Journal of Chromatography, 215 (1981) 243–254
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,935

AROMATIC INTERACTION CHROMATOGRAPHY

ACRIFLAVIN GEL FOR SEPARATION OF NUCLEOTIDES, OLIGONUCLEOTIDES AND NUCLEIC ACIDS

JEAN-MARC EGLY
U-184 INSERM, LGME, Faculté de Médecine, 11 Rue Humann, 67085 Strasbourg Cedex (France)

SUMMARY

Using aromatic ligands anchored on insoluble polysaccharide matrices, it is possible to separate many classes of synthetic and naturally occurring substances according to their aromaticity, hydrophobicity or electrostatic character. The strength of the interactions, mainly charge-transfer interactions which occur between ligand and solute, are functions of (i) the electron donor–acceptor properties of the ligand (type, number and electron-attracting or -releasing substituents on the aromatic ring), (ii) the coupling method, (iii) the degree of substitution and (iv) the adsorption and desorption conditions of the solute sample.

Different applications of this type of chromatography to the separation of nucleotides, oligonucleotides and nucleic acids are presented. With acriflavin–dextran, methods have been developed for measuring cyclic AMP in cells pre-labelled with radioactive adenine and a rapid direct assay of 3′,5′-cyclic nucleotide phosphodiesterase activity. With highly substituted acriflavin–agarose it is possible to separate a series of oligonucleotides. The low acriflavin ligand density on agarose permits the separation of single-stranded from double-stranded nucleic acids.

INTRODUCTION

In molecular biology and genetics, aromatic–aromatic interactions involving two aromatic or pseudo-aromatic molecules are of great importance. Such interactions, involving among others electron sharing or electron donation or accepting between molecules, result in the formation of more or less stable molecular complexes (for a review, see ref. 1). One of the components of the complex is generally an amino acid, a nucleoside, a nucleotide or a nucleic acid. In the last case, the second component may be an aromatic compound such as a complementary nucleotide base, an intercalating agent, a mutagen, an antibiotic or a carcinogen, which are known to interact with nucleic acids.

This property of nucleic acids of interacting with aromatic compounds led to the development of chromatographic techniques for their isolation. Poly-A containing mRNA was separated from the other cellular RNAs by affinity chromatography on

0021-9673/81/0000–0000/$02.50

oligo-dT cellulose[2] or poly-U agarose[3] through base-pairing interactions, or by adsorption on nitrocellulose filters[4] or unmodified[5] cellulose (the last adsorption technique was explained by aromatic–aromatic interactions between the nucleic acid and the lignin component of cellulose). Other techniques based on base-pairing interactions have also been developed for the isolation of DNA strands or cDNA–RNA hybrid components.

On the basis of these observations and of the developments in Porath's laboratory, we decided to develop some new chromatographic techniques based on charge-transfer interactions where one of the aromatic components is anchored to an insoluble matrix, and the other is, in this instance, a nucleoside, nucleotide, oligonucleotide or nucleic acid.

In this paper, we shall review the general considerations and the experimental data that led us to choose acriflavin as the ligand to be insolubilized on a polysaccharide matrix. In addition we present some useful applications for the determination of enzymes involved in the synthesis or degradation of nucleosides and nucleotides. We also report a method for the separation of chemically or enzymatically synthesized oligonucleotides and for the separation of single-stranded from double-stranded nucleic acids.

CHOICE OF THE LIGAND

We shall not consider the theory in depth, but refer to Mulliken and Person's work[6]. The principle of molecular complex formation was studied by Porath and coworkers[7,8] and suggested that the adsorption phenomenon does not appear to be related to ionic or molecular sieving effects, but may be due to a charge-transfer effect. This effect should be very useful as a new principle of separation[9–11].

The most important point in such chromatographic procedures is the proper selection of the ligand gel adsorbent. The selection of a particular reagent for introduction into the matrix is based on the following: it should be (i) capable of interaction with nucleotides, (ii) chemically reactive in order to be anchored to a suitable matrix and (iii) chemically stable under the chromatographic conditions. The interaction between the ligand and the nucleotides may be of variable specificity. Many strengths of interaction are involved in the complex formation between the two components. The most important is the π–π interaction between the two components of the complex where the electron donor or acceptor properties of the ligand towards the nucleic acid are a function of the conjugated system (type and number of aromatic rings) and of the electron-attracting or -releasing substituents on the aromatic ring. These parameters will determine the adsorption conditions of the sample on the substituted gels. As an example, planar dyes such as phenyl neutral red are bound to DNA by intercalation[12]; consequently, the fixation of the ligand on the gel and the conditions of chromatography have to be determined in such a way as to favour the intercalation of the dye between the base pairs of the DNA molecule. As discussed previously[13], other types of hypothetical charge-transfer interaction might be involved, such as an n–π complex between the lone-pair electron of oxygen or sulphur and the aromatic ring, or a σ–π complex between the hydrogen of a polyhydroxylic gel and the aromatic ring[7]. Hydrophobic effects and/or electrostatic forces also take place and could be used to our advantage. The choice of the ligand, as in affinity

