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Affinity adsorbents consisting of nucleic acids immobilized via bisoxirane activated polysaccarides

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ABSTRACT

An easy and efficient procedure for the immobilization of polynucleotide ligands to bisoxirane activated insoluble polysaccharides has been elaborated and is described in this paper. The resulting materials have been applied to the chromatography of DNA polymerase I, and RNA polymerase from E.coli. Because of their extraordinary stability to temperature, formamide, and alkaline conditions they seem to be particularly useful adsorbents for nucleic acid hybridization.

INTRODUCTION

Chromatography of proteins as well as nucleic acids on adsorbents containing polynucleotide ligands has recently received wide application in molecular biology (1-10) and a variety of methods for immobilization of different nucleic acid species has been described (11-16). DNA binding proteins have been fractionated successfully on high molecular weight DNA attached to cellulose by adsorption (17) but the low stability of the DNA-cellulose complex to desorbing conditions causes limitations in the applicability of the material.

On the other hand, immobilization of nucleic acids to insoluble supports by covalent interactions with the matrix often leads to materials which contain the polynucleotide ligand in but low concentration (15,18) or seriously damaged (19,20).

In order to overcome these disadvantages we set out to develop an affinity adsorbent containing large amounts of nucleic acid covalently immobilized to an insoluble support by an efficient and nondestructive procedure. As bisoxirane activated polysaccharide matrices seemed to provide ideal supports for the linkage of polynucleotide ligands we were particularly interested in this reaction. We therefore elaborated a procedure which allows immobilization of nucleic acids to such supports and investigated the resulting adsorbents for their potential applicability to affinity chromatography. The results of our investigations are summarized in this paper.

EXPERIMENTAL SECTION

1,4-Butanediol diglycidylether was purchased from Aldrich, Middlesex, U.K., Sepharose 6 B and epoxyactivated Sepharose 6 B from Pharmacia, Uppsala, and microcrystalline cellulose of the type CC 41 from Whatman, Maidstone, U.K. Calf thymus DNA, type II was obtained from Sigma, St. Louis, a soluble RNA preparation from baker's yeast, DNA- and RNA polymerase from E.coli MRE 600 as well as nucleoside triphosphates from Boehringer, Mannheim. All other chemicals were of analytical grade.

Activation of matrices:

Bisoxirane activation of Sepharose 6 B was carried out according to a suggestion by Porath (21). For the activation of cellulose 10 g were suspended in 30 ml 1 M NaOH containing 60 mg sodium borohydride. 20 ml of 1,4-butanediol diglycidylether were added and the mixture shaken vigorously for 20 h at room temperature. After filtration under suction the cellulose matrix was washed extensively with cold water until the effluent had reached neutrality. After lyophilization the material was stored desiccated at -20 °C. Thus activated cellulose matrices contained approximately 10-15 mmoles of reactive groups/g.

Immobilization of the polynucleotide:

The desired amount of the polynucleotide was dissolved in 20 mM KCl (5 to 10 ml per g of dry matrix) containing 10% 1,4-dioxane, and the pH of the solution was adjusted to 12.0 by addition of triethylamine. After adding to the activated matrix the mixture was spread over the surface of a petri-dish and allowed to evaporate slowly over a period of approximately 60 h at 35 °C in an atmosphere of about 30% rel. humidity. In order to inactivate unreacted epoxy groups, the material was resuspended in a small amount of 0.01 M NaOH and left to stand at room temperature for 5 days.

Desorption of the nonreacted ligand:

As nucleic acids and polynucleotides of high mol.wt. interact nonspecifically with polysaccharide matrices (15,22) it is essential to remove the nonreacted ligand by thoroughly washing the material in a desorbing solution. The adsorbent was therefore suspended in a solution of low ionic strength (preferentially deionized water) containing 20-50% of a hydrophilic organic solvent such as glycerol or formamide and gently agitated for 2 days. After washing with deionized water the material can be stored in any neutral buffer solution (preferentially citrate buffers) or in lyophilized form at 0-5 °C.

