Review Letter

AFFINITY CHROMATOGRAPHY ON COLUMNS CONTAINING NUCLEIC ACIDS

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1. Introduction

Chromatography on affinity adsorbents containing polynucleotide ligands dates back to 1968 when successful purification of micrococcal DNA polymerase on a column containing DNA immobilized onto cellulose after irradiation with ultraviolet light was described by Rose Litman [1]. In the same year both Bruce Alberts [2] and Peter Gilham [3] independently suggested new methods for the preparation of adsorbents containing nucleic acids following earlier attempts mainly with oligonucleotides [4,5] or heavily damaged DNA [6,7] linked to different insoluble supports. Since that time, affinity chromatography using immobilized nucleic acids has established itself as a useful technique [8–10] which has recently received diverse applications in molecular biology [11–20].

The following discussion summarizes the most significant methods of immobilization of polynucleotides and analyses applications of the affinity adsorbents obtained.

2. Immobilization of nucleic acids onto solid supports

2.1. *Adsorption onto cellulose*

Attachment of single- and double-stranded DNA to inert cellulose matrices by lyophilization as introduced [2] leads to fairly stable DNA–cellulose complexes which have been widely used in the study of proteins involved in cellular polynucleotide synthesis and degradation. Besides being very simple this method allows linkage of large amounts of DNA in yields around 50%. Due to the mild and non-destructive procedure damage to the ligand is kept to a minimum. DNA–Celluloses with similar properties but still higher ligand concentrations have been prepared by drying the polynucleotide to the cellulose matrix in an evacuated desiccator at room temperature over silica gel (H. P., unpublished observation). The lyophilization step can be omitted in this case.

Since DNA columns prepared by adsorption of the polynucleotide ligand to cellulose as described above exhibit only limited stability to desorbing conditions such as elevated temperatures, hydrophilic organic solvents (e.g., formamide), and low ionic strengths of the buffers used, they cannot be recommended for nucleic acid hybridization. But they provide excellent results in the chromatography of proteins with affinity for DNA. In their initial paper Alberts and Herrick [9] described the purification of T4 gene 32 protein, *E. coli* RNA polymerase, T4 DNA polymerase (gene 43 protein), as well as other DNA-binding proteins, the latter not having been identified as to their biological functions. The method has subsequently been used to prepare affinity adsorbents for the isolation of single-strand specific DNA binding proteins from different organisms [21–28].

RNA of sufficiently high molecular weight can also form similar adsorption complexes with cellulose and columns containing RNA of bacteriophage R_{17} have been successfully applied to the fractionation of RNA binding proteins from an RNAase-deficient strain of *E. coli* [9]. Attempts to bind tRNA failed, however, probably because the much smaller molecule has fewer sites of interaction with the matrix.

2.2. *Ultraviolet-irradiation technique*

Nucleic acids can be linked very efficiently to

insoluble supports such as cellulose, agarose, and fibreglass by short irradiation with ultraviolet light as proposed by Britten [7]. Using this technique Litman developed an improved procedure for immobilization of native DNA to an inert cellulose matrix in apparently large amounts and high yields [1]. The resulting DNA–cellulose complexes are very stable even to strong desorbing conditions and elevated temperatures. Such complexes have successfully been applied to the chromatography of DNA polymerases [1,29,30] and DNA-binding proteins [31,32]. Although for these proteins the capacity of the column is low since irradiation causes considerable damage to the ligand, particularly due to dimerization of adjacent pyrimidine bases, DNA–celluloses prepared by this method should be of considerable value in the chromatography of nucleases specific for ultraviolet-irradiated DNA.

The irradiation technique can also be used to immobilize RNA or synthetic polyribonucleotides to cellulose [33–35] and fibreglass [36] leading to materials which are widely used for the fractionation of RNA-binding proteins [35,37].

2.3. *Activation of phosphate termini*

Columns containing polynucleotides have been prepared from nucleoside 5′-phosphates [4] or synthetic polynucleotides [38] and cellulose by activation of terminal phosphate groups with carbodiimides in anhydrous solutions. This method which introduces stable phosphodiester bonds between the matrix and the ligand has been extended to nucleic acids and improved by Gilham [3] who used water-soluble carbodiimide in aqueous solution at pH 6 thus avoiding modification of the bases by the action of carbodiimide. Both single- and double-stranded DNA as well as RNA can be immobilized in satisfactory yields and damage to the ligand is minimal as the reaction conditions are very mild.

The technique is not restricted to cellulose only since other matrices such as Sephadex [39] have been used. It is, however, essential that the molecular weight of the polynucleotide does not exceed a limit above which non-specific interactions of the ligand with the polysaccharide matrix cannot be avoided. Because of the exceptional stability of the resulting adsorbents to temperature and formamide they are particularly useful materials for the chromatography of nucleic acids which hybridize to the ligand [5,38,40] and furthermore they have been used as insoluble templates for DNA- and RNA polymerase and initiators of the terminal deoxynucleotidyltransferase [41].