chromatography, is restricted as it must react chemically with the activated gel. Both chemical reactions, ligand modification and the coupling procedure, have to be effected in such a way that they do not modify extensively the association constant because a decrease or increase in the association constant will condition the adsorption or desorption, respectively, of the solute from the gel.

CHOICE OF ACRIFLAVIN

Acridine drugs induce several biological effects: they have an antiviral and antibacterial effect known as photodynamic action, and they inhibit DNA and RNA synthesis. The biological effects of a wide variety of drugs, including acridine derivatives, have their primary origin in an intimate association with genetic material[14–16]. The first direct evidence for the formation of complexes between purine and flavin was obtained by Weber[17], who showed that adenosine and caffeine quench the fluorescence of riboflavin. Generally, the flavins, derived from 6,7-dimethylisoalloxarine, are very avid complexing agents, and show good electron-acceptor properties. Quantum mechanical calculations suggest that on complexing with purines, a charge-transfer band should be visible in the region of 400 nm[18,19]. On the addition of nucleoside molecules to riboflavin solutions, spectral changes are observed[19]. It is possible to isolate solid purine–flavin complexes[20]. Such complexes are usually strongly coloured, much more so than riboflavin alone[1,13]. The visible spectrum of such a complex dispersed in potassium bromide is different to that of riboflavin, notably in the region around 500 nm[1].

There seems to be good evidence, contrary to the generally held view, that charge-transfer forces are important in the stabilization of nucleotide–flavin complexes in solution and probably when applied to affinity chromatographic techniques. Thus, we chose acridine derivatives and particularly acriflavin as a ligand for developing a chromatographic technique for application to nucleoside compounds.

COMPARISON OF CONSTANTS FOR CHARGE-TRANSFER COMPLEXES

Chromatographic parameters of some nucleosides and nucleotides are compared with other values characteristic of a charge-transfer complex in Table I. Several features are apparent. First, there is a good correlation in the order but not an obvious correlation in the size between the ionization potentials, *i.e.*, the energy of the highest filled molecular orbital (HOMO), the association constants and the V_E/V_T values. This indicates that the complexing is associated with the conjugated electron system of the nucleotide base. The V_E/V_T values of the different nucleotides, when chromatographed on an unsubstituted gel, are below 1.1, which indicates a poor retention of the solute on the gel[21].

Purines and pyrimidines must be considered separately because a purine constituted by two aromatic rings will have different overlapping of its donor orbitals with a flavin than will pyrimidine. Hence, for a given HOMO energy they will have larger association constants (see Table I). This is reflected by the V_E/V_T values, which are higher for purines.

Other features of the association constants and ionization potentials can be noted. Methylated purines, such as caffeine, should be better electron donors than the

TABLE I

COMPARISON OF DIFFERENT VALUES CHARACTERISTIC OF A CHARGE-TRANSFER COMPLEX

K_c = association constant for riboflavin complexes[22]; k = values of the highest filled molecular orbital[23] calculated according to the equation $\Sigma = \alpha + k\beta$ (ref. 24), where α and β are the coulombic and resonance orbitals; V_E/V_T = reduced elution volume (V_E, elution volume; V_T, total volume of the column) on acriflavin–Sephadex G-25 in 0.1 *M* ethylmorpholine buffer (pH 7) containing 0.3 *M* sodium chloride. Column, 6 × 1 cm I.D.; flow-rate, 10 ml/h; temperature, 20°C.