RESULTS

Nucleic acids and polynucleotides are easily and efficiently immobilized to bisoxirane activated polysaccharides under the conditions as suggested in the Experimental Section. The efficiency of the reaction procedure depends mainly on pH, temperature, and activity of the matrix as indicated in fig. 1. The molecular weight of the polynucleotide is less significant; in most of our experiments we used 19 S denatured calf thymus DNA and a mixed tRNA preparation from baker's yeast as model substances but similar results were obtained with sonicated DNA or high molecular weight RNA. The dependance of the coupling efficiency on input and nature of the polynucleotide as well as ligand concentrations of the resulting matrices are summarized in table 1.

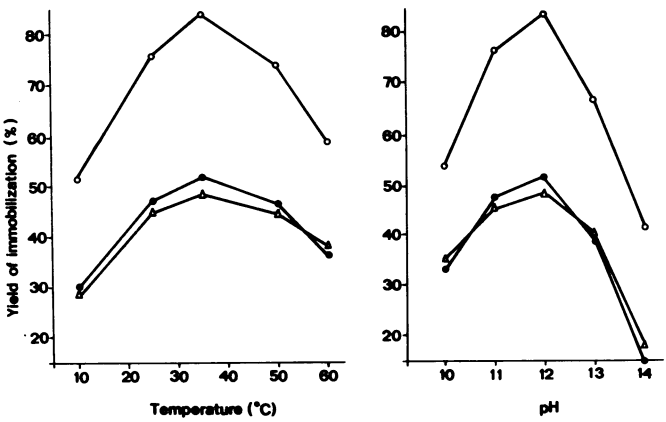

Figure 1

Effect of temperature and pH on the efficiency of the immobilization procedure; the reaction mixtures contained 10 mg of yeast soluble RNA per g matrix.

(o——o) epoxyactivated Sepharose 6 B

(●——●) commercial preparation of activated Sepharose 6 B

(Δ——Δ) activated cellulose CC 41

Affinity adsorbents containing polynucleotide ligands immobilized onto bisoxirane activated cellulose are particularly stable to temperature, formamide, and alkaline conditions. Polynucleotide celluloses were incubated for several hours at 90°C in solutions of high pH (up to 12.5) containing formamide concentrations up to 70% without any detectable decrease in ligand concentration. For this reason they seem to provide particularly useful adsorbents for nucleic acid hybridization. In a representative experiment a column containing 5.2 mg of poly(U) retained 3.8 mg of poly(A) which could be eluted quantitatively

Table 1

Immobilization of (a) yeast soluble RNA, and (b) calf thymus DNA to different bisoxirane activated supports

(a) input mg	immobilized mg	yield %	ligand concentration mg/ml	(b) input mg	immobilized mg	yield %	ligand concentration mg/ml
epoxyactivated Sepharose 6 B				epoxyactivated Sepharose 6 B			
5	4.8	96	1.3	5	4.4	88	1.2
10	8.8	88	2.4	10	8.2	82	2.3
15	13.0	87	3.6	15	11.7	78	3.4
20	17.2	86	4.8	20	14.8	74	4.3
25	21.3	85	5.9	25	17.6	70	5.0
30	25.2	84	7.0	30	20.2	67	5.7
commercial preparation of activated Sepharose				commercial preparation of activated Sepharose			
5	3.2	64	0.9	5	2.9	58	0.8
10	5.3	53	1.5	10	4.9	49	1.1
15	7.0	47	2.0	15	6.4	43	1.8
20	8.2	41	2.3	20	7.7	39	2.1
25	9.8	39	2.7	25	8.7	35	2.4
30	11.1	37	3.1	30	9.8	33	2.7
activated cellulose CC 41				activated cellulose CC 41			
5	3.7	74	2.5	5	3.2	64	2.1
10	5.1	51	3.4	10	4.6	46	3.1
15	7.4	49	4.9	15	5.6	37	3.7
20	9.4	47	6.2	20	6.1	31	4.0
25	11.2	45	7.5	25	6.2	25	4.1
30	12.6	42	8.4	30	6.3	21	4.2

with solutions containing high concentrations of formamide.

