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AFFINITY CHROMATOGRAPHY FOR THE PURIFICATION OF LECTINS (A REVIEW)

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SUMMARY

Three major types of biospecific adsorbents have been described for the purification of lectins by affinity chromatography: (1) polysaccharides, either native or modified; (2) matrix-bound glycoproteins and glycopeptides; and (3) matrix-bound mono- and disaccharides. In this review the various adsorbents used and the methods for their synthesis, as well as their respective advantages and disadvantages, are discussed.

INTRODUCTION

No other single technique has had such a far reaching impact on the development of methods for the purification of naturally occurring materials as affinity chromatography. This impact has been particularly pronounced is research on lectins and their application in biology and medicine. Lectins are sugarbinding and cell-agglutinating proteins of non-immune origin that are widely distributed in nature, particularly in plants[1–4]. They exhibit many interesting biological properties, such as specificity for human blood types, preferential agglutination of malignant cells and mitogenic stimulation of lymphocytes. As a result, lectins are being utilized extensively as macromolecular carbohydrate-specific reagents for probing the structure, organization and function of cell-surface glycoconjugates and the changes they undergo during cell growth and malignant transformation[5–7]. The ability of lectins to combine with carbohydrates specifically and reversibly permits not only their facile purification on immobilized sugar derivatives, but also their use for the isolation and characterization of carbohydrate-containing compounds and for cell fractionation. In this article we shall deal only with the purification of lectins by affinity chromatography. The use of lectins for the separation of glycoproteins, glycopeptides and cells has recently been reviewed[8–12].

In principle, purification of lectins by affinity chromatography is not different from that of other biopolymers with specific combining sites, although it is generally much simpler. This is because lectins do not modify the compounds with which they interact, nor is the interaction very strong (the association constant, K_a, of lectins with monosaccharides is usually in the range 10^2–10^4)[3,4]. As a result most lectins can readily be displaced from the affinity columns by the sugars for which they are spe-

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cific. It is also very easy to establish the specificity of a lectin from hapten inhibition of hemagglutination, using simple sugars and crude lectin preparations. Knowledge of the specificity then permits the design of a suitable purification procedure.

A large variety of affinity adsorbents for the purification of lectins has been described. Many of these originate from the laboratory of Professor Jerker Porath, who showed an early interest in the study of lectins and in the development of methods for their isolation. The biospecific adsorbents for lectins can be divided into three major types: (1) polysaccharides, either native or modified; (2) matrix-bound glycoproteins and glycopeptides; and (3) matrix-bound mono- and disaccharides (Table I). Of the first type, the most widely used are the commercially available

TABLE I

METHODS FOR AFFINITY CHROMATOGRAPHY OF LECTINS

Matrix	*Ligand*	*Source of lectin* and ref.***
Type 1: Polysaccharides		
Sephadex	—	Jack bean, lentil, garden pea[13], *Vicia faba*[14], *Vicia sativa*[15]
Sepharose	—	*Abrus precatorius*, *Ricinus communis*, *Bauhinia purpurea alba*[16], *Pseudomonas aeruginosa*[17], *Dictyostelum discoideum*[18]
Acid-treated Sepharose	—	*Crotolaria juncea*[19], *Bauhinia purpurea alba*[20], *Sophora japonica*[20], soybean[20], *Wistaria floribunda*[20], *Tridacna maxima*[21]
Chitin	—	*Bandeiraea simplicifolia* II
Polysaccharide from *Aspergillus niger* micellium	—	*Datura stramonium*[22]
Insolubilized guaran	—	Soybean[23], *Ricinus communis*[23], *Echinocytis lobata*[23], *Bandeiraea simplicifolia*[23,25]
Cross-linked arabinogalactan		*Ricinus communis*[26,27], soybean[27], *Crotolaria juncea*[27], *Echinocytis lobata*[27], *Momordica charantia*[27]
Type 2: Matrix-bound glycoproteins and glycopeptides		
Polyleucine	Hog blood group substances A + H	*Dolichos biflorus*, lima bean, *Sophora japonica*, *Evonymus europeus*[28], tomato[29], *Helix pomatia*, *Aaptos papillata*[30]
Bovine serum albumin	Hog blood group substances A + H	*Vicia cracca*[31]
Sepharose	Glycopeptides from hog gastric mucin	Jack bean[32], soybean[32], *Ricinus communis*[32], *Lotus tetragonolobus*[32], *Ulex europeus*[32], *Limulus polyphemus*[32]
	Human blood group substance A	*Vicia cracca*[33]
	Thyroglobulin	*Phaseolus vulgaris*[34]
	Ovomucoid	Potato[35]
	Fetuin	Jack bean[36], pea[36], *Datura stramonium*[37]
	Bovine submaxillary mucin	*Limulus polyphemus*[38,39]
	Human erythrocyte stroma	*Phaseolus vulgaris*[40]
	Desialylated human erythrocyte glycopeptide	Pokeweed[41]

