Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Exploiting lectin affinity chromatography in clinical diagnosis

Posetthalli R. Satish, Avadhesha Surolia*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Abstract

Lectin affinity chromatography (LAC) offers a tool that aids purification of cell surface glycoconjugates in sufficient quantities so that studies addressing their structural elucidation could be carried out. It has several advantages over the conventional biochemical methods, such as immunoprecipitation and/or immunoaffinity chromatography, used for the purification of various glycoconjugates. Serial LAC (SLAC) not only helps establish the identity of a glycoprotein or allows purification of a glycoprotein to homogeneity from among a mixture of glycoproteins, but it also successfully resolves the microheterogeneity in these glycoproteins, which is an otherwise impracticable problem to address. Specific cases of the altered expression and maintenance of microheterogeneity of some of the glycoproteins in pathological conditions vis a vis during normal biology are presented. The application of LAC in (i) itself, (ii) a serial fashion, and (iii) conjunction with other techniques such as two-dimensional electrophoresis, capillary electrophoresis, mass spectrometry, etc. in the diagnosis of certain pathological conditions, and the possibility of using this knowledge in designing treatments for various diseases, is discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Lectin affinity chromatography; Serial lectin affinity chromatography; Glycoprotein microheterogeneity; Human chorionic gonadotropin; α-Foetoprotein; Mass spectrometry

1. Introduction (lectins as affinity ligands)

Lectins by definition are multivalent proteins of non-immune origin that bind to sugars rather specifically, agglutinate cells, and display no catalytic activity [1] (however, see also Refs. [2,3]). The ability of lectins to detect subtle variations in carbohydrate structures found on cell surface glycoproteins and glycolipids has made them a paradigm for protein–carbohydrate recognition [4]. Lectins have been implicated, among
other things, in defense against invading organisms, symbiotic association between
nitrogen-fixing bacteria and the roots of leguminous plants, recognition of host cells by
viruses [5], cellular adhesion [6], cellular recognition [7] cell growth and differentiation
[8], histochemical detections of sugar chains on the cell surface, staining and structural
estimation of electrophoretically separated membrane glycoconjugates, and also separa-
tion of immunocyte subsets and cells in different differentiation stages. On account of
their ability to specifically bind cell surface carbohydrates which play important roles in
biological recognition, lectins have, during the recent past, found extensive applications
for differentiating between cells, i.e. blood typing, diagnosis and prognosis of cancer,
elucidation of the architecture and dynamics of cell surface carbohydrates, glycoconju-
gate purification and structural characterization. The reason why lectins find applications
across such a broad spectrum is that they are not only ubiquitously distributed in nature,
and occur in abundance in plants, especially seeds of legumes, but also because of the
ease of their purification to homogeneity and the wide repertoire of carbohydrate
specificities that they exhibit. It is therefore not surprising that lectins find extensive
applications also in many aspects of biology and medicine, such as cell-selection
processes, blood typing, analysis of oligosaccharides [9], and isolation of glycoconju-
gates on a preparative scale (purification of glycoproteins) by affinity chromatography.

Cell surface glycoconjugates constitute surface markers which control and determine
cell–ligand and cell–cell interactions in phenomena such as cellular signaling and
intercellular signal transduction. Studies addressing the molecular mechanisms of these
processes essentially hinge on the structural elucidation of membrane glycoconjugates.
Sufficient quantities of the glycoconjugates are required for this purpose; their isolation
by conventional methods is very difficult, and this is further complicated by the
occurrence of microheterogeneities in them [10].

That sugars have an affinity for lectins (10^2–10^6 M⁻¹) which is not as high as that
for the carbohydrate-specific antibodies (10^3–10^8 M⁻¹) is of immense practical utility
while designing strategies for purification of glycoconjugates. If carbohydrate-specific
antibodies are used as affinity ligands, unphysiologically harsh conditions need to be
employed to elute the proteins that get adsorbed onto the matrix during the purification
of glycoconjugates; also, retention of the biological activity of the proteins purified
under such circumstances is not guaranteed. Lectins, on the contrary, when immobilized
and employed as the affinity ligands for the purification of the said glycoconjugates,
offer an advantage since only mild conditions need be applied to elute the protein of
interest. Moreover, because of their ubiquitous distribution and abundant presence, lectin
purification per se is not as arduous a task as raising the carbohydrate-specific
monoclonal antibodies.

Taken together, the above facts imply that lectin affinity chromatography by itself
offers a powerful tool to address the purification of membrane glycoconjugates in
sufficient quantities.

2. Lectin affinity chromatography

Historically, one of the problems which could not be appropriately addressed using
the conventional means of purification was the obtainment of sufficient quantities of cell
surface glycoconjugates despite the central role they play in cellular adaptation or signal transduction, either during the course of normal development or pathological situations. The advent of the lectin affinity chromatography has not only enabled the purification in sufficient quantities of the cell surface glycoconjugates, but also has afforded a way to fractionate and resolve a mixture of glycoproteins (which exhibit microheterogeneity in their sugars) even to the level of a single component, which falls beyond the scope of conventional methods.

In principle, lectin affinity chromatography is similar to other types of affinity chromatography. A mixture of heterogeneous glycoproteins is chromatographed on a matrix with a particular lectin immobilized on it. The glycoprotein with a specific sugar sequence gets adsorbed onto the matrix because of its interaction with the immobilized lectin, while other glycoproteins are washed off by the buffer and collected as the breakthrough. Washing is continued until no more proteins come out of the column by using the buffer alone, as monitored by the absorbance at a particular wavelength, against a suitable blank. The adsorbed glycoprotein is eluted from the column using a sugar with a complementary structure, its purity confirmed by gel electrophoresis and characterized further. The sugar specificity and properties of some common lectins and their applications in affinity chromatography are listed in Table 1 [11–30].