Compound	*Association constant, K_c (l/mole)*	*HOMO, k*	*Chromatographic parameter, V_E/V_T*
Uracil	0.52	0.60	1.10
Thymine	2.44	0.51	1.25
Cytidine	–	–	1.12
Adenine	7.65	0.486	3.29
Caffeine	52.6	–	2.52
Guanine	14.20	0.307	3.41
Adenosine	120	–	2.45
Cytosine	1.82	0.60	1.28
AMP	–	–	1.88
CMP	–	–	0.95
ADP	–	–	1.90

non-methylated purines (adenine and guanine); however, their V_E/V_T values do not reflect their association constants. On the contrary, purines substituted in the 6-position are particularly good complexers[22]. Adenine is a better complexer than adenosine, which in turn is better than adenosine monophosphate. The difference between adenine and adenosine may be the result of either the hydrophilicity or the steric hindrance of the ribose molecule. Further alkylation of the 6-amino group increases the complexing ability in the order ethyl > methyl > propyl[25]. These discrepancies may be explained by steric considerations: in the former instance the methyl group directly bound to the aromatic ring may prevent the approach of the two components, whereas alkylation of the amino group does not exert an effect as it is farther from the ring and may reinforce the electron-donor power of such substituted purines.

PROPERTIES OF ACRIFLAVIN GEL

Acriflavin was covalently bound to polysaccharide gel using epichlorohydrin as an activating agent, as described elsewhere[26]. Then a systematic study of this gel was carried out in order to determine the optimal chromatographic conditions.

Influence of structure on interactions

In order to elucidate the nature of the interactions between nucleotides and acriflavin, we chromatographed different solutes on acriflavin gel (Table II). We observed a strong interaction between acriflavin and adenine (V_E/V_T = 3.25). Glucose did not interact significantly (V_E/V_T = 0.87), although slight adsorption

occurred when a phosphate group was introduced into the solute (glucose-6-phosphate, $V_E/V_T = 1.16$). Analogous observations were made with the amino group of serine or nucleotides[21]. However, when the chromatography was performed at low ionic strength, the phosphate groups of adenosine mono-, di- and triphosphate interacted electrostatically with the positive charge of acriflavin (at the N-9 position). When the ionic strength was increased, the V_E/V_T values decreased. Further, when several adsorption centres were introduced into the solute, the strength of adsorption increased considerably. The high V_E/V_T values for pApApApApA ($V_E/V_T = 9$) and for poly-A, which is completely retained on the gel, are explained by a multi-point attachment effect[27], which is favoured either by the size of the polynucleotide or by an increase in the degree of substitution of the acriflavin on the gel.

TABLE II

CHROMATOGRAPHY OF DIFFERENT COMPOUNDS ON ACRIFLAVIN GEL AT LOW IONIC STRENGTH [0.1 *M* ETHYLMORPHOLINE BUFFER (pH 7)] AND AT HIGH IONIC STRENGTH [0.1 *M* AMMONIUM ACETATE BUFFER (pH 6)–0.3 *M* SODIUM CHLORIDE]

Compound	V_E/V_T	
	Low ionic strength	*High ionic strength*
Glucose	0.87	1.02
Glucose-6-phosphate	1.16	1.02
Adenosine	3.25	3.25
Adenine	2.45	2.50
AMP	4.48	1.88
ADP	9.25	1.95
ATP	14	1.98
ApApC	12	4.50
pApApApApA	n.d.	9
Poly-A	retained	retained

Physico-chemical properties

To characterize the properties of the acriflavin gel, we performed a systematic study of the different parameters which may affect the adsorption properties[21]. Table III summarizes the following properties of acriflavin gel when adenosine and AMP are used as solutes.

An increase in ionic strength on adding 0.2 *M* sodium chloride solution does not affect the acriflavin–adenosine interaction and the V_E/V_T value remains constant ($V_E/V_T = 2.45$), which is not the case in hydrophobic interaction chromatography[28]. On the contrary, the V_E/V_T value of AMP diminishes considerably by neutralization of electrostatic interactions between the phosphate of the nucleotide and the positive charge on acriflavin. The action of the ions of neutral salts on the ligand–solute interaction corresponds to the Hoffmeister series, *i.e.*, an increasing chaotropic effect reduced the interaction.

Amine buffers are often volatile, which is interesting for the recovery of the chromatographed molecules. Addition of amine to a buffer system does not affect the adenosine–acriflavin interaction, whereas the V_E/V_T value of AMP–acriflavin de-

TABLE III

INFLUENCE OF DIFFERENT PARAMETERS ON THE RETENTION OF ADENOSINE AND AMP ON ACRIFLAVIN GEL

Column characteristics as in Table I.