TABLE I *(continued)*

Matrix	*Ligand*	*Source of lectin* and ref.***
Type 3: Matrix-bound mono- and disaccharides		
Sepharose	β-Aminocaproyl-N-glycosylamine of:	
	(a) L-fucose	*Lotus tetragonolobus*
	(b) galactose	Soybean
	p-Aminobenzyl 1-thio-glycoside of N-acetylglucosamine	Potato[35], *Bandeiraea simplicifolia* II[42]
	p-Aminophenyl O-glycosides of:	
	(a) L-fucose	*Lotus tetragonolobus*[43]
	(b) N-acetylgalactosamine	Soybean[44], *Caragana arborescens*[45]
	(c) galactose	*Bandeiraea simplicifolia* I[42]
CH-Sepharose***	Galactosamine	Soybean[46]
	3-O-Methyl-glucosamine	*Vicia faba*
	N-Acetylgalactosamine	*Vicia cracca*[47]
Epoxy-activated Sepharose***	N-Acetylgalactosamine	Soybean[48], *Amphicarpaea bracteata*[44]
	L-Fucose	*Ulex europeus* I[49,50], *Sarathamnus scoparius*[50]
Divinylsulfone-activated Sepharose	Galactose	*Erythrina cristagalli*[51]
	Mannose	*Vicia ervilia*[52]
Polyacrylamide	Allyl glycoside of:	
	(a) L-fucose	*Ulex europeus* I
	(b) galactose	*Ononis spinosa*[53], *Fomens fomentarius*[53], *Marasmus oreades*[53], *Griffonia simplicifolia Baill*[53]
	(c) glucose	Jack bean[53]
	(d) N-acetylgalactosamine	*Vicia cracca*[53], lima bean[53], soybean[53], *Dolichos biflorus*[53], *Maclura pomifera*[53], *Clitocybe nebularis*[53], *Sarothamus scoparius*[53 §]
	(e) lactose	*Ononis hircina*[54]
Aminoethylpolyacrylamide	Melibiose	*Bandeiraea simplicifolia* I[55]
	Lactose	*Ricinus communis*[56,57]
	Maltose	Jack bean[57], lentil[57]
Other types		
Formalinized erythrocytes	–	Jack bean[58], lima bean[58], *Ulex europeus*[58], *Robinia pseudoacacia*[59]

* Methods for the purification of wheat germ agglutinin and of peanut agglutinin are listed in Tables II and III, respectively.

** *Methods in Enzymology*. Vols. 28, 34 and 50, contain compilations of methods for affinity chromatography of lectins. Only references to lectins (or methods) not mentioned in these volumes are given in the table.

*** Commercial product of Pharmacia, Uppsala, Sweden.

§ It is not clear whether this is the same lectin as that described in ref. 50.

Sephadex and Sepharose. The former, which is a cross-linked dextran, is suitable for the purification of lectins that are specific for glucose*, such as concanavalin A (the lectin from jack bean) and the lentil lectin, whereas lectins that bind galactose [*e.g.*, those from the castor bean (*Ricinus communis*) and from *Bauhinia purpurea alba*] have been purified on Sepharose (modified agarose). However, not all lectins that are specific for galactose are adsorbed to Sepharose. This may be due to the inability of these lectins to interact with the internal residues of the linear galactan chains of the polysaccharide. In such cases adsorption is made possible by increasing the number of galactosyl end groups by mild acid hydrolysis under conditions that split the galactan chains in a number of places without completely degrading the interlocking network[19].