3. Serial lectin affinity chromatography

While fractionating a heterogeneous mixture of glycoproteins by lectin affinity chromatography if the breakthrough obtained from one matrix-immobilized lectin affinity column (as outlined in the previous section) is rechromatographed on a second lectin-affinity column, and the process is repeated for different lectins in a serial manner, the process is called serial lectin affinity chromatography (SLAC). In addition to achieving the purification to apparent homogeneity of each component of a mixture of glycoproteins, SLAC also subserves the purpose of resolution of the microheterogeneity present in the carbohydrate part of the apparently homogenous glycoprotein. Primarily, by subjecting a mixture of heterogeneous glycoproteins to affinity chromatography on a matrix with a particular lectin immobilized on it, the nature of the sugar and the linkage pattern of its constituent monosaccharides is either confirmed or ruled out depending on whether the glycoprotein binds to the column or comes out in the flow through. The flow through is chromatographed on a second lectin-immobilized affinity matrix, specificity analysis is repeated, and so on. Thus, SLAC has been one of the chief means by which structural elucidation of sugars on the glycoproteins is carried out. In combination with exoglycosidase digestion and methylation analysis, one can put to use the SLAC for complete structural elucidation of the sugars. Many SLAC studies have been carried out after the first report of Cummings and Kornfeld [31,32], and subsequently, by Endo et al. [11].

4. Glycoform microheterogeneity (GM) and its resolution

Glycosylation is a complex posttranslational modification that can result in extensive heterogeneity for glycoproteins produced by eukaryotic systems. The carbohydrate
Table 1
The properties of some common lectins and their applications in affinity chromatography

| Source of lectin       | Specificity                                      | Properties/applications/remarks                                                                 |
|------------------------|--------------------------------------------------|-------------------------------------------------------------------------------------------------|
| *Datura stramonium*    | binds 2,6 branched tri/tetraantennary oligosaccharides | Binds the human urinary chorionic gonadotropin Asn-linked sugar chains of patients with invasive mole or choriocarcinoma, but not in that of normal pregnant women or patients with hydatidiform mole [11]; metastasized carcinomas contain more than twice as much DSA-binding oligosaccharides as the normal gland, primary carcinomas contain an intermediate amount [12]. |
| Garlic lectin          | recognizes monosaccharides in mannosyl configuration | Distinguishes between (Glc,Man$_{5-7}$,GlcNAc$_2$) and (Man$_{5-7}$,GlcNAc$_2$) chains; ligand potencies for the lectin increase in the order mannobiose < triose < pentaose < (man)$_9$ oligosaccharide; addition of 2 GlcNAc residues at the reducing end of triose or pentaose enhances their potency significantly; substitution at the nonreducing end increases their potency only marginally. However, the best mannoooligosaccharide ligand is Man$_9$GlcNAc$_2$AsN, which bears several α:1-2 linked mannose residues. Man$_{20}$GlcNAc exhibits highest binding affinity; no other lectin is known to show such specificity [13]. |
| Jack fruit lectin      | 1-β-galactopyranosyl-3-(α-2-acetamido-2-deoxyGalactopyranoside) in O-linked oligosaccharides [14] | Distinguishes between ‘O’-linked glycans and N-linked glycans; generally used for SLAC purification of O-linked oligosaccharides or the glycoconjugates bearing these sugars [15], and for purification of immunoglobulins from rabbits infected intraduodenally with *Vibrio cholerae* [16], and from normal human serum [17–20]. |
*Maackia amurensis* \( \alpha,2\text{--}3 \) linked sialic acid

Amino terminal octapeptide from human glycoporphin A having three Neu5Ac(\( \alpha 2\text{--}3 \)Gal\( \beta 1\text{--}3 \)Neu5Ac(\( \alpha 2\text{--}6 \)GalNAc tetrasaccharide chains designated as CB-II has an extremely strong affinity for *M. amurensis* hemagglutinin (MAH) [21]; used as probe for analysis of sialic acid containing cell surface glycoconjugates by flow cytometry [22]; potent leukoagglutinin for the mouse lymphoma cell line BW5147 [23]; weak hemagglutinin in of human erythrocytes. Generally, interactions with sugars are not dependent on either branching pattern of mannose residues or presence of poly-N-acetyllactosamine sequences.

*Phaseolus vulgaris* distinguishes between biantennary and tetraantennary sugar chains

Hepatocellular carcinoma, extra-hepatic malignancy including yolksac tumors produce increased amounts of erythroagglutinating phytohemagglutinin (viz PHA-E4)-binding proteins, asialo-\( \alpha \)-foetoprotein being one of them [24,25]; human hepatoma \( \gamma \)-glutamyl transpeptidase associated with malignant transformation is specifically detected by *P. vulgaris* erythroagglutinating lectin agarose [26].

*Psophocarpus tetragonolobus* distinguishes H-antigenic structures from the non-H antigenic structures

Winged Bean Agglutinin (WBA I) reacts with the antigenic determinants of blood groups A and B, WBA II reacts with that of group ‘O’ [27]. WBA II binds to the H-and T-antigenic determinants on the human erythrocytes [28].

*Sambucus nigra* \( \alpha\text{-NeuNAc}2 \rightarrow 6 \text{gal/galNAc} \)

Highly specific, does not bind \( \alpha\text{-NeuNAc}2 \rightarrow 3 \text{gal/galNAc} \) or the glycoconjugates bearing the structure; *S. nigra* agglutinin immobilized on Sepharose 4B resolves two oligosaccharides/glycopeptides based on the number of Neu5Ac[alpha 2–6]Gal units present and also reveals the presence of microheterogeneity in fetuin and orosomucoid [29].

