capital letters, according to the papers cited, the maps in Figure 8 are obtained; these are compared to the conventional genetic map of lambda at the top. We have used our results for construction of corresponding maps which show the variation of average base composition within the complete lambda DNA molecule. For this purpose we numbered the different fragments according to their sequence in the elution profiles from A·T-specific malachite green columns. When the sequence of elution is further illustrated by thickness of bars representing the single fragments, very similar maps for both digests are obtained (Figure 8). In both digests the largest fragment is (G+C) rich and situated at the left hand end of the lambda DNA molecule. In the middle part the fragments with relatively high (A+T) content are concentrated. A further but less pronounced accumulation of (A+T)-rich sequences is found at the right hand end of lambda DNA molecule. This base distribution pattern is in reasonably good agreement with published data (21).

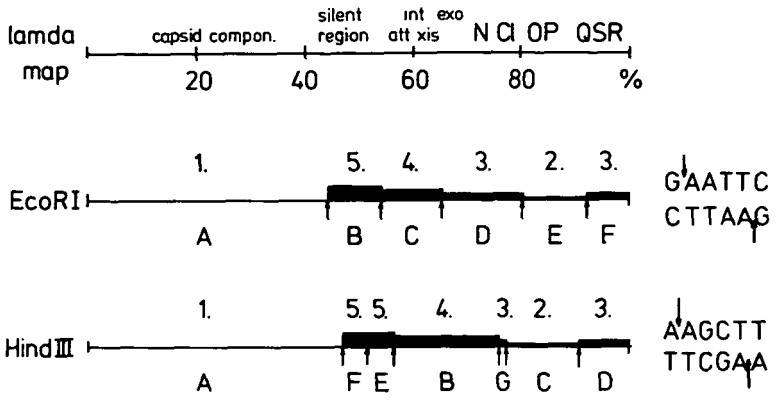


Figure 8: Illustration of the variation of average base composition within the lambda genome. The thicker the bar for a DNA fragment the later its elution from a malachite green column (Fig. 7) and the higher its (A+T) content respectively.

DISCUSSION

For the discussion of the results, which we obtained from separations with both types of adsorbants as well as with different DNA samples it is helpful to distinguish between a 'real' and an 'apparent' resolving power of a chromatographic method. By the 'real resolving power' of base pair specific affinity chromatography of DNA we define the minimum difference in the base composition of two DNA molecules, which is sufficient for their complete separation at a 2-3 times larger bed volume than necessary for quantitative absorption of a given amount of DNA. Both DNA molecules should have the same molecular weight and a base composition close to 50% G+C. (The latter limitation facilitates a normalization for those materials which show binding specificities for sequences of neighbouring base pairs of the same kind.) If DNA species can be characterized by their average base composition and their average molecular weight only, the real resolving power must be substituted by the 'apparent resolving power'. This magnitude is defined as the minimum difference in the average base composition of two DNA species of the same molecular weight distribution which is sufficient for their complete separation. It is clear to us that these limiting conditions for a correct evaluation of the resolving power are only fulfilled in a few of our cases.

Nevertheless in our first example of the comparative separations on A·T-specific and G·C-specific materials (Fig. 2) the apparent resolving power can be determined, since three bacterial DNAs of different average base composition but similar molecular weight distribution were investigated.

The apparent resolving power of the malachite green substituted polymer (Fig. 2a) amounts to about 20% difference in G·C content. The corresponding value for the phenyl neutral red substituted polymer (Fig. 2b) must be substantially larger; a reasonable estimation of this value is not possible, however, due to the strong tailing of the peaks. The reasons for this different behaviour of two dye-polymers with similar specificity and affinity parameters of their effective groups (see materials and methods) are not well understood. A possible explanation of this phenomenon may arise from the differences in the rate constants for complex formation and dissociation for the two dyes. DNA-complexes of planar dyes such as phenyl neutral red, which are bound to DNA by intercalation, have a life time in the range of milliseconds (Crothers, personal communication). If one DNA molecule on the column is simultaneously bound by more than one intercalating dye, effective life times in the range of seconds or minutes may result. Under these conditions the dissociation of DNA-polymer complexes can become slow compared to the elution rate and the observed tailing phenomena may result. Since life time of DNA-complexes of non-intercalating dyes such as malachite green can be expected to be 1-2 orders of magnitude shorter, the effective life times of the DNA-particle complexes should be small compared to the flow rate and therefore no tailing should be observed.

The separations of high molecular weight DNA of Halobacterium (Fig. 5) and Calf thymus (Fig. 4) verify that a higher molecular weight as well as a lower complexity of a DNA sample improve the apparent resolving power of the malachite green column. For example less than 10% difference in the base composition of satellites I and II is sufficient for their complete separation (Fig. 4). Due to their low complexities the satellites are eluted as much sharper peaks than the 'main' DNA which contains the DNA of high complexity.

The evaluation of the real resolving power of the malachite green substituted polymer is possible by evaluation of the elution profiles obtained for a mixture of two defined DNA fragments with 9% difference in their base composition (Fig. 6) and a mixture of 6 defined DNA fragments with average differences of about 2-3% in their base composition (Fig. 7). Although the separation for DNA fragments of lambda is less complete than the corresponding separation for the plasmid fragments, we estimate that the real resolving power of our malachite green column amounts to about 3%. A difference of about 2% in base composition should be sufficient for the discrimination of two DNA molecules in an elution profile. If other dyes

with specificities for more than two neighbouring base pairs are immobilized in the same way, adsorbants for affinity chromatography of DNA should be obtained which allow the separation of defined DNA fragments with differences of 1% or even less in their base composition.

It is clear that the application of this type of substituted polymer is not limited to the fractionation of double stranded DNA molecules. For example, substitution of intercalating dyes with no base specificity yields a column material suited for rapid and efficient separation of supercoiled DNA from nicked and linear material (25,26). To what extent other biopolymers can be fractionated depends mainly on the availability of acrylamino derivatives of biospecific compounds such as coenzymes etc. To our preliminary knowledge (25) the macroreticular pore size structure of the cross-linked bisacrylamide support favours its application for affinity chromatographic purposes of proteins.

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