Buffer system	*4°C*		*20°C*	
	Adenosine	*AMP*	*Adenosine*	*AMP*
Ethylmorpholine, 0.1 *M* (pH 7)	3.24	7.22	2.45	4.80
+ NaCl, 2 *M*	1.83	1.54	2.45	1.25
+ Ethanolamine, 0.2 *M*	3.14	2.58	2.50	1.75
+ Urea, 3 *M*	1.98	3.47	1.75	2.23
+ Ethylene glycol, 50%	1.56	4.07	1.71	2.78

creases to 1.75 at room temperature[21]. It may be suggested that the different amines (ethylmorpholine, ethanolamine, triethylamine, diethylenetriamine) interact through a mixed mechanism where the electron-acceptor property of the amino group and the decrease in ionic strength affect the complex association constant of the complex.

In aqueous buffer systems, on increasing the temperature adenosine– and AMP–acriflavin interactions decrease. The temperature effect supports the hypothesis that electron charge transfer is more important for the system than the hydrophobic effect[10].

When the polarity of the eluent is lowered by including ethylene glycol, the gel–solute interaction decreases. In addition, the importance of the electron density of compounds that form charge-transfer complexes was observed[29] with urea and formamide, which are good candidates for disturbing charge-transfer complexes, as is the case for acriflavin–adenosine or pentachlorophenyl–adenosine[30].

APPLICATIONS

Separation of nucleosides and nucleotides

As previously observed, the solutes molecules may interact with the acriflavin gel through both charge-transfer and electrostatic interactions. The latter effect may be abolished by increasing the ionic strength. This interesting property was used to separate cyclic AMP from AMP and adenine derivatives. At low ionic strength, all of the adenine derivatives are separated as a function of their aromatic–aromatic interaction and of the number of phospho groups they contain; their elution is in the order adenosine > adenine > AMP > ADP > ATP (see Table I). In this case cyclic AMP is co-eluted with AMP. In contrast, at high ionic strength, electrostatic interactions are neutralized between phosphate and the positive charge of acriflavin. Thus, both compounds are separated as a function of their ability to interact with acriflavin; AMP is less retarded than the cyclic nucleotide (Table II). Fig. 1 represents the possible role of the phosphate group in the formation of the charge-transfer complex. We suggest that at high ionic strength the phosphate group does not interact electrostatically but is related to the configuration of the nucleotide molecule. In this instance, an intramolecular rearrangement could be favoured in which the phospho group in monoester form can partially prevent the adenine–acriflavin interaction. The

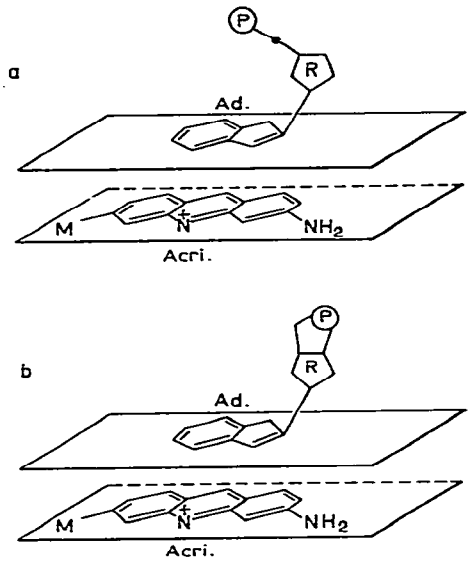


Fig. 1. Schematic representation of interaction at high ionic strength of (a) AMP–acriflavin and (b) cyclic AMP–acriflavin. M = Matrix; P = phosphate group; R =ribose; Ad. = adenine.

phospho group might partially affect the electron-donor capacity of adenine base, thus decreasing the association constant of the complex. On the other hand, the mobility of the phosphate may sterically prevent the complex formation. In contrast, with cyclic AMP the phosphate is covalently bound to the ribose through two phosphodiester bonds, suppressing its mobility and permitting a close approach of the two aromatic components.

Determination of 3′,5′-cyclic nucleotide phosphodiesterase activity. This procedure provides a rapid, sensitive and specific method for the determination of phosphodiesterase activity[31]. In this instance unreacted cyclic nucleotides can be separated from their corresponding 5′-nucleotides once enzymatic assay has been effected. The separation is performed on acriflavin gel at high ionic strength. A large number of assays can be run in parallel. In this instance, 5′-nucleotidase action is not necessary[32]. The assay is quantitative as some possible secondary products are taken into account (Figs. 1 and 3 in ref. 31): first, 5′-IMP resulting from a 5′-nucleotide deaminase action is co-eluted with 5′-AMP and/or 5′-GMP; secondly, when 5′-nucleotidase, nucleosidases and/or nucleoside deaminases are present in the tested sample, their products can be evaluated by measuring the hydrolysed products of ^{14}C-labelled 5′-AMP or 5′-GMP included in the assay mixture before the incubation test. As an example[31], we showed that such purine-metabolizing enzymes were absent from semi-purified calmodulin-dependent phosphodiesterase preparations from rat pancreas but were present in crude extracts of various tissues.