*Calreticulin* distinguishes between (Glc\( _1\text{Man}_7\text{GalNAc}_2 \)) and (Glc\( _2\text{Man}_9\text{GalNAc}_2 \))

Exquisitely specific for the monoglucosyl Man\( _7\text{Man}_9 \) structures [30].
| Serial no. | Origin and nature of glycoprotein microheterogeneity | Technique(s) used for heterogeneity resolution | Applications/analysis/conclusions/remarks | Reference |
|-----------|-----------------------------------------------|---------------------------------------------|--------------------------------------------|-----------|
| 1         | α1-antichymotrypsin microheterogeneity         | immunoaffinoelectrophoresis with free Concanavalin A (Con A) in the first dimension; Con A Sepharose Affinity Chromatography (Con A-SAC) and high resolution $^1$H-NMR spectroscopy | Con A-SAC separates the protein into 3 fractions: a Con A-reactive form with 4 triantennary glycans, a Con A weakly reactive form with 3 triantennary and 1 diantennary glycans, and a Con A non-reactive form with 1 triantennary and 3 diantennary glycans. There is an increased proportion of Con A non-reactive form in patients developing a systemic disease (systemic lupus erythematosus, rheumatoid arthritis, temporal arteritis). | [37] |
| 2         | Glycosylation status of serum transferrin as a biochemical index of carbohydrate-deficient glycoprotein syndrome type I | Capillary zone electrophoresis and a novel HPLC strategy for quantification of glycans released by exoglycosidase treatment | Hexa-, penta-, and tetrasialoforms of human serum transferrin are present in both normal and carbohydrate-deficient glycoprotein syndrome type I serum samples. In addition, the carbohydrate-deficient glycoprotein syndrome type I transferrin also contained a disialoform, representing a glycoform in which one of the two N-glycosylation sites is unoccupied, and non-glycosylated form where both remain unoccupied. This could be used as a rapid diagnostic test for the carbohydrate-deficient glycoprotein syndromes group of diseases. | [38] |
| 3         | Fel d1 (cat allergen 1) | HPLC and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) | The allergen is a 38-kDa dimer of two 19 kDa subunits, each of which comprises a light α-chain and a heavy β-chain containing an N-linked oligosaccharide on Asn33; Fel d1 is found to be partially truncated and to exist in several isoforms; the glycan is a heterogeneous triantennary complex type structure; and the heterogeneity is caused by terminal sialic acid and a fucose residue attached to a β-galactose residue. | [39] |
Transferrin, α1-antitrypsin, haptoglobin β-chain, and α1-acid glycoprotein microheterogeneity in serum and liver of patients with carbohydrate-deficient glycoprotein syndrome type I

High-resolution two-dimensional electrophoresis (2-DE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Serum glycoproteins in all patients showed a cathodal shift and decreased mass. The two-dimensional pattern of immunodetected precursors of serum proteins in liver cells from patients with CDGS showed abnormal low-mass precursors and absence precursors normally found in controls. These results suggest that these abnormal precursors accumulate during early oligosaccharide processing of the nascent protein-bound oligosaccharides and that glycoprotein precursors undergo an altered intracellular transport while the post-translational processing along the normal pathway is still apparently functioning in patients with CDGS.

Glycoform heterogeneity of follicle stimulating hormone (FSH) and luteinizing hormone (LH) through the normal menstrual cycle and in the post-menopausal state

Con A-SAC

The changes in gonadotropin glycoforms occur through the menstrual cycle which are related to changes in the prevailing steroid environment. Following the menopause oestrogenic loss resulted in acidic, relatively, simple glycoforms.

Sugar sequence and branch structure of the oligosaccharides in RNase B

GCC-LC/MS in the positive ion mode and (LC/MS/MS)

These techniques can be used for elucidation of the distribution of oligosaccharides too.

Armadillidium vulgare androgenic hormone glycoforms (AH1 and AH2)

HPLC and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF)

Amino acid (aa) sequence of the two chains A (29-aa long) and B (44-aa long) of the two glycoforms are identical; Asn18 of chain A is N-glycosylated.
Table 2 (continued)

| Serial no. | Origin and nature of glycoprotein microheterogeneity                                                                 | Technique(s) used for heterogeneity resolution                                                                 | Applications/analysis/conclusions/remarks                                                                 | Reference |
|------------|-------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------|
| 8          | Glycosylation sites and preliminary glycosylation pattern in erythropoietin (EPO) and the detailed site-specific carbohydrate heterogeneity | Liquid chromatography (LC) mass spectrometry (MS) with graphitized carbon column (GCC), coupled with tandem mass spectrometry (LC/MS/MS) | The di- and trisialylated tetraantennary oligosaccharides are attached to Asn24, 38, and 83, whereas their isomers, di- and trisialylated triantennary oligosaccharides containing N-acetyl lactosamines, are combined with Asn24. 32 new complex-type glycans are characterized Oligomannose-type glycans range from Man\textsubscript{5} GlcNAc\textsubscript{2} to Man\textsubscript{8}GlcNAc\textsubscript{2}. Di-, tri-, and tetraantennary complex-type structures are present, both neutral and (alpha2–3)-sialylated (up to tetrasialo), comprising 24% and 59%, respectively, of the total carbohydrate moiety. The self-aggregation of IgA1 is closely connected with the glycoform of a mucin-type sugar chain on its hinge portion (HGP33). Jacalin affinity chromatography separated the normal human serum IgA1 into two subfractions as: the monomeric form (eluted by 0.25 mM galactose and abundant in the sialic acid-rich components) and the aggregated form (eluted by 0.8 mM galactose, and abundant in the sialic acid-poor components). Application of CE analysis to HGP33 indicated that the monomeric IgA1 was composed of a relatively complete molecule with respect to the glycoform rather than the aggregated IgA1. | [44]      |
| 9          | Glycosylation pattern of human epidermal growth factor receptor (EGFR)                                                   | Con A-SAC, anion exchange chromatography, HPLC and high pH anion-exchange chromatography; NMR spectroscopy and mass spectrometry |                                                                                                           | [45]      |
| 10         | Microheterogeneity of the IgA1 hinge glycopeptide (HGP33) having multiple O-linked oligosaccharides                     | Jacalin affinity chromatography and capillary electrophoresis                                                |                                                                                                           | [46]      |
moiety of a glycoprotein may affect the immunogenicity, half-life, bioactivity, and stability of a potential therapeutic product [33]. This puts glycoproteins among the most challenging of products to characterize because of the extreme and fine nature of the sample microheterogeneities [34]. In nature, microheterogeneity in glycoproteins is a condition that arises due to variations in their carbohydrate moiety [35]. The variation is either due to an incomplete biosynthetic sequence or due to post-synthetic degradation within the cell or during isolation of the protein.