Affinity matrices with a high capacity for galactose-specific lectins have been obtained by cross-linking naturally occurring, galactose-containing polysaccharides with epichlorhydrin or divinylsulfone. The polysaccharides are arabinogalactan[26,27] and guaran (guar gum)[23,24], a galactomannan. A bioadsorbent in which the latter is the active component has also been prepared by entrapment of the polysaccharide in polyacrylamide gels[25,60]. Guaran, in contrast to Sepharose and arabinogalactan, contains α-linked galactose residues and can therefore be used for the isolation of a lectin from *Bandeiraea* (*Griffonia*) *simplicifolia* with an anomeric preference for α-galactose[24,25]. An example of a polysaccharide that is used for the affinity purification of lectins (*e.g.*, wheat germ agglutinin) in its native form is chitin, an insoluble polymer of N-acetylglucosamine[61].

In cases of lectins with sugar specificities for which no ready-made adsorbents are available, affinity columns are prepared by coupling the appropriate carbohydrate residue to an insoluble support. The carbohydrate can be introduced in the form of a glycoprotein or glycopeptide (Type 2, Table I) or a suitable synthetic derivative of the specific sugar (Type 3, Table I).

In contrast to the affinity columns described above (and those of Type 3, to be described), where each adsorbent interacts only with lectins of the same sugar specificity, columns made with glycoproteins possess a wider complement of saccharides and the same adsorbent can therefore by used for the purification of lectins with different sugar specificities. The most notable examples of such "general affinity adsorbents" are matrix-bound fetuin, hog gastric blood group A + H substance and glycopeptides from hog gastric mucin. Thus, fetuin–Sepharose was employed for the isolation of the agglutinins from wheat germ, jack bean, pea, horseshoe crab (*Limulus polyphenus*) and several other sources[36]. Some of these lectins were also purified on immobilized glycopeptides from hog gastric mucin, as were soybean agglutinin, lima bean agglutinin and others[32].

Affinity adsorbents that can be considered as a form of immobilized glycoproteins are cross-linked erythrocytes[58,59] and entrapped glutaraldehyde-treated erythrocyte membranes[41]. These adsorbents bind most lectins, including many of those that do not interact with simple sugars. In the latter case, desorption is usually achieved with solutions of low pH (*e.g.*, 0.1 *M* acetic acid or glycine–HCl buffer, pH 2.5) and therefore the system is not suitable for lectins that are denatured under these conditions. Even with lectins for which specific sugar inhibitors are available, elution

* All sugars are of the D-configuration, unless otherwise stated.

may present difficulties. This is because adsorption of lectins to erythrocytes involves secondary, non-specific interactions (*e.g.*, hydrophobic), in addition to specific binding to carbohydrates.

The widest range of affinity adsorbents for the purification of lectins is found among the matrix-bound mono- and disaccharides (Table I, Type 3). Many of these adsorbents use Sepharose as matrix. In principle, Sepharose–carbohydrate conjugates are easily synthesized by the reaction of cyanogen bromide-activated Sepharose with a carbohydrate derivative containing an amino function. However, very few naturally occuring saccharides contain an amino group. Moreover, direct coupling of a mono- or disaccharide to the carrier will often result in an adsorbent in which the ligand is not easily accessible to the binding site of the lectin. As a rule, it is necessary to separate the ligand from the matrix by a spacer group, thereby minimizing steric hindrance of the protein–ligand interaction. Examples of such spacers are 6-aminohexyl and ε-aminocaproyl groups. Thus the major problem in the preparation of Type 3 affinity columns is the synthesis of the appropriate carbohydrate derivative.

