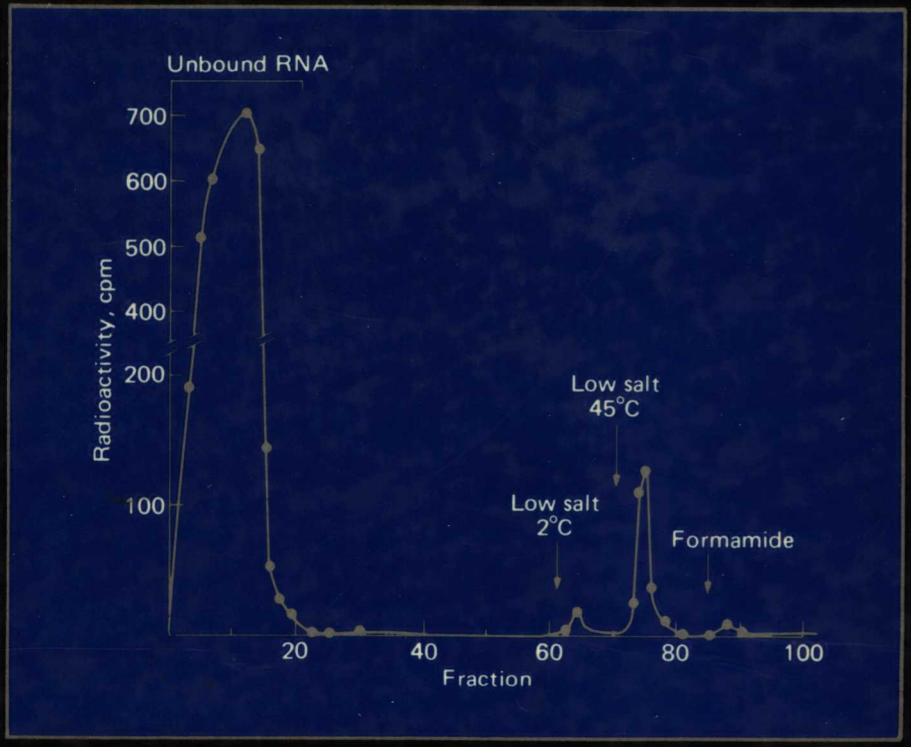
CHROMATOGRAPHIC SCIENCE SERIES VOLUME 27

AFFINITY CHROMATOGRAPHY

Template Chromatography of Nucleic Acids and Proteins



Herbert Schott

Affinity Chromatography

TEMPLATE CHROMATOGRAPHY OF NUCLEIC ACIDS AND PROTEINS

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MARCEL DEKKER, INC. New York and Basel

Library of Congress Cataloging in Publication Data

Schott, Herbert, [date]
Affinity chromatography.

(Chromatographic science ; v. 27)
Bibliography: p.
Includes index.
1. Affinity chromatography. 2. Nucleic acids--Separation. 3. Proteins--Separation. I. Title. II. Series. [DNLM: 1. Chromatography, Affinity. 2. Nucleic Acids--isolation & purification. 3. Proteins--isolation & purification. W1 CH943 v.27 / QU 25 S375a]
QP519.9.A35S34 1984 574.19'245 84-17461
ISBN 0-8247-7111-7

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Preface

Affinity chromatography, which uses the specific interactions between natural products for their separation, purification, and isolation, has provided an essential contribution to the rapid development of molecular biology and, especially, genetic engineering. The huge number of publications, especially in these current fields, ranges from the isolation of nucleic acids, genes, oligonucleotides, and nucleic acid fragments to the purification of special proteins and enzymes as well as investigations of protein-nucleic acid interactions. Since this interesting field has not been previously summarized sufficiently in books on affinity chromatography, it seems the right time to describe the published material more extensively in a book. Since the template functions of nucleic acids, specifically the base-pairing mechanism, are used for chromatography in almost all papers, so "Template Chromatography of Nucleic Acids and Proteins" seemed to be the obvious subtitle for the book.

The book is arranged as follows. First, various techniques are shown for immobilization of nucleic acid fragments, polynucleotides, and nucleic acids by which the desired separation of materials can be achieved. The main part of the book summarizes examples of applications. The isolation of DNA and its fragments, as well as RNA, is shown in various examples, with ample treatment of the isolation of mRNA. A chapter on protein isolations is followed by a chapter on enzymic synthesis or degradation of polynucleotides. The book ends with studies of peptide nucleotide interactions.

In the given context it is impossible to describe all published results. It is the purpose of this book to inform the reader about the practical methods, possibilities, and limits of template chromatography as well as to lead graduate students, young scientists, and those beginning to interest themselves in molecular biological questions to the original papers.