2.4. *Immobilization onto different cellulose derivatives*

In a number of attempts to develop column materials containing large amounts of tightly immobilized nucleic acids, different cellulose derivatives have been examined for their ability to provide such adsorbents. Materials consisting of polynucleotides linked to phosphocellulose [6,40], nitrocellulose [42,43], aminoethylcellulose [8], and *m*-diazobenzoyloxymethylcellulose [44] have been developed and applied mainly to nucleic acid hybridization.

Recently the immobilization of nucleic acids onto carboxymethylcellulose by an easy efficient reaction procedure has been reported [45,46]. The resulting adsorbents contain large amounts of the polynucleotide and exhibit stability to elevated temperatures and to high formamide concentrations but only limited stability to alkaline conditions. DNA–Celluloses prepared by this technique provide excellent adsorbents for the chromatography of DNA polymerases [14,45] and other proteins with affinity for DNA. It has been shown that ion-exchange effects of non-reacted carboxyl groups do not interfere with the affinity chromatography of such enzymes when appropriate conditions are employed.

2.5. *DNA–agar procedure*

Immobilization of DNA by entrapment in agarose gel and attempts to apply the resulting materials to nucleic acid fractionation have been reported [47]. The method which involves trapping of single-stranded DNA molecules in a 4% agar during gel formation has been improved by Schaller et al. [48] who described successful chromatography of *E. coli* DNA polymerase I, RNA polymerase, exonuclease III, as well as T4 polynucleotide kinase [48,49]. It has been shown that relatively high amounts of proteins are retained by DNA–agarose columns due to non-specific interactions with the gel. This problem can be overcome by carrying out several cycles of adsorption and elution [48] or better beading the DNA–agar gel via addition of a hydrophilic organic solvent.

The agar procedure also allows immobilization of circular double-stranded DNA [50]; linear double-stranded DNA, however, is more efficiently entrapped in polyacrylamide gels [51].

2.6. *Immobilization onto polysaccharides via CNBr-activation*

Since CNBr-activated Sepharose provides an ideal matrix for the linkage of ligands containing nucleophilic groups [52,53] immobilization of different nucleic acid species and synthetic polynucleotides to such supports has been attempted and well discussed [29,54]. It has been shown that single-stranded DNA and RNA can be immobilized efficiently; native double-stranded DNA, however, does not react except after introduction of single-stranded ends by partial degradation with exonuclease III. Arndt-Jovin et al. [55] reported a modification of this method which allows linkage of DNA in ratios about 50 times higher by using reaction solutions of lower ionic strengths. DNA–Sepharose columns prepared by this technique have been applied to the chromatography of *E. coli* DNA polymerase I and RNA polymerase and can also be recommended for nucleic acid hybridization. RNA preparations of low molecular weight such as tRNA can be linked to CNBr-activated agarose preferentially after introduction of a spacer-arm as suggested by Robberson and Davidson [56]. The method involves periodate oxidation of the nucleic acid which is subsequently coupled to an agarose matrix containing hydrazide functions at the end of a 5-membered hydrophobic spacer-arm. One might question the long term stability of such linkages.

2.7. *Immobilization onto polysaccharides via bis-oxirane activation*

Although polysaccharides activated with bifunctional epoxides have proven excellent supports for the linkage of ligands containing nucleophilic groups [57], immobilization of nucleic acids via this method has not until recently been described [58]. The procedure which is both simple and efficient allows preparation of derivatized celluloses and beaded agarose gels containing single-stranded polynucleotide ligands in particularly high concentrations. DNA–Sepharose prepared by this method is an excellent adsorbent for the fractionation of DNA- and RNA polymerases and should also be applicable to the chromatography of single-strand specific DNA-binding proteins. Because of their stability to temperature, formamide, as well as alkaline conditions polynucleotides immobilized via the bis-oxirane method could be of considerable value to nucleic acid hybridization. The immobilization techniques discussed above are summarized in table 1.

3. Application of immobilized nucleic acids to affinity chromatography

Considerable interest has recently been shown in the application of immobilized macromolecules to affinity chromatography. In particular adsorbents consisting of polynucleotides linked to insoluble supports (in many cases derivatized polysaccharides) have received wide use in molecular biology:

1. Several enzymes involved in polynucleotide synthesis and repair such as DNA-dependent DNA- and RNA polymerase [1,14,29,30,37,45,48,55,58], RNA-dependent DNA polymerase [59–61], polynucleotide kinase [49], exonuclease III [49] and correndonuclease II [62] have been purified by fractionation on such adsorbents.

2. Besides hybridization on nitrocellulose filters, affinity chromatography of nucleic acids on polynucleotides covalently immobilized onto different matrices has been successfully applied to their base-specific separation [7,40,44,55].

3. A class of single-strand specific DNA-binding proteins with an ability to destabilize the double helix of native DNA has recently led to exciting reflections on the DNA replication problem [63]. These together with a variety of other cellular or virus-induced proteins, amongst them a class of DNA-binding enzymes which denature double-stranded DNA by an ATP-dependent mechanism [64,65], are highly susceptible to affinity fractionation on DNA columns.