Several strategies have been developed for the synthesis of such derivatives and for their coupling to the matrix. Four types of derivatives (O- and S-glycosides, N-acylglycosylamines and N-acylamino sugars) of the general structure shown in Fig. 1 have been described in the literature. The complexity of the procedures involved in their preparation is illustrated in Figs. 2 and 3, which show the steps leading to the synthesis of 6-amino-1-hexyl-2-acetamido-2-deoxy-β-glucopyranoside[62] (an O-glycoside employed in the purification of wheat germ agglutinin) and of N-(ε-aminocaproyl)-β-galactopyranosylamine[63] (an N-acylglycosylamine used for the purification of soybean agglutinin and peanut agglutinin), respectively.

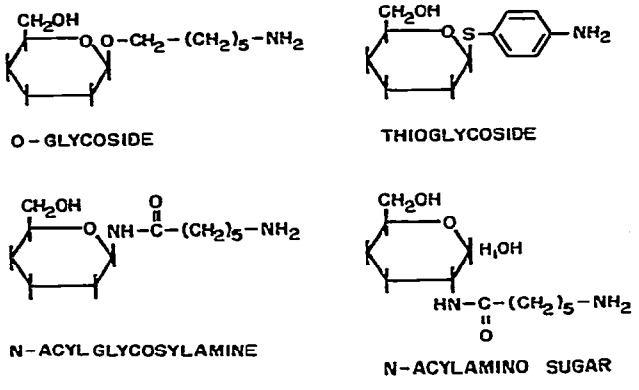

Fig. 1. Monosaccharide derivatives used for the synthesis of Sepharose-carbohydrate conjugates.

In the examples quoted above, the ligand is coupled to the spacer group prior to being immobilized on the matrix. Alternatively, the spacer group may be bound to the matrix and the ligand coupled to the immobilized spacer. This approach has been utilized for the synthesis of affinity columns for the purification of the lectins from *Bandeiraea simplicifolia*[42]. The procedure consisted of the following steps: (a) attachment of hexane-1,6-diamine to cyanogen bromide-activated Sepharose; (b) treatment

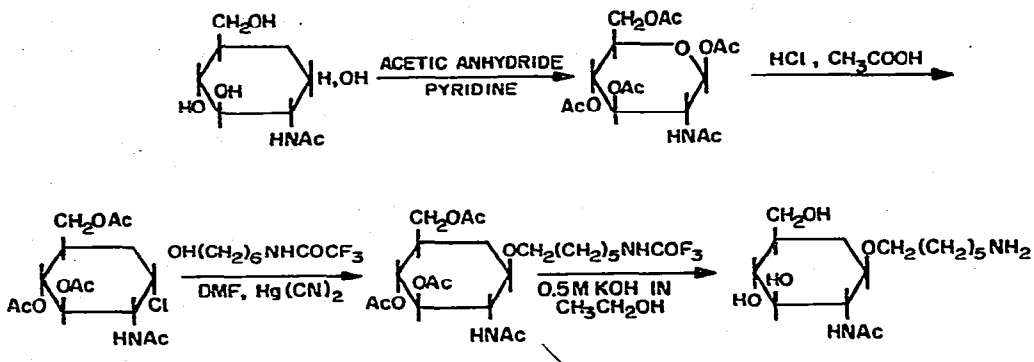

Fig. 2. Synthesis of 6-amino-1-hexyl-2-acetamido-2-deoxy-β-glucopyranoside[62]

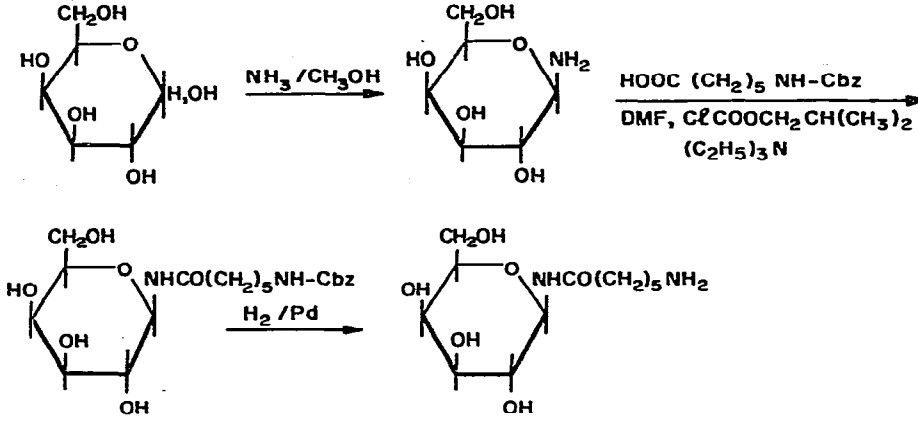

Fig. 3. Synthesis of N-(ε-aminocaproyl)-β-galactopyranosylamine[63].