Determination of cyclic AMP in cells pre-labelled with radioactive adenine. Acriflavin gels were used for the determination of the relative amounts of cyclic AMP newly synthesized from ATP in cells pre-labelled with adenine[33]. The cyclic AMP formed from ATP was extracted with perchloric acid. The neutralized perchloric acid was applied to an acriflavin column equilibrated in low ionic strength buffer. After

elution of adenine, the ionic strength is increased for desorption of the other adenosine-related nucleotides. This latter fraction is concentrated by lyophilization and chromatographed on the same acriflavin gel pre-equilibrated with a high ionic strength buffer. Under these conditions, cAMP is separated from AMP, ADP and ATP and up to 95% of the cyclic nucleotide is recovered.

Separation of oligonucleotides

The separation of chemically or enzymatically synthesized oligonucleotides is of a growing interest. Usually the separation of oligonucleotides requires ion-exchange resins; however, owing to the small difference in charge between two consecutive oligomers, the separations are often incomplete. Previous work has shown that when several adsorption centres are introduced into the solute molecule, cooperative π–π interactions will occur with an increase in the strength of the interactions[21]. Similarly, an increase in the degree of substitution of acriflavin on the gel will increase the number of interactions between the ligand and nucleoside base, and consequently the strength of adsorption. Thus a highly substituted acriflavin–agarose permits the simple and efficient analytical and preparative separation of oligonucleotides[34]. In addition to aromatic interactions, the separation power is improved by some electrostatic effects. Fig. 2 shows an elution profile of an oligo-I series; the sample is adsorbed at low ionic strength, and once the mononucleotides have eluted one applies a salt gradient (sodium chloride or ammonium acetate as a volatile buffer) which permits a good separation of the different oligonucleotides.

In view of its preparative possibilities, low cost and rapidity (we are able to perform the chromatography in 3 h), this novel type of chromatography is a serious competitor for high-performance liquid chromatography (HPLC).

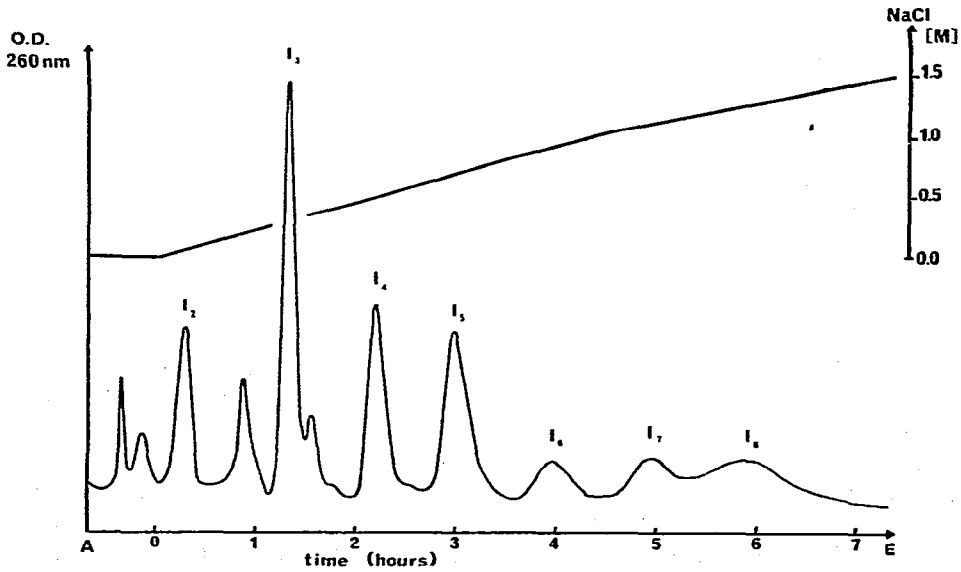


Fig. 2. Separation of oligo-I series on highly substituted acriflavin–agarose. Column, 40 × 0.38 cm I.D.; flow-rate, 19.5 ml/h; room temperature (20°C); buffer, 0.1 *M* sodium acetate–acetic acid (pH 4.5).

