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# Exploiting lectin affinity chromatography in clinical diagnosis

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## 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.

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## 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

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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 separation 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, glycoconjugate 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 glycoconjugates 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 ($10^4$–$10^8$ $M^{-1}$) 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 ($Glc_1Man_{5-7}GlcNAc_2$) 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 mannooligosaccharide ligand is $Man_9GlcNAc_2$ AsN, which bears several $\alpha$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* | α,2–3 linked sialic acid | Amino terminal octapeptide from human glycophorin A having three Neu5Ac(α 2–3) Gal(β 1–3)Neu5Ac(α 2–6)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 hemagglutin 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-α-foetoprotein being one of them [24,25]; human hepatoma γ-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* | α-NeuNAc(2 → 6)gal/galNAc | Highly specific, does not bind α-NeuNAc(2 → 3)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 ($Glc_1Man_{7-9}GlcNAc_2$) and ($Man_{7-9}GlcNAc_2$) | Exquisitely specific for the monoglucosyl $Man_7$–$Man_9$ structures [30]. |

Table 2
Analysis/resolution of glycoprotein microheterogeneity using other techniques in conjunction with lectin affinity chromatography

| 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: Con A-non reactive form (with 4 triantennary glycans), a Con A weakly reactive form (with 3 triantennary and 1 diantennary glycans) and a Con A 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 carbo hydrate 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] |

| | | | | |
|---|---|---|---|---|
| 4 | Transferrin, α1antitrypsin, 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 oligossaccharide 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. | [40] |
| 5 | 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. | [41] |
| 6 | Sugar sequence and branch structure of the oligosaccharides in RNase B | GCC-LC/MS in the positive ion mode and (LC/MS/MS) | Identification of 1 $Man_5GlcNAc$, 3 $Man_6GlcNAc$, 3 $Man_7GlcNAc$, 3 $Man_8GlcNac$, 1 $Man_9GlcNac$, and an oligosaccharide having six hexose units (Hex) and 2 *N*-acetylhexosamine units (HexNAc). These techniques can be used for elucidation of the distribution of oligosaccharides too. | [42] |
| 7 | *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. | [43] |

(continued on next page)

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 oligo saccharides are attached to Asn24, 38, and 83, whereas their isomers, di- and trisialylated tri-antennary oligosaccharides containing *N*-acetyl lactosamines, are combined with Asn24. | [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 | 32 new complex-type glycans are characterized Oligomannose-type glycans range from $Man_5GlcNAc_2$ to $Man_8GlcNAc_2$. Di-, tri′- and tetra-antennary complex-type structures are present, both neutral and (alpha2–3)-sialylated (up to tetrasialo), comprising 24% and 59%, respectively, of the total carbohydrate moiety. | [45] |
| 10 | Microheterogeneity of the IgA1 hinge glycopeptide (HGP33) having multiple O-linked oligosaccharides | Jacalin affinity chromatography and capillary electrophoresis | 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. | [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 radioiodinated 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 mircoheterogeneity.

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), leukoagglutinating 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* leukoagglutinin (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-D-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 diesease [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 chemotheraphy 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 ±NeuAcα2 → 3Galβ1 → 4GlcNAcβ1 → 4(±NeuAcα2 → 3Galβ1 → 4GlcNAcβ1 → 2)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 goups. All oligosaccharides with either the Galβ1 → 4GlcNAcβ1 → 6(Galβ1 → 4GlcNAcβ1 → 2)Man group or the Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 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 Galβ1 → 4GlcNAcβ1 → 4(Galβ1 → 4GlcNAcβ1 → 2)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.

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