of the aminohexaminyl-Sepharose thus obtained with succinic anhydride; (c) coupling of the appropriate sugar derivative (*i.e.*, *p*-nitrophenyl-β-galactoside or *p*-aminobenzyl-1-thio-N-acetylglucosaminide) with carbodiimide as the condensing agent.

Obviously, the preparation of these adsorbents, too, is rather laborious and demands considerable expertise in organic synthesis. Preparation of affinity columns with bound carbohydrate derivatives has been greatly simplified by the introduction of commercially available matrices with spacer groups already attached. With such supports, preparation of the adsorbent requires only addition of the carbohydrate, which is often achieved by a simple one-step coupling reaction. Thus, CH-Sepharose, formed by the covalent attachment of 6-aminohexanoic acid to the carrier, contains a six-carbon-long spacer arm terminating in a carboxyl group, to which amino groups can be easily bound by the carbodiimide method. Columns containing galactosamine, glucosamine and 3-O-methyl glucosamine have been prepared in this manner and used for the purification of soybean agglutinin[46], wheat germ agglutinin[46] and of *Vicia faba* lectin[64], respectively.

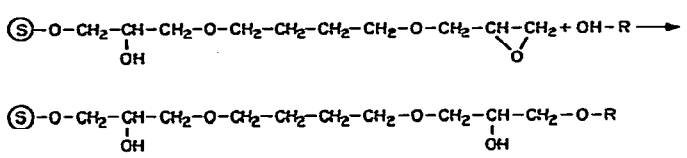

Fig. 4. Coupling of hydroxyl compounds to epoxy-activated Sepharose.

Epoxy-activated Sepharose, obtained by reacting the matrix with 1,4-bis(2,3-epoxypropoxy)butane[65,66], provides an extended (equivalent to twelve carbon atoms) hydrophilic spacer and an active oxirane (epoxy) group for the direct coupling of hydroxyl groups via stable ether bonds (Fig. 4). A disadvantage of this matrix is that binding of the ligand requires rather high pH (12–13) and temperatures above 40°C to achieve a high degree of substitution. The use of such columns is therefore limited to sugar derivatives that are stable to the alkaline conditions required in the coupling reaction. Moreover, hydroxylic compounds differ greatly in their reactivity towards the oxirane group. The optimal conditions for coupling may therefore have to be worked out for each individual sugar. To date, the binding of N-acetylgalactosamine, N-acetylglucosamine and L-fucose to epoxy-activated Sepharose and the successful application of the adsorbents thus obtained to the purification of a number of lectins have been described[44,48–50,66].

A very simple and convenient way for the attachment of a spacer with the simultaneous introduction of a group highly reactive with hydroxylic compounds is the divinylsulfone coupling method developed by Porath and Sundberg[67] (Fig. 5).

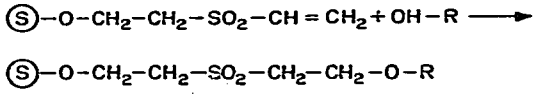

Fig. 5. Coupling of hydroxyl compounds to divinylsulfone-activated Sepharose.

The vinyl groups are more reactive than the oxirane groups and coupling will therefore take place at lower pH and temperature than with epoxy-activated Sepharose. Unfortunately, the link formed is unstable in alkaline solutions and studies must often be made to optimize the coupling conditions in each particular case. Both galactose and mannose have been coupled in this manner to give very satisfactory adsorbents that have been employed for the purification of the lectins from *Crotolaria juncea*[68] and *Vicia ervilia*[52], respectively.