Separation of single-strand from double-strand nucleic acid

Acridine dyes have been shown to bind double-stranded DNA by two different types of interactions[35]. One, a strong binding process, becomes saturated when a dye molecule is bound by four or five nucleotides. The other is a weak outside-binding mechanism which involves mainly electrostatic interactions between the positive charge of the drugs and the DNA phosphate.

These observations provide evidence for a model in which strong binding occurs when cationic acridine molecules intercalate between two adjacent base layers in the DNA helix[36]. In chromatography, whatever the degree of substitution of acriflavin on the gel, at low ionic strength DNA molecules are adsorbed. With an increase in ionic strength, DNA alone is retained on highly substituted gel[37]. In solution, ethidium bromide does not interact with DNA at high salt concentrations[38]. In contrast, when the chromatography is performed on a low-substituted gel DNA is not adsorbed, first because electrostatic interactions are neutralized and second because high salt concentrations increase the contribution of the water extrusion to base pairing of the complementary strands by enhancing the stability of the helix[39]. Under these conditions the intercalation of acriflavin is prevented. Nevertheless, acridines can form complexes with RNA but the binding with RNA is weaker[40]. At high salt concentrations, RNA molecules are adsorbed on the gel through a stacking effect and multi-point attachment[27], although electrostatic interactions are neutralized. This property was used to discriminate single-stranded from double-stranded nucleic acid[37]. Fig. 3 shows the chromatography of DNA on acriflavin gel. Less than 4% of the non-adsorbed DNA fraction is hydrolysed by nuclease S1, which digests single-stranded nucleic acid. In contrast, the retained fraction contains 61% S1-sensitive material, which indicates that the retained fraction contains a large amount of single-strand regions. Similar separations may occur to obtain only the RNA–DNA hybrid[37].

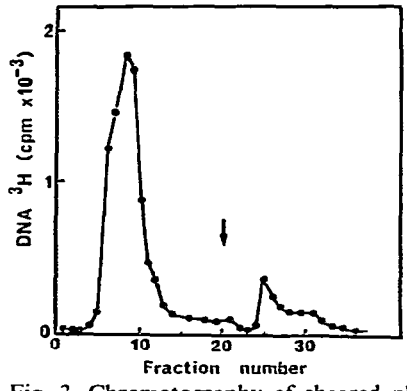


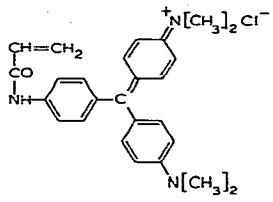
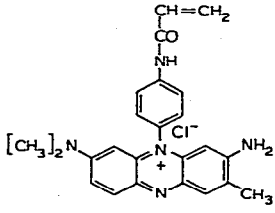
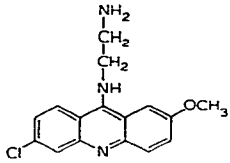
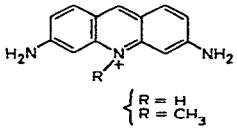
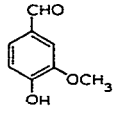
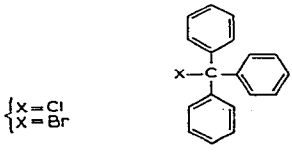
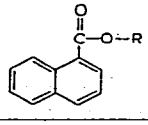
Fig. 3. Chromatography of sheared plasmacytoma DNA on acriflavin agarose. A 200-μg amount of [^{3}H]DNA is adsorbed on 1 ml of acriflavin gel packed in a Pasteur pipette. The adsorption is effected in 10 m*M* Tris–hydrochloric acid buffer (pH 7.6)–0.5 *M* potassium chloride solution and the elution in 10 m*M* Tris–hydrochloric acid buffer (pH 7.6)–0.5% sodium dodecyl sulphate.

TENTATIVE DEFINITION OF AROMATIC INTERACTION CHROMATOGRAPHY

To our knowledge, the separation of nucleosides, nucleotides, oligonucleotides and nucleic acids using a substituted matrix has not been fully developed elsewhere.