Since N-acetylneuraminic acid forms the terminal sugar in most glycoproteins, desialylation leads to exposure of the galactose residues. A striking role for the exposed galactose residues on the survival time and fate of these glycoproteins in vivo has been demonstrated by Ashwell and Morell [36]. Moreover, the rate and extent of the uptake of these proteins by liver parenchymal cells is determined by the number of galactose residues exposed on the surface of these glycoproteins [36]. Therefore, isolation of sialylglycoproteins at different stages of sialylation–desialylation will help delineate quantitatively their uptake on the basis of the number of the galactose sites exposed. In one of the pioneering studies addressing GM resolution, the artificial generation and demonstration of the microheterogeneity subsequent to amplification by binding with monovalent galactose specific lectin ricin has been elegantly shown by Surolia et al. [35] using simple techniques of lectin affinity chromatography followed by gel filtration. In this study, microheterogeneous populations of the glycoprotein fetuin were found to bind molecules of the galactose-binding lectin ricin in proportion to the degree of desialylation of the former and the number of galactose moieties exposed as a result. Monitoring the difference in molecular weight of the fetuin–ricin complexes formed from radiiodinated ricin, native fetuin could be resolved into different microheterogeneous groups. This method could find application not only in purification of several other glycoproteins to homogeneity, but also in resolution of their microheterogeneity.

In addition to lectin affinity chromatography, many techniques such as mass spectrometry, ion-exchange chromatography, different forms of capillary electrophoresis, and high resolution two-dimensional electrophoresis have been put to use in resolving the GM. A few of the techniques most generally used in conjunction with lectin affinity chromatography are listed in Table 2 [37–46]. The mass spectral techniques, especially, are an indispensable tool for the detailed analysis of the covalent structures of proteins, particularly those that are refractory to standard approaches of protein chemistry [47]. In Table 3, application of SLAC pioneered by Cummings and Kornfeld and exploited with a great degree of success by Kobata et al. in studying the altered glycosylation in, and hence, the diagnosis of certain diseases, is depicted [12,31,32,48–55]

5. GM in normal vs. clinical conditions

It is clear from the foregoing that the studies of factors responsible for the sustenance of GM per se in healthy organisms and alterations in some of them that manifest in clinical conditions are of central importance in understanding not only its generation, but also in determining the prospects of using it in diagnosis and treatment of pathological conditions. Although not much was made of the phenomenon earlier [56], GM has
### Table 3

Application of serial lectin affinity chromatography (SLAC) in resolution of the glycoprotein microheterogeneity

| Serial no. | Sequence of matrix-immobilized lectin columns used for SLAC | Source of glycoprotein under investigation | Reference |
|------------|-------------------------------------------------------------|------------------------------------------|-----------|
| 1          | Concanavalin A (Con A), pea lectin (PSA), leuкоagglutinating phytohemagglutinin and D. stramonium agglutinin (DSA) | Different types of Asn-linked oligosaccharides in mouse lymphoma BS5147 cells | [31,32] |
| 2          | Aleuria aurantia lectin (AAL), Ricinus communis agglutinin (RCA)-120-WG003, E4-phytohemagglutinin (PHA-E4) | 16 different biantennary complex-type Asn-linked sugar chains | [48] |
| 3          | AAL, Con A, PHA-E4, and DSA | Asparagine-linked sugar chains of rat kidney aminopeptidase N and dipeptidylpeptidase IV | [49] |
| 4          | Con A and DSA | N-linked oligosaccharides released from normal human esophageal epithelium and esophageal squamous carcinoma | [12] |
| 5          | Con A, S. nigra agglutinin (SNA), and P. vulgaris leuкоagglutinin (LPHA) | N-glycosylation site mapping of human serotransferrin | [50] |
| 6          | Con A, PHA-E and jacalin lectin | Sugar-chain heterogeneity of human urinary chorionic gonadotropin (hCG) | [51] |
| 7          | Con A, PHA-E, PSA and wheat germ agglutinin (WGA) | Sugar-chain structures of gamma-glutamyltransferase in human renal cell carcinoma | [52] |
| 8          | Con A, WGA | Asn-linked sugar-chain structure of prostatic acid phosphatase (differences between human prostate carcinoma and benign prostatic hyperplasia) | [53] |
| 9          | Con A, WGA | Asn-linked sugar-chain structures of N-acetyl beta-t-hexosaminidase A (Hex A) (in human renal oncogenesis) | [54] |
| 10         | Con A, PHA-E4 and PHA-L4 | Asn-linked sugar-chain structures of prostate-specific antigen (PSA) | [55] |
received due attention during the recent past. Work on various glycoproteins such as the α1-acid glycoprotein, transferrin, ferritin, S-peplomer glycoprotein, etc. has shown that the expression and maintenance of GM in clinical conditions are altered. The variation in GM of the α1-acid glycoprotein is implicated in a wide variety of pathological conditions such as acute inflammation [57], lung disease [58], chronic alcohol abuse [59], alcoholic cirrhosis [60], rheumatoid arthritis [61–64], depressive disorder [65], systemic lupus erythematosus [66], malignant mesothelioma [67] and renal failure [68]. The acute encephalomyelitis (and subsequent death) and demyelinating disease in the surviving animals, which is attributed to the JHM strain of the mouse hepatitis virus (a neurotropic coronavirus), is known to be mediated by and reflected in the altered expression of the microheterogeneity of the S-peplomer glycoprotein [69].