In addition to adsorbents utilizing Sepharose as matrix, many columns for lectin purification have been prepared based on polyacrylamide gels. Fully synthetic affinity adsorbents have been obtained by the copolymerization of alkenyl O-glycosides[69] (or S-glycosides[70]) with acrylamide and N,N′-methylenebisacrylamide. By a suitable choice of glycosides and by varying the amounts of the components in the polymerization reaction, it is possible to prepare adsorbents with specified anomeric configuration and ring form, to introduce spacer chains of different type and length and to regulate both the sugar content and pore size of the copolymer (Fig. 6). These adsorbents also have a higher resistance to chemical and bacterial degradation than affinity columns derived from natural substances. Their disadvantages are: unfavor-

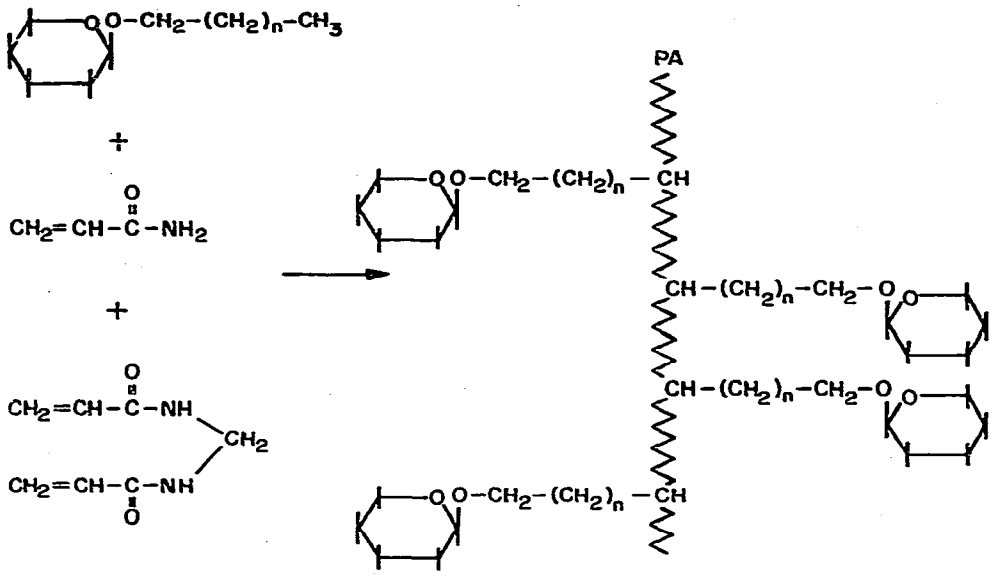

Fig. 6. Schematic representation of the synthesis of O-glycosyl polyacrylamide gels.

able flow properties, relatively low mechanical stability, limited exclusion range of molecular weights, and last but not least the fact that synthesis of alkenyl glycosides, whether O- or S-glycosidic, is a complex process.

In a variation of the above approach, polymerization of the acrylamide gel was carried out in the presence of active esters of acrylic acid,N-succinimidyl acrylate and phthalimidyl acrylate. The resulting "active" gels reacted readily with ligands containing primary amine groups[71].

Other adsorbents based on polyacrylamide supports take advantage of commercially available Bio-Gels in bead form, thus ensuring rapid flow in column techniques. Bio-Gel can be easily derivatized with ethylenediamine to give aminoethyl derivatives to which carbohydrates can be coupled via the functional amino group. One coupling method relies on the ability of the cyanoborohydride anion to reduce selectively the Schiff base formed between the aldehyde group of a reducing sugar and a free amine group[56,57]. However, the rate of coupling, which varies with different saccharides, is rather slow as a rule. The introduction of about 12 μmoles of galactose, for example, per ml of gel required up to two weeks, while with N-acetylgalactosamine only 1.5 μmoles/ml of gel were coupled after 45 days[57]. Moreover, since the ring form of the reducing sugar is destroyed, disaccharides with the sugar determinant in the non-reducing end must be used. Thus, gels containing β-galactose (for the purification of peanut agglutinin and the lectin from castor bean) have been obtained starting from lactose, α-galactose (for the purification of *Bandeiraea simplicifolia* lectin) starting from melibiose, α-glucose (for concanavalin A and lentil lectin) from maltose and N-acetylglucosamine (for wheat germ agglutinin) starting from N-acetylchitobiose.