TABLE IV

AROMATIC-SUBSTITUTED GELS FOR SEPARATION OF NUCLEIC ACID-RELATED COMPOUNDS

Ligand		*Specificity*	*Ref.*
Name	*Formula*		
Malachite green		A–T base pair specific sheared DNA	12
Phenyl neutral red		G–C base pair specific sheared DNA	12
Acridine yellow		Retention of open, circular and linear DNA. Covalently closed circular DNA is not retained	42
Acriflavin		Aromatic–aromatic interactions. Separation of nucleotides oligonucleotides. Discrimination between single-stranded and double-stranded nucleic acid	31, 33 34, 37
Lignin (MN-cellulose)		Aromatic–aromatic interactions. Poly-A retention	5
Trityl		Hydrophobic stacking type bonds. Poly-A retention	43
Naphthoyl		Hydrophobic bond. tRNA separation	44

In every case, the fixed ligand is an aromatic molecule (Table IV). The nature of the interaction is not always well discussed. Nevertheless, it seems that the bases of the nucleic acid play an important role in the adsorption procedure. For example, malachite green or phenyl neutral red gel are used for base-pair specific fractionation of sheared DNA[12]. Acridine drug separates nucleic acid-related compounds as a function of its intercalating property[37–42] and of the base composition of the solute[31,33,34]. Trityl gel and MN-cellulose show a certain specificity for the poly-A region of mRNA[5,43], whereas a naphthoyl matrix separates nucleic acids through a mixed mechanism in which the nature of the base and the secondary structure of nucleic acid are involved[44]. The authors are not in agreement about the nature of the interaction. In fact, as shown by several studies, charge-transfer (π–π) interactions play an important role, as suggested by the formation of coloured complexes[10,13], by the electron donor–acceptor properties of the ligand[11,13], by some spectral modifications[17–19] and by the temperature effect[10,21]. Nevertheless, other types of interaction may also take place, as follows.

Hydrophobic effects occur. First, the release and ordering of the bound water molecules can significally stabilize the complex through a solvation effect[39,45]. Secondly, Van der Waals forces may occur between two non-polar groups involved in the complex formation; addition of a polar solvent such as ethylene glycol decreases the hydrophobic effect.

Water may also affect the adsorption process, presumably by formation of hydrogen bonds. Hydrogen bonding occurs equally between two complementary bases[11,39].

Electrostatic interactions, as for some acridine drugs, are present and may either stabilize or disturb complex formation[33,34,37].

We therefore think that the term "charge-transfer chromatography" is ambiguous and too restricted to one kind of interaction between an electron donor and an electron acceptor molecule. As observed, several types of interaction are present, and their extent may be altered by variation of the ligand molecule and of the environment determined by the buffer composition. The importance of these different forces varies as a function of the nature of both molecules which are associated in the complex, and of the environment determined by the buffer composition. For these reasons, we prefer to use the term "aromatic interaction chromatography" (AIC), which includes the important part of the charge-transfer effect and also does not exclude the other types of interaction that may occur between two aromatic or pseudo-aromatic molecules.

In conclusion, it may be noted how a good knowledge of a technical problem, which in our case was the systematic study of the numerous parameters involved in aromatic interaction chromatography (with, *e.g.*, acriflavin gel), permits in a second more fruitful step the development of several applications for the separation of nucleotide-related compounds. There are numerous further promising applications for the separation of aromatic compounds. The rapidity of this type of chromatography, the good separation power and the possibility of using it for large-scale separations make it a serious competitor for other types of chromatography such as molecular sieving and ion-exchange and high-performance liquid chromatography.

ACKNOWLEDGEMENTS

It is a pleasure to thank Drs. E. Boschetti and C. Rochette-Egly for their important contributions to this work. Thanks are also due to Professors P. Chambon and J. Kempf for their continuous interest in the work and to J. L. Plassat for his inestimable technical assistance.