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Abbreviations

A	adenosine
Ade	adenine
ADP	adenosine-5'-diphosphate
AE	aminoethyl
AMP	adenosine-5'-monophosphate
ATP	adenosine-5'-triphosphate
bp	base pairs
br^8ATP	8-bromoadenosine-5'-triphosphate
C	cytidine
cDNA	complementary deoxyribonucleic acid
cl^6ITP	6-chloroinosine-5'-triphosphate
CM	carboxymethyl
CMP	cytidine-5'-monophosphate
CNBr	cyanogenbromide-activated
CTP	cytidine-5'-triphosphate
Cyt	cytosine
dA	deoxyadenosine
DBM	diazobenzyloxymethyl
dC	deoxycytidine
DEAE	diethylaminoethyl
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
ds	double-stranded
dT	deoxythymidine

EDTA	ethylenediaminetetraacetic acid
G	guanosine
GMP	guanosine-5'-monophosphate
GTP	guanosine-5'-triphosphate
Gua	guanine
H	heavy
HnRNA	heterogeneous nuclear ribonucleic acid
I	inosine
IMP	inosine-5'-monophosphate
kb	kilobases
L	light
mol wt	molecular weight
mRNA	messenger ribonucleic acid
mt	mitochondrial
mt DNA	mitochondrial DNA
N terminus	amino terminus
oligo(dT)	oligodeoxythymidylate
P-cellulose	phosphocellulose
poly(A)	polyadenylate
poly(C)	polycytidylate
poly(G)	polyguanylate
poly(I)	polyinosylate
poly(U)	polyuridylate
PVAL	poly(vinyl alcohol)
RNA	ribonucleic acid
RNase	ribonuclease
RNP	ribonucleoprotein
rRNA	ribosomal ribonucleic acid
SDS	sodium dodecyl sulfate
s^6ITP	6-thioinosine-5'-triphosphate
TCA	trichloroacetic acid
Thy	thymine
T_m	melting temperature
tRNA	transfer ribonucleic acid
U	uridine
UDP	uridine-5'-diphosphate
UMP = pU	uridine-5'-monophosphate
UTP	uridine-5'-triphosphate
UV	ultraviolet

Contents

1

Introduction

It is known that the exact replication of genetic information is controlled by the base pair mechanism described by Watson and Crick. This mechanism depends on the fact that Ade and Cyt undergo specific interaction with Thy and Gua, respectively.

The base pairs Thy·Ade and Cyt·Gua are thus referred to as complementary base pairs. Single strands of nucleic acids posessing complementary base sequences will aggregate to double strands under certain conditions. The association is not restricted to complementary single strands of nucleic acids but can, under certain conditions, also be observed among poly-, oligo-, and mononucleotides; nucleosides, and nucleobases.

Since base pairing is specific and reversible, it recommends itself as the basis of a form of affinity chromatography suitable for the separation and isolation of nucleic acids and their fragments. This is accomplished in practice by immobilizing defined nucleic acids, polynucleotides, oligonucleotides, or nucleic acid residues on a stationary phase. If the conditions of base pairing are fulfilled, the complementary partner in the mobile phase is strongly adsorbed by the stationary complementary partner, noncomplementary partners being retarded to only a small extent or not at all. After the elution of the noncomplementary components, the conditions of elution are altered such that base pairing is eliminated, and the adsorbed compounds are desorbed.

These immobilized compounds have the same template function as DNA, since only the nucleotide sequence of the immobilized

compound determines which partner is complementary and which is thus adsorbed. The template function can be used additionally for the isolation of enzymes and other natural substances if they are capable of undergoing specific interactions with stationary nucleotide sequences.

This special form of affinity chromatography, which has shown rapid development in the last few years, may thus be termed *template chromatography*. The method has established itself as a useful technique which has recently received diverse applications in molecular biology as well as in the study of enzymes. The use of template chromatography in the study of enzymes is not surprising since about 30% of the approximately 2000 enzymes found in the cell participate in reactions involving nucleotides/nucleotide coenzymes.

The requirements of a suitable system for template chromatography are dictated by the moiety to be purified, the coupling of ligand to matrix, and the working milieu. For many research purposes details of molecular parameters may be vital: knowledge and reproducibility of coupling modes, matrix characteristics, and ligand-matrix-medium interactions then assume paramount importance. For large-scale production in particular, mechanical stability, rapid flow rate, resistance to microbial attack, and reuse are important factors. It will often be necessary to space the ligand from its support. This may be achieved either by coupling the ligand to one end of an "arm," the other end of which is subsequently attached to the carrier, or by coupling it to an arm already modifying the matrix. This precaution will be superfluous if the ligand is bulky enough to make its binding site(s) available.