Other glycoproteins exhibiting a disease-state-altered character of microheterogeneity are ferritin which serves as an additional tool for the diagnosis of Still’s disease, an acute systemic inflammatory disorder [70] and transferrin, implicated in chronic alcohol abuse and major depression [71]; in fact, the appearance of desialo-transferrin (De-TF) in serum has been reported to be a biochemical marker of chronic alcoholism and alcoholic liver disease. The patterns of variability in transferrin structure in pregnancy, iron deficiency anemia, women using oral contraceptives, nonanaemic rheumatoid arthritis, iron deficient rheumatoid arthritis and anemia of the chronic diseases have been reported too [71–74].

6. LAC as a supplementary diagnostic tool

In addition to the above, application of lectin affinity chromatography as a supplement to other conventional diagnostic tools is not uncommon. One case in point is the human chorionic gonadotropin hCG, a glycoprotein hormone produced by trophoblasts of the placenta. High levels of hCG in the blood and urine are also detected in patients with various trophoblastic diseases such as hydatidiform mole and choriocarcinoma. Therefore, urinary and serum hCGs have been measured as useful markers for the diagnosis and prognosis of trophoblastic diseases as well as normal pregnancy.

Many a sensitive method to determine the level of hCG in biological material have been developed, but none provides a way to discriminate hCGs from normal pregnant women and those from various trophoblastic diseases. Differential diagnosis of trophoblastic diseases is essential because it is indispensable for the correct treatment of these diseases. Hydatidiform mole is considered to be essentially a benign lesion, although the rate of incidence of choriocarcinoma in patients with this disease is much higher than in normal pregnancy. Some hydatidiform moles show apparently more malignant characteristics than others, such as invasion into the surrounding tissues and metastasis, and are discriminated from typical moles by naming them invasive moles. Although prophylactic chemotherapy is effective to reduce the development of persistent gestational trophoblastic diseases such as invasive mole and choriocarcinoma, the use of chemotherapy at the time of molar evacuation is controversial because of the drug toxicity.Thus, any method to discriminate invasive mole from hydatidiform mole would be useful to avoid indiscriminate prophylactic chemotherapy [11].
Investigation of the structures of the carbohydrate moieties of hCGs purified from the urine of patients with various trophoblastic diseases reveals that although all hCGs contain four asparagine-linked sugar chains in one molecule, their structures are different depending on the disease. Accordingly, a method that specifically detects the hCGs containing the \( \text{Gal}β1 \rightarrow 4\text{GlcNAc}β1 \rightarrow 4(\pm \text{NeuAc}α2 \rightarrow 3\text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow 2\text{Man} \) group in their sugar moieties could be used to discriminate patients with invasive mole or choriocarcinoma from pregnant women or patients with hydatidiform mole. Endo et al. [11] found that a \( D. stramonium \) agglutinin (DSA)-Sepharose column fractionates the oligosaccharides into three groups. All oligosaccharides with either the \( \text{Gal}β1 \rightarrow 4\text{GlcNAc}β1 \rightarrow 6(\text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow 2\text{Man} \) group or the \( \text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow 3\text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow \) group in the non-substituted form are bound to the column and eluted with buffer containing \( β-N\)-acetylglucosamine oligomers. Oligosaccharides with the non-substituted \( \text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow 4(\text{Galβ1} \rightarrow 4\text{GlcNAc}β1 \rightarrow 2\text{Man} \) group are retarded in the column and eluted with buffer only. Oligosaccharides which contain none of the groups described above or the groups in either sialylated or fucosylated form pass through the column without interaction. The binding specificity of the DSA-Sepharose column is thus useful in distinguishing malignant hCGs from the normal counterpart.

Another example of applying LAC as a supplementary diagnostic tool is the detection and isolation of human \( α \)-foetoprotein from the serum. \( α \)-Foetoprotein is normally produced by foetal hepatocytes and is detectable in serum up to 2 weeks after birth. In certain liver diseases, it may reappear in the serum during the first year of life. This glycoprotein is also synthesized by primary liver cell carcinoma, and its presence in serum is therefore of a great diagnostic significance. The concentration of albumin in normal serum is very high as compared to any single glycoprotein, and specifically, it is about 100 times as high as that of the human \( α \)-foetoprotein.

The primarily difficult protocol of purifying the \( α \)-foetoprotein due to its lesser relative abundance is further compounded by the similarity of the foetoprotein and albumin with regard to their physicochemical properties such as molecular weight, isoelectric point, and the electrophoretic mobility. They are thus almost inseparable. Whereas other immunochemical precipitation methods used for the resolution of the two proteins are known to be efficient so far as the separation is concerned, they involve long and tedious processes, and subsequently, resolution of the difficulty posed by similarity is impossible using the conventional methods. Con A Sepharose affinity chromatography as part of a two-step chemical method for the purification of the human \( α \)-foetoprotein free of albumin, as reported by Page [75], successfully separates the two components. Other lectin-Sepharoses which help distinguish between glycoproteins with very minor microheterogeneities and their applications in medicine are listed in Table 1.