Affinity chromatography of proteins interacting specifically with polynucleotides of defined sequence (e.g., restriction endonucleases) has not yet been well discussed. This might be due to the fact that chemical

Table 1
Immobilization techniques and properties of the resulting affinity adsorbents

Method	Nucleic acid	Ligand (mg/ml)	Yield (%)	Applications (fractionation)	Ref.
Adsorption to cellulose	Native and denatured DNA	1.5	50	proteins	[2,9] [21–28]
Ultraviolet-irradiation technique	DNA	4.0	90	proteins	[1,7] [29–37]
	RNA	2.0	40		
Carbodiimide-activation	DNA	0.5	50	proteins, nucleic acids	[3–5] [38–41]
	tRNA	[a]	30		
Linkage to CM-cellulose	DNA	3.5	50	proteins	[14,45,46]
	rRNA	2.5	40		
Entrapment in agarose gel	Single-stranded DNA	3.5	75	proteins nucleic acids	[47–51]
CNBr-activated polysaccharides	DNA	2.5	80	proteins, nucleic acids	[29,52–56]
	RNA and polyribonucleotides	1.0	95		
Bis-oxirane -activated polysaccharides	Single-stranded DNA	5.0	70	proteins	[57,58]
	tRNA	7.0	85	nucleic acids	

[a]Data not available

and enzymatic syntheses of such sequences in amounts necessary for affinity chromatography presents a major problem.

Since in affinity chromatography, interactions between the components should mimic free solution, careful consideration has to be given to the nature of the support, the ligand, and the method of immobilization. The selection of the ligand (molecular weight and type of the polynucleotide) is generally determined by the problem and need not be further discussed here.

3.1. *The choice of the matrix*

In general the nature of the matrix should be such that optimal performance of the column combines with minimal non-specific interactions. The ideal matrix should therefore be an insoluble porous network which permits unimpaired movement of macromolecules. It should have an abundant supply of chemical groups which can be modified to allow covalent immobilization of the ligand but must not interfere with affinity chromatography through non-specific interactions with macromolecules to be purified. Good chemical and mechanical stability are essential [66].

A variety of insoluble supports, amongst them cellulose, polystyrene, crosslinked dextrans, polyacrylamide, porous glass and agarose have successfully been used as matrices in affinity chromatography. Not all of them have yet been exploited for the immobilization of polynucleotide ligands, the materials most widely applied for the preparation of such adsorbents being polysaccharides such as cellulose, agarose, and their derivatives. The advantage of cellulose as affinity matrix is its high chemical, mechanical and thermal stability. It therefore provides an ideal support for nucleic acid hybridization. For protein fractionation cellulose and its derivatives combine good mechanical properties with high flow rates. Because of its inhomogeneous structure, however, undesirable side effects reducing the capacities of the columns are likely to arise. Beaded agarose

gels exhibit many of the properties ideal for protein fractionation but their low mechanical stability often causes restrictions in the applicability of the adsorbents. Spherical cellulose derivatives [67] and cross-linked agarose such as CL-Sepharose, Sephacryl (Pharmacia) and Ultrogel (LKB) might lead to an improvement but they have not yet been investigated with regard to nucleic acid immobilization.

3.2. *The choice of the method of immobilization*

In spite of the demand for good affinity adsorbents containing polynucleotide ligands no really optimal method for the preparation of such materials has been elaborated at present. Adsorbents containing nucleic acids adsorbed to the matrix are highly sensitive to desorbing conditions which causes restrictions of their applicability with regard to temperature, pH and ionic strength of elution buffers. Contamination of the eluate with ligand molecules is difficult to avoid. In addition to these drawbacks, the performance of the columns is often inadequate. Other materials with large amounts of polynucleotide immobilized via ultraviolet-irradiation contain heavily damaged ligand, the latter adsorbents being useful only for the fractionation of endonucleases specific for pyrimidine dimers [62,68]. Adsorption of nucleic acids to CM-cellulose leads to materials with high stability and good performance but the mechanism of the immobilization reaction is not well understood [45,46].

Affinity adsorbents with covalently immobilized polynucleotide ligands suffer from a low ligand concentration [40] or the introduction of additional charges on the matrix as a result of CNBr activation [29,55]. Small polynucleotides are best immobilized after introduction of a spacer-arm [56] which can on the other hand give rise to undesirable hydrophobic interactions with proteins. Immobilization of nucleic acids onto polysaccharides via bis-oxirane activation as recently suggested in our laboratory [58] seems an adequate approach to overcome some of these disadvantages. Nevertheless the relatively high pH of the procedure necessary for an efficient reaction is far from being ideal since denaturation of double-stranded DNA and partial degradation of polyribonucleotides can hardly be avoided.

This brief article is intended to promote interdisciplinary discussion between chemists who design affinity adsorbents, and biochemists who wish to apply such materials to particular separation problems.

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