The stability of polynucleotide-Sepharoses to temperature and formamide is less pronounced which is due to the limited tolerance of the matrix to such conditions. Low ionic strengths as well as alkaline conditions up to pH 13, however, do not affect the materials. Since as compared to cellulose nonspecific interactions of proteins with beaded agarose matrices are minimal, such materials are most favourably employed for protein fractionation. We therefore investigated the potential applicability of DNA-Sepharose to the chromatography of proteins involved in cellular polynucleotide synthesis. Figure 2 shows the elution profiles of (a) DNA polymerase I, and (b) RNA polymerase from E.coli on an agarose matrix containing 4.3 mg of denatured calf thymus DNA per ml column volume. Both enzymes were recovered from the column in yields higher than 80%. In a typical experiment E.coli DNA polymerase I purified through step 5 according to a published procedure by Jovin et al. (23) was fractionated on DNA-Sepharose as above resulting in a 20-fold purification of the enzyme.

CONCLUSION

Activation of polysaccharide matrices with bifunctional epoxides as introduced by Porath (21) provides excellent supports for the immobilization of low- as well as high mol.wt. ligands containing nucleophilic functions. Whereas immobilization of proteins, polysaccharides and low mol.wt. ligands

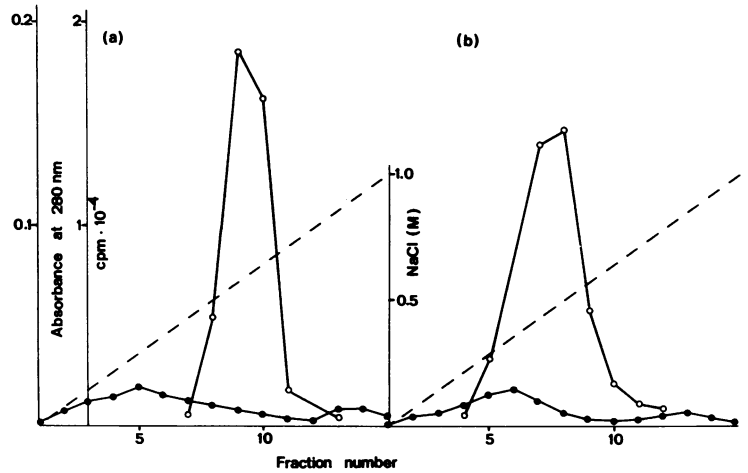

Figure 2

Fractionation of (a) DNA polymerase I, and (b) RNA polymerase from E.coli; 4.6 units of DNA polymerase and 9.3 units of RNA polymerase respectively were applied to DNA-Sepharose columns of 1 ml containing 2.4 mg of denatured calf thymus DNA. Elution was carried out with a linear gradient of 0.0 - 1.0 M NaCl (5 ml) in a 20 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 0.5 mM DTT, and 5% (v/v) glycerol. Fractions of 0.33 ml were collected and tested for polymerase activity. Column operations were carried out at 4°C at a flow rate of 3 ml/h.

(●——●) protein concentrations as measured at 280 nm

(o——o) enzyme activity as measured by incorporation of nucleotides into acid precipitable material

broken line: molar concentration of NaCl

After elution columns were regenerated in situ by washing with 0.1 N NaOH. Enzyme assays were carried out according to published procedures (12,23).

has been well discussed, nucleotide and polynucleotide ligands have not until now been coupled to such supports. Since the immobilization of polynucleotide ligands to insoluble matrices presents a major problem which has been dealt with by a variety of laboratories (11-20) we set out to investigate the reaction of nucleic acids with materials containing insolubilized oxirane functions. Optimal conditions have been elaborated for the immobilization of tRNA and denatured DNA and are described in this paper. The resulting materials which contain the ligand at the end of a 12 membered, slightly hydrophobic spacer arm have been successfully applied to the fractionation of proteins involved in cellular polynucleotide synthesis as well as for nucleic acid hybridization. The advantages over other polynucleotide containing adsorbents

are a good flow rate of the columns even at extraordinary high ligand concentrations and the high stability of the materials to both heat and alkaline conditions.

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