Melibiose has also been attached to aminoethyl-Bio-Gel, after oxidation of the

TABLE II

METHODS FOR AFFINITY PURIFICATION OF WHEAT GERM AGGLUTININ

Matrix	*Ligand*	*Coupling method*	*Ref.*
Chitin	–		61
Kieselguhr	Ovomucoid	Cyanogen bromide activation	72
Sephsrose	Ovomucoid	Cyanogen bromide activation	73
	Glycopeptides from hog gastric mucin	Cyanogen bromide activation	32
	ε-Aminocaproyl N-glycosylamine of N-acetylglucosamine	Cyanogen bromide activation	74
	6-Aminohexyl O-glycoside of N-acetylglucosamine	Cyanogen bromide activation	62
CH-Sepharose	Glucosamine	Carbodiimide coupling	46
Epoxy-activated Sepharose	N-Acetylglucosamine	Direct coupling at high pH	48
	Asparaginyl-N-acetylglucosamine	Carbodiimide coupling via succinic anhydride	75
Aminoethylpoly-acrylamide gel	N-Acetylchitobiose	Reduction with cyano-borohydride	57
Ultrogel A4	*p*-Aminobenzyl 1-thio-glycoside of N-acetyl-glucosamine	Carbodiimide coupling via succinic anhydride	76
Copolymers of acrylamide and active esters of acrylic acid	6-Aminohexyl-N-acetylglucosamine	Copolymerization	71
Formalinized erythrocytes	–	–	58

TABLE III

METHODS FOR THE AFFINITY PURIFICATION OF PEANUT AGGLUTININ

Matrix	*Ligand*	*Coupling method*	*Ref.*
Insolubilized guaran	–	–	24, 60
Cross-linked arabinogalactan	–	–	27, 82
Cross-linked desialylated erythrocyte stroma	–	–	77
Sepharose	Desialylated fetuin	Cyanogen bromide activation	78
	ε-Aminocaproyl N-glycosylamide of galactose	Cyanogen bromide activation	79
Divinylsulfone-activated Sepharose	Galactose	Direct coupling at high pH	80
Aminoethylpoly-acrylamide gel	Lactose	Reduction with cyano-borohydride	56, 57
Acrylamide gel	Alkyl glycoside of galactose	Copolymerization	60

disaccharide to the corresponding aldonic acid, by condensation in the presence of carbodiimide[55].

As can be seen from the above compilation, there is a wide range of adsorbents for the biospecific purification of lectins. It should be noted, however, that most of these adsorbents also bind glycosidases. Since glycosidases are frequently present in biological extracts, especially from plants, lectins purified by affinity chromatography may be contaminated by such enzymes. In some cases, especially when working with lectins of a very restricted specificity, adsorbents have to be "tailor made", as for example Sepharose-bound asialoglycophorin for the purification of the N blood type-specific lectin from *Vicia graminea*[81]. Many lectins, on the other hand, can be isolated by a variety of techniques. This is best exemplified in Tables II and III, in which different approaches to the purification of two widely used lectins, wheat germ agglutinin and peanut agglutinin, have been summarized. Faced with the bewildering selection of supports, ligands and methods of attachment now available, it is not always easy to decide which to choose. Ideally, a support should be insoluble, of suitable particle form, resistant to chemical and biochemical attack, non-adsorbing and easily derivatized. The coupling procedure should be simple, non-destructive, utilize readily available ligands and give a stable linkage and a high degree of substitution. None of the methods described fulfills all of the above requirements. Some of the disadvantages of the various adsorbents have already been discussed. To this should be added leakage (*i.e.*, solubilization) of ligands bound to cyanogen bromide-activated Sepharose. Nevertheless, with most lectins one or another of the columns described in the literature will be found suitable for the problem at hand. That this is so, is largely due to the foresight and pioneering work of Jerker Porath.

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