References

[1] Rani PG, Bachhawat K, Reddy GB, Oscarson S, Surolia A. Isothermal titration calorimetric studies on the binding of deoxytrimannoside derivatives with artocarpin: implications for a deep-seated combining site in lectins. Biochemistry 2000;39:10755–60.
[2] del Campillo E, Shannon LM, Hankins CN. Molecular properties of the enzymic phytohemagglutinin of mung bean. J Biol Chem 1981;256:7177–80.
[3] Hankins CN, Shannon LM. The physical and enzymatic properties of a phytohemagglutinin from mung beans. J Biol Chem 1978;253:7791–7.
[4] Sharon N. Lectin–carbohydrate complexes of plants and animals: an atomic view. Trends Biochem Sci 1993;18:221–6.
[5] Suguna K, Surolia A, Vijayan M. Structural diversity and carbohydrate specificity of plant lectins. In: Vijayan M, Yathindra N, Kosalkar AS, editors. Perspectives in structural biology. Hyderabad: Universities Press; 1999. p. 367–79.
[6] Moore KL, Varki A, McEver RP. GMP-140 binds to a glycoprotein receptor on human neutrophils: evidence for a lectin-like interaction. J Cell Biol 1991;112:491–9.
[7] McEver RP, Moore KL, Cummings RD. Leukocyte trafficking mediated by selectin–carbohydrate interactions. J Biol Chem 1995;270:11025–8.
[8] Vijayan M, Chandra N. Lectins. Curr Opin Struct Biol 1999;9:707–14.
[9] Donaldson MS, Shuler ML. The use of lectins to select subpopulations of insect cells. Biotechnol Bioeng 1999;64:616–9.
[10] Osawa T, Tsuji T. Fractionation and structural assessment of oligosaccharides and glycopeptides by use of immobilized lectins. Annu Rev Biochem 1987;56:21–42.
[11] Endo T, Ino K, Nozawa S, Iizuka R, Kobata A. Immobilized Datura stramonium agglutinin column chromatography, a novel method to discriminate the urinary hCGs of patients with invasive mole and choriocarcinoma from those of normal pregnant women and patients with hydatidiform mole. Jpn J Cancer Res 1988;79:160–4.
[12] Hiraizumi S, Takasaki S, Nishihira T, Mori S, Kobata A. Comparative study of the N-linked oligosaccharides released from normal human esophageal epithelium and esophageal squamous carcinoma. Jpn J Cancer Res 1990;81:363–71.
[13] Dam TK, Bachhawat K, Rani PG, Surolia A. Garlic (Allium sativum) lectins bind to high mannose oligosaccharide chains. J Biol Chem 1998;273:5528–35.
[14] Hortin GL. Isolation of glycopeptides containing O-linked oligosaccharides by lectin affinity chromatography on jacalin-agarose. Anal Biochem 1990;191:262–7.
[15] Hortin GL, Trimpe BL. Lectin affinity chromatography of proteins bearing O-linked oligosaccharides: application of jacalin-agarose. Anal Biochem 1990;188:271–7.
[16] Kabir S. Simultaneous isolation of intestinal IgA and IgG from rabbits infected intraduodenally with Vibrio cholerae 01 by combined lectin affinity chromatography involving jacalin and protein A. Comp Immunol Microbiol Infect Dis 1993;16:153–61.
[17] Haun M, Incledon B, Alles P, Wasi S. A rapid procedure for the purification of IgA1 and IgA2 subclasses from normal human serum using protein G and jackfruit lectin (jacalin) affinity chromatography. Immunol Lett 1989;22:273–9.
[18] Aucouturier P, Pneau N, Preud'Homme JL. A simple procedure for the isolation of human secretory IgA of IgA1 and IgA2 subclass by a jackfruit lectin, jacalin, affinity chromatography. Mol Immunol 1988;25:321–2.
[19] Kondoh H, Kobayashi K, Hagiwara K. A simple procedure for the isolation of human secretory IgA of IgA1 and IgA2 subclass by a jackfruit lectin, jacalin, affinity chromatography. Mol Immunol 1987;24:1219–22.
[20] Kondoh H, Kobayashi K, Hagiwara K, Vaerman JP. Separation of human slgA1 and slgA2 by affinity chromatography on the jackfruit lectin, jacalin. Adv Exp Med Biol 1987;216B:1193–7.
[21] Konami Y, Yamamoto K, Osaka T, Irimura T. Strong affinity of Maackia amurensis hemagglutinin (MAH) for sialic acid-containing Ser/Thr-linked carbohydrate chains of N-terminal octapeptides from human glycoporphin A. FEBS Lett 1994;342:334–8.
[22] Kaku H, Mori Y, Goldstein II, Shibuya N. Monovalent, monovalent derivative of Maackia amurensis leukoagglutinin. Preparation and application to the study of cell surface glycoconjugates by flow cytomtry. J Biol Chem 1993;268:13237–41.
[23] Wang WC, Cummings RD. The immobilized leukoagglutinin from the seeds of Maackia amurensis binds with high affinity to complex-type Asn-linked oligosaccharides containing terminal sialic acid-linked alpha-2,3 to penultimate galactose residues. J Biol Chem 1988;263:4576–85.
[24] Taketa K, Ichikawa E, Akamatsu K, Ohuta Y, Sekiya C, Namiki M, et al. Increased asialo-alpha-fetoprotein in patients with alpha-fetoprotein-producing tumors: demonstration by affinity electrophoresis with erythroagglutinating phytohemagglutinin of Phaseolus vulgaris lectin. Tumour Biol 1985;6:533–44.

[25] Taketa K, Ichikawa E, Nakabayashi H, Sato J, Kato K, Akai S, et al. Further resolution of human alpha-fetoprotein by affinity electrophoresis with erythroagglutinating phytohemagglutinin of Phaseolus vulgaris lectin. Tumour Biol 1985;6:519–31.

[26] Hitoi A, Yamashita K, Ohkawa J, Kobata A. Application of a Phaseolus vulgaris erythroagglutinating lectin agarose column for the specific detection of human hepatoma gamma-glutamyl transpeptidase in serum. Gan`o 1984;75:301–4.

[27] Kirkeby S, Singha NC, Surolia A. Localized agglutinin staining in muscle capillaries from normal and very old atrophic human muscle using winged bean (Psophocarpus tetragonolobus) lectin. Histochem Cell Biol 1997;107:31–7.

[28] Patanjali SR, Sajjan SU, Surolia A. Erythrocyte-binding studies on an acidic lectin from winged bean (Psophocarpus tetragonolobus). Biochem J 1988;252:625–31.

[29] Shibuya N, Goldstein JJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ. Fractionation of sialylated oligosaccharides, glycopeptides, and glycoproteins on immobilized elderberry (Sambucus nigra L.) bark lectin. Arch Biochem Biophys 1987;254:1–8.