The basic problem in template chromatography, that of immobilizing the nucleic acids and their fragments on a suitable support in order to form the stationary phase, may be solved in several ways, as will be shown.

2

Immobilization of Nucleoside Phosphates, Nucleosides, and Nucleobases

It is the aim of this chapter to review the basic strategies of immobilizing nucleoside phosphates, nucleosides, and nucleobases to insoluble supports. The affinity between the covalently bound nucleic acid component and the partner of the mobile phase to be isolated is highly stereospecific. The interaction does not only lead to a complex formation. The subsequent displacement of the molecule from the complex will depend (a) on the three-dimensional orientation, (b) on the group and position on the ligand molecule through which it is immobilized to the support, (c) to some extent on the chemical method by which the column is made, and (d) on the spacer distance between the ligand and the supporting matrix. Moreover, the exact mechanism by which the immobilized ligand interacts with the partner of the mobile phase is very important for the design of the optimal matrix.

Columns with immobilized nucleic acid components were prepared particularly with a view to using them to investigate the wide field of enzymes that interact with nucleic acids and/or their components. The variety of these enzymes required template columns in which different positions of the nucleic acid components are bound to polymer support.

I. IMMOBILIZATION OF NUCLEOSIDE PHOSPHATES

Figure 1, for example, demonstrates the possible positions on AMP that can be functionalized for the immobilization to a polymer sup-

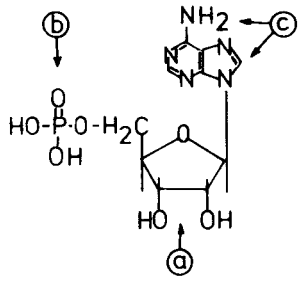


Figure 1 Positions of AMP that can be derivatized: (a) ribosyl-linked; (b) phosphate-linked; (c) N^6-linked and C-8-linked. (From Ref. 3.)

port. In essence, these can be classified into (a) phosphate-linked and (b) sugar-linked derivatives having an unsubstituted nucleobase, and (c) base-linked nucleoside phosphates containing free sugar and phosphate groups.

An immobilization of nucleotides in which several positions of the ligand are simultaneously involved, for example, amide linkage through an amino group, esterification of sugar hydroxyls, or anhydride formation with the phosphoric acid residue, results in an ill-defined matrix. Rather, it is better to aim for methods that result in a more defined matrix, the interaction of which can be more exactly predicted and controlled.

A lot of literature has been concerned with designing simplified chemical methods for preparing polymer support derivatized with nucleic acid components. The immobilization of ligands to several supports (e.g., CNBr-Sepharose, AE-Sepharose, CM-cellulose, AE-cellulose, and functionalized nylon) is described [1] and the yields of several reactions are summarized in Table 1.

The most exploited procedure for immobilization of nucleic acid components and their derivatives is the CNBr method, which has been reported by many laboratories. The results described, however, are very different [3-6]. The major problem in the preparation of adsorbents suitable for template chromatography is the design and synthesis of nucleotide ligands to be attached to the insoluble support.

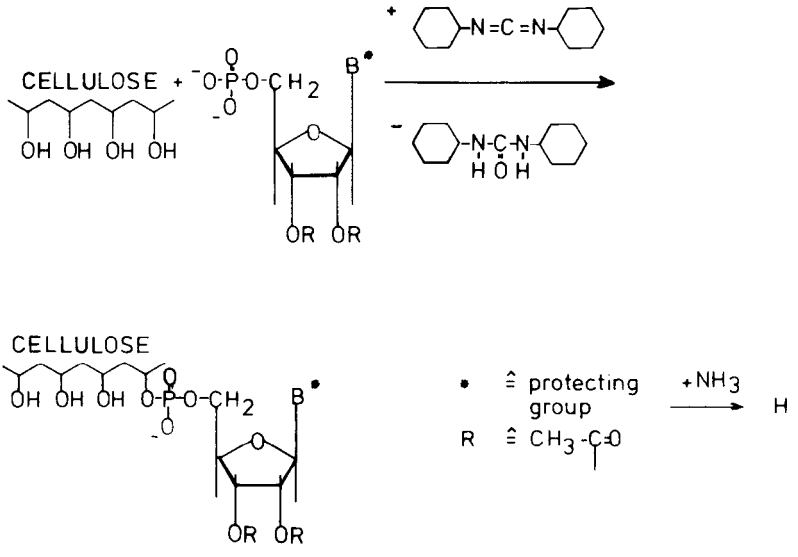
Table 1 Methods of Linking Nucleoside Phosphates to Insoluble Supports