REFERENCES

1 M. A. Slifkin, *Charge Transfer Interactions of Biomolecules*, Academic Press, London, 1971.
2 H. Nakazato and M. Edmonds, *J. Biol. Chem.*, 247 (1972) 3365–3367.
3 U. Lindberg and T. Persson, *Eur. J. Biochem.*, 31 (1972) 246–254.
4 S. Y. Lee, J. Mendecki and G. Brawermann, *Proc. Nat. Acad. Sci. U.S.*, 68 (1971) 1331–1336.
5 P. A. Kitos, G. Saxon and H. Amos, *Biochem. Biophys. Res. Commun.*, 47 (1972) 1426–1433.
6 R. S. Mulliken and W. B. Person, *Molecular Complexes*, Wiley-Interscience, New York, 1969.
7 D. Eaker and J. Porath, *Separ. Sci.*, 2 (1967) 507–511.
8 J. Porath, *Proc. Nobel Symp.*, 3 (1967) 302–315.
9 J. Porath and K. Dahlgren Caldwell, *J. Chromatogr.*, 133 (1977) 180–183.
10 J. M. Egly and J. Porath, in O. Hoffmann-Ostenhof, M. Breitenbach, F. Koller, D. Kraft and O. Scheiner (Editors), *Affinity Chromatography (Proc. Int. Symp., Vienna, Sept. 20–24, 1977)*, Pergamon Press, Oxford, New York, 1978, pp. 5–22.
11 J. Porath and B. Larsson, *J. Chromatogr.*, 155 (1978) 47–68.
12 H. Bunemann and W. Muller, *Nucl. Acids Res.*, 5 (1978) 1059–1074.
13 J. Porath, *J. Chromatogr.*, 159 (1978) 13–24.
14 W. Muller and D. M. Crothers, *J. Mol. Biol.*, 35 (1968) 251–290.
15 H. M. Sobel and S. J. Jain, *J. Mol. Biol.*, 68 (1972) 21–34.
16 C. Craig and I. Isenberg, *Biopolymers*, 9 (1970) 689–696.
17 G. Weber, *Biochem. J.*, 47 (1950) 114–121.
18 P. S. Song, *J. Amer. Chem. Soc.*, 91 (1969) 1850–1858.
19 M. A. Slifkin, *Biochim. Biophys. Acta*, 103 (1965) 365–373.
20 J. F. Pereira and G. Tollin, *Biochim. Biophys. Acta*, 143 (1967) 79–88.
21 J.-M. Egly and J. Porath, *J. Chromatogr.*, 168 (1979) 35–47.
22 J. C. M. Tsibris, D. B. McCormick and L. D. Wright, *Biochemistry*, 4 (1965) 504–510.
23 A. Szent-Gyorgyi, *Introduction to a Submolecular Biology*, Academic Press, New York, 1960.
24 B. Pullmann and A. Pullmann, *Rev. Mod. Phys.*, 32 (1960) 428–434.
25 J. A. Roth and D. B. McCormick, *Photochem. Photobiol.*, 6 (1967) 657–664.
26 J. M. Egly, *FEBS Lett.*, 99 (1978) 369–372.
27 H. P. Jennissen, *Biochemistry*, 14 (1975) 754–760.
28 S. Påhlman, J. Rosengren and S. Hjertén, *J. Chromatogr.*, 131 (1977) 99–108.
29 V. Gutmann, *The Donor–Acceptor Approach to Molecular Interaction*, Plenum Press, New York, 1978.
30 J. L. Ochoa, J. Porath, J. Kempf and J.-M. Egly, *J. Chromatogr.*, 188 (1980) 257–261.
31 C. Rochette-Egly and J. M. Egly, *J. Cyclic Nucl. Res.*, 6 (1980) 335–345.
32 W. J. Thompson, G. Brooker and M. M. Applemann, *Methods Enzymol.*, 38 (1974) 205–212.
33 C. Rochette-Egly, J. Kempf and J. M. Egly, *J. Cyclic Nucl. Res.*, 5 (1979) 397–406.
34 E. Boschetti, P. Girot and J. M. Egly, *FEBS Lett.*, submitted for publication.
35 J. Peacoxe and N. Skerrett, *Trans. Faraday Soc.*, 52 (1956) 261–279.
36 L. S. Lerman, *J. Mol. Biol.*, 3 (1961) 18–30.
37 J. M. Egly, J. L. Plassat and E. Boschetti, *Anal. Biochem.*, submitted for publication.
38 J. B. Le Pecq and C. Paoletti, *J. Mol. Biol.*, 27 (1967) 87–106.
39 S. Lewin, *Displacement of Water*, Academic Press, London, 1974.
40 T. Tinkelstein and J. B. Weinstein, *J. Biol. Chem.*, 242 (1967) 3763–3768.
41 G. Bernardi, *Nature (London)*, 206 (1965) 779–780.
42 W. S. Wincent, III and E. S. Goldstein, *Anal. Biochem.*, 110 (1981) 123–127.
43 P. Cashion, G. Sathe, A. Javed and A. Kuster, *Nucl. Acids Res.*, 8 (1980) 1167–1185.
44 S. Hjerten, V. Hellmann, I. Svenson and J. Rosengreen, *J. Biochem. Biophys. Method*, 1 (1979) 263–273.
45 R. Forster, *Organic Charge Transfer Complexes*, Vol. 15, Academic Press, London, 1969.