[30] Patil AR, Thomas CJ, Surolia A. Kinetics and the mechanism of interaction of the endoplasmic reticulum chaperone, calreticulin, with monoglucosylated (Glc\_Man\_GlcNAc\_\_\_\_) substrate. J Biol Chem 2000;275:24348–56.

[31] Cummings RD, Kornfeld S. The distribution of repeating [Gal beta 1.4GlcNAc beta 1.3] sequences in asparagine-linked oligosaccharides of the mouse lymphoma cell lines BW5147 and PHAR 2.1. J Biol Chem 1984;259:6253–60.

[32] Cummings RD, Kornfeld S. Fractionation of asparagine-linked oligosaccharides by serial lectin-agarose affinity chromatography. A rapid, sensitive, and specific technique. J Biol Chem 1982;257:11235–40.

[33] Hooker AD, James DC. Analysis of glycoprotein heterogeneity by capillary electrophoresis and mass spectrometry. Mol Biotechnol 2000;14:241–9.

[34] Apffel A, Chakel J, Udaiyar S, Swedberg S, Hancock WS, Souders C, et al. Application of new analytical technology to the production of a “well-characterized biological”. Dev Biol Stand 1998:96:11–25.

[35] Surolia A, Appukuttan PS, Pain D, Bachhawat BK. Monovalent lectin as a novel tool for the resolution of microheterogeneity in glycoproteins. Anal Biochem 1980;105:436–40.

[36] Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv Enzymol Relat Areas Mol Biol 1974;41:99–128.

[37] Laine A, Hachulla E. Glycoforms of serum alpha 1-antichymotrypsin studied by immunoaffino-electrophoresis. From the fundamental aspect to clinical applications. Ann Biol Clin (Paris) 1991;49:359–66.

[38] Inour O, Mattu TS, Mian N, Keir G, Winchester B, Dwek RA, et al. The identification of abnormal glycoforms of serum transferrin in carbohydrate deficient glycoprotein syndrome type I by capillary zone electrophoresis. Glycoconjugate J 1996;13:1031–42.

[39] Kristensen AK, Schou C, Roepstorff P. Determination of isoforms, N-linked glycan structure and disulfide bond linkages of the major cat allergen Fel d1 by a mass spectrometric approach. Biol Chem 1997;378:899–908.

[40] Henry H, Tissot JD, Messerli B, Markert M, Muntau A, Skladal D, et al. Microheterogeneity of serum glycoproteins and their liver precursors in patients with carbohydrate-deficient glycoprotein syndrome type I: apparent deficiencies in clusterin and serum amyloid P. J Lab Clin Med 1997;129:412–21.

[41] Anobile CJ, Talbot JA, McCann SJ, Padmanabhan V, Robertson WR. Glycoform composition of serum gonadotrophins through the normal menstrual cycle and in the post-menopausal state. Hum Reprod 1998;4:631–9.

[42] Kawasaki N, Ohuta M, Hyuga S, Hashimoto O, Hayakawa T. Analysis of carbohydrate heterogeneity in a glycoprotein using liquid chromatography/mass spectrometry and liquid chromatography with tandem mass spectrometry. Anal Biochem 1999;269:297–303.

[43] Martin G, Sorokine O, Moniatte M, Bulet P, Huet R, Van Dorsselaer A. The structure of a glycosylated protein hormone responsible for sex determination in the isopod, Armadillidium vulgare. Eur J Biochem 1999;262:727–36.
spectrometry and liquid chromatography with tandem mass spectrometry to the analysis of the site-specific carbohydrate heterogeneity in erythropoietin. Anal Biochem 2000;285:82–91.

[45] Stroop CJ, Weber W, Gerwig GJ, Nimtz M, Kamerling JP, Vliegenthart JF. Characterization of the carbohydrate chains of the secreted form of the human epidermal growth factor receptor. Glycobiology 2000;10:901–17.

[46] Iwase H, Tanaka A, Hiki Y, Kuboku T, Sano T, Ishii-Karakasa I, et al. Analysis of the microheterogeneity of the IgA1 hinge glycopeptide having multiple O-linked oligosaccharides by capillary electrophoresis. Anal Biochem 2001;288:22–7.

[47] Bezoskuska K, Sklenar J, Novak P, Halada P, Havlicek V, Kraus M, et al. Determination of the complete covalent structure of the major glycoform of DQH sperm surface protein, a novel trypsin-resistant boar seminal plasma O-glycoprotein related to pB1 protein. Protein Sci 1999;8:1551–6.

[48] Harada H, Kamei M, Tokumoto Y, Yui S, Koyama F, Kochibe N, et al. Systematic fractionation of oligosaccharides of human immunoglobulin G by serial affinity chromatography on immobilized lectin columns. Anal Biochem 1987;164:374–81.

[49] Yamashita K, Tachibana Y, Matsoula Y, Katunuma N, Kochibe N, Kobata A. Comparative studies of the sugar chains of aminopeptidase N and dipeptidylpeptidase IV purified from rat kidney brush-border membrane. Biochemistry 1988;27:5565–73.

[50] Fu D, van Halbeek H. N-glycosylation site mapping of human serum transferrin by serial lectin affinity chromatography, fast atom bombardment-mass spectrometry, and 1H nuclear magnetic resonance spectroscopy. Anal Biochem 1992;206:53–63.

[51] Sakai H, Yamagishi F, Miura M, Hata K, Koyama I, Sakagishi Y, et al. Sugar chain heterogeneity of human urinary chorionic gonadotropin determined by serial lectin affinity chromatography: difference between benign and malignant disease. Tumour Biol 1994;15:230–5.

[52] Yoshida K, Sumi S, Honda M, Hosoya Y, Yano M, Arai K, et al. Serial lectin affinity chromatography demonstrates altered asparagine-linked sugar chain structures of gamma-glutamyltransferase in human renal cell carcinoma. J Chromatogr, B: Biomed Sci Appl 1995;672:45–51.