Ligand	Coupling yields	
	μmol	%
Coupling of ligands to 500 mg of Sepharose by the CNBr method:		
p-Aminophenyl ATP		20
6-Aminohexanoyl ATP		90
s^6 ITP		43
Coupling of ligand to 500 mg of CM-cellulose by the mixed carbonic anhydride method:		
ATP	6.0	16
Coupling of halogen-substituted ligands to 200 mg of AE-cellulose:		
br^8 ATP	2.0	40
cl^6 ITP	24	72
Coupling of ligand to 500 mg of AE-Sepharose by the glutaraldehyde method:		
ATP	4.1	20
Coupling of ligands to 500 mg of AE-cellulose by bisimidate cross-linking:		
AMP	2.8	20
ATP	2.5	22
Coupling of ligand to 200 mg of nylon 6 powder by the methylation procedure:		
ATP	4.0	80

Source: Data from Ref. 1.

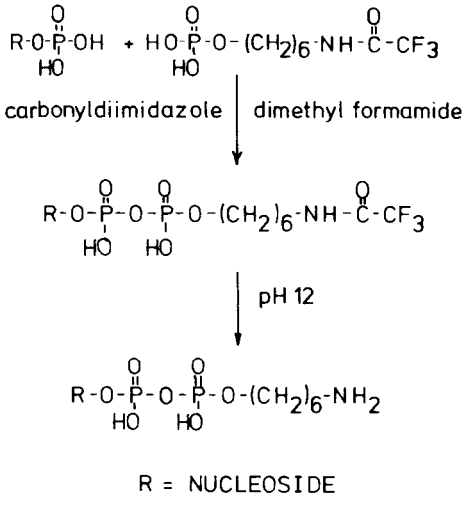
A. Phosphate-Linked Nucleoside Phosphates

Linkage through the phosphate groups is a method applicable to all nucleotides. The early approaches were based on the formation of phosphate esters (e.g., the synthesis of cellulose contain-



Scheme 1a

ing covalently bound nucleotides (Scheme 1a). For instance, thymidylate, adenylate, cytidylate, and guanylate cellulose are obtained by the condensation of the corresponding 5'-mononucleotides with hydroxyl groups of the cellulose support using dicyclohexylcarbodiimide in pyridine [7,8]. A similar carbodiimide reaction was used to link a 6-p-nitrobenzamido-hexan-1-ol spacer arm to the 5'-phosphate of 2'-deoxyuridylic acid, prior to attachment to agarose [9]. Immobilization through p-aminophenyl esters of the nucleotides has also been used [10]. Coincidental production of symmetrical pyrophosphate is a potential problem during the synthesis of these phosphate esters. Anion displacement [11] and imidazolide synthesis [12] are proposed to overcome this undesired side reaction. According to Scheme 1b, nucleoside phosphate can be derivatized to a ligand that contains one more phosphate group than the starting material [2]. The amino terminal of this nucleoside phosphate derivative can be used for attachment to CNBr-agarose. The synthesis of the P^1-(6-aminohex-1-yl)-P^2-(5'-nucleosyl)pyrophosphate derivative of uridine [13], adenosine [14], deoxyguanosine [15], and cytidine [16] was based on this procedure. Adsorbents containing uridine-5'-phos-



Scheme 1b

phate or pyrophosphate residues linked to Sepharose through phosphoamide bond were prepared through reaction of N^1-(uridine-5'-phosphoryl)- or N^1-(uridine-5'-pyrophosphoryl)-1,6-diaminohexanes with CNBr-activated Sepharose [34].

B. Sugar-Linked Nucleoside Phosphates

Linkage through the sugar hydroxyl groups is the easiest way of immobilizing nucleotides. Initially, carbodiimide-promoted condensation reactions were used [17], but this technique has been superseded by the hydrazone procedure, which involves the reaction of specifically modified groups of the ribonucleotides, and thus ensures a more defined chemical linkage (Scheme 2a). The 2'- and 3'-hydroxyl groups of ribonucleotides are oxidized to aldehyde functions, which are then linked to Sepharose [2,18]- or agarose [19]-bound hydrazide. The immobilization is carried out under mild conditions, the reaction is fast, and the binding capacity is very high. This method has been employed extensively and has proved to be the most useful of the ribosyl linkages. Table 2 shows some of the nucleotides immobilized by this method.