[53] Yoshida KI, Honda M, Arai K, Hosoya Y, Moriguchi H, Sumi S, et al. Serial lectin affinity chromatography with concavalin A and wheat germ agglutinin demonstrates altered asparagine-linked sugar-chain structures of prostate acid phosphatase in human prostate carcinoma. J Chromatogr, B: Biomed Sci Appl 1997;695:439–43.

[54] Yoshida K, Moriguchi H, Sumi S, Horimi H, Kitahara S, Umeda H, et al. Alterations of asparagine-linked sugar chains of N-acetyl beta-D-hexosaminidase during human renal oncogenesis: a preliminary study using serial lectin affinity chromatography. J Chromatogr, B: Biomed Sci Appl 1999;723:75–80.

[55] Sumi S, Arai K, Kitahara S, Yoshida K. Serial lectin affinity chromatography demonstrates altered asparagine-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma. J Chromatogr, B: Biomed Sci Appl 1999;727:9–14.

[56] Zanetta JP, Reeker A, Vincendon G, Gombos G. Synaptosomal plasma membrane glycoproteins: II. Isolation of fucosyl-glycoproteins by affinity chromatography on the Ulex europeus lectin specific for L-fucose. Brain Res 1977;138:317–28.

[57] Iijima S, Shiba K, Kimura M, Nagai K, Iwai T. Changes of alpha1-acid glycoprotein microheterogeneity in acute inflammation stages analyzed by isoelectric focusing using serum obtained postoperatively. Electrophoresis 2000;21:753–9.

[58] Hansen JE, Larsen VA, Bog-Hansen TC. The microheterogeneity of alpha 1-acid glycoprotein in inflammatory lung disease, cancer of the lung and normal health. Clin Chim Acta 1984;138:41–7.

[59] Henry H, Froehlich F, Perret R, Tissot JD, Etters-Messerli B, Lavanchy D, et al. Microheterogeneity of serum glycoproteins in patients with chronic alcohol abuse compared with carbohydrate-deficient glycoprotein syndrome type I. Clin Chem 1999;45:1408–13.

[60] Biou D, Chanton P, Konan D, Sete N, N'Guyen H, Feger J, et al. Microheterogeneity of the carbohydrate moiety of human alpha 1-acid glycoprotein in two benign liver diseases: alcoholic cirrhosis and acute hepatitis. Clin Chim Acta 1989;186:59–66.

[61] Elliott MA, Elliott HG, Gallagher K, McGuire J, Field M, Smith KD. Investigation into the concanavalin A reactivity, fucosylation and oligosaccharide microheterogeneity of alpha 1-acid glycoprotein expressed in the sera of patients with rheumatoid arthritis. J Chromatogr, B: Biomed Sci Appl 1997;688:229–37.
[62] Hrycaj P, Sobieska M, Mackiewicz S, Muller W. Microheterogeneity of alpha 1 acid glycoprotein in rheumatoid arthritis: dependent on disease duration? Ann Rheum Dis 1993;52:138–41.
[63] Hrycaj P, Sobieska M, Mackiewicz S, Muller W. Microheterogeneity of alpha 1-acid glycoprotein in early and established rheumatoid arthritis. J Rheumatol 1993;20:2020–4.
[64] Mackiewicz A, Pawlowski T, Mackiewicz-Pawlowska A, Wiktrowicz K, Mackiewicz S. Microheterogeneity forms of alpha 1-acid glycoprotein as indicators of rheumatoid arthritis activity. Clin Chim Acta 1987;163:185–90.
[65] Sluzewska A, Rybakowski JK, Sobieska M, Wiktrowicz K. Concentration and microheterogeneity glycophorms of alpha-1-acid glycoprotein in major depressive disorder. J Affective Disord 1996;39:149–55.
[66] Mackiewicz A, Marcinkowska-Pieta R, Ballou S, Mackiewicz S, Kushner I. Microheterogeneity of alpha 1-acid glycoprotein in the detection of intercurrent infection in systemic lupus erythematosus. Arthritis Rheum 1987;30:513–8.
[67] Herve F, Duche JC, Jaurand MC. Changes in expression and microheterogeneity of the genetic variants of human alpha1-acid glycoprotein in malignant mesothelioma. J Chromatogr, B: Biomed Sci Appl 1998;715:111–23.
[68] Vasson MP, Roch-Arveiller M, Couderc R, Baguet JC. Effects of alpha-1 acid glycoprotein on human polymorphonuclear neutrophils: influence of glycan microheterogeneity. Clin Chim Acta 1994;224:65–71.
[69] Oleszak EL, Knisley K, Rodkey LS, Leibowitz JL. Microheterogeneity of S-glycoprotein of mouse hepatitis virus temperature-sensitive mutants. J Virol Methods 1992;38:103–12.
[70] Tsutsumi M, Wang JS, Takada A. Microheterogeneity of serum glycoproteins in alcoholics: is desialo-transferrin the marker of chronic alcohol drinking or alcoholic liver injury? Alcohol Clin Exp Res 1994;18:392–7.
[71] Ghosh P, Okoh C, Liu QH, Lakshman MR. Effects of chronic ethanol on enzymes regulating sialylation and desialylation of transferrin in rats. Alcohol Clin Exp Res 1993;17:576–9.
[72] Wang JS, Tsutsumi M, Ueshima Y, Takase S, Matsuda Y, Takada A. Analysis of the characteristics of microheterogeneity of various serum glycoproteins in chronic alcoholics. Alcohol Alcohol Suppl 1993;1A:21–8.
[73] de Jong G, van Noort WL, Feelders RA, de Jeu-Jaspars CM, van Eijk HG. Adaptation of transferrin protein and glycan synthesis. Clin Chim Acta 1992;212:27–45.
[74] Page M. α-Foetoprotein: purification on sepharose-linked concanavalin A. Can J Biochem 1973;51:1213–5.