ELSEVIER

PII: S0144-8617(97)00034-9

Carbohydrate Polymers 33 (1997) 27–32

0144-8617/97/$17.00 + .00

Immobilized *Cratylia mollis* lectin as a potential matrix to isolate plasma glycoproteins, including lecithin-cholesterol acyltransferase

Vera L.M. Lima[a], Maria T.S. Correia[a], Yêda M.N. Cechinel[a], Claudio A.M. Sampaio[b], James S. Owen[c]* and Luana C.B.B. Coêlho[a]

[a]*Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego, s/n, Cidade Universitária, 50.670-420, Recife, PE, Brazil*
[b]*Departamento de Bioquímica, Escola Paulista de Medicina (EPM), Rua Três de Maio, no. 100, São Paulo, SP, Brazil*
[c]*University Department of Medicine, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK*

(Received 1 October 1996; revised version received 28 November 1996; accepted 4 February 1997)

A crude seed extract from the native Brazilian forage, *Cratylia mollis* Mart., and its purified lectin (termed Cra), were found to precipitate glycoproteins from serum. An affinity column of Cra lectin coupled to Sepharose CL-4B was prepared and its ability to isolate glycoproteins from human plasma compared to that of a commercial immobilized lectin, Concanavalin (Con) A-Sepharose. Although both lectins are of the α-D-mannose/α-D-glucose binding class, clear differences in the type and amount of serum glycoproteins adsorbed were seen on analysis by denaturing polyacrylamide gel electrophoresis. Similarly, when a semipurified preparation of the plasma glycoprotein, lecithin-cholesterol acyltransferase (LCAT, EC 2.3.1.43) was applied to the columns some differences were evident; most LCAT was not retained by either matrix but when the bound fractions were eluted and analyzed electrophoretically the LCAT isolated by the Cra-Sepharose column was much purer. These findings suggest that immobilized Cra lectin has the potential for use in studies both to isolate and to characterize certain serum glycoproteins. © 1997 Elsevier Science Ltd

INTRODUCTION

Lectins are versatile, widely distributed proteins which bind carbohydrate structures in oligosaccharides, glycoproteins or glycoconjugates (Goldstein *et al.*, 1980; Barondes, 1988; Sharon and Lis, 1990). Plant lectins have received particular attention because of their potential application to a broad range of chemical and laboratory analyses (Ryder *et al.*, 1992; Sarkar *et al.*, 1991); they have different carbohydrate specificities and are readily immobilized to inert supports (Hock *et al.*, 1980; Gioannini *et al.*, 1982; Tsuji *et al.*, 1983; Torres and Smith, 1988; Delanghe *et al.*, 1989; Sarkar *et al.*, 1991). Indeed, commercial lectin affinity matrices are widely used in the purification of membrane glycoproteins (Torres and Smith, 1988) or glycolipids (Delanghe *et al.*, 1989), and in studies to characterize enzymes (Gioannini *et al.*, 1982) and receptors (Hock *et al.*, 1980; Tsuji *et al.*, 1983).

*To whom correspondence should be addressed.

Production of lectins by forage legume plants from semi-arid regions has received little attention, although we have recently purified and characterized a new lectin from a native plant in northeast Brazil: *Cratylia mollis* Mart. (Correia and Coelho, 1995). This lectin, hereafter termed Cra lectin, was strongly inhibited by methyl α-D-mannoside and belongs therefore to the mannose/glucose binding class of lectins, similar to those isolated from *Canavalia ensiformis* (Concanavalin A, Con A) and *Lens culinaris* (Lentil lectin). However, it is not known whether immobilized Cra has the ability to bind glycoconjugates; the affinity of immobilized lectins towards free and/or conjugated sugars is unpredictable and may differ from findings with the lectin in solution (Montreuil *et al.*, 1994).

The enzyme lecithin-cholesterol acyltransferase (LCAT, EC 2.3.1.43), a plasma glycoprotein (Collet and Fielding, 1991) with one-quarter of its apparent molecular mass (68 kDa) as four *N*-linked oligosaccharide chains (Chung *et al.*, 1979; Hill *et al.*, 1993), is responsible for synthesis of almost all plasma cholesteryl esters

in humans (Glomset, 1968). The carbohydrate content of LCAT is about 5.0% glucosamine, 9.2% mannose, 6.0% galactose and 3.9% sialic acid (14), a composition consistent with a variety of *N*-glycans (Dwek, 1995). Recently, affinity chromatography on a Con A-Sepharose column has been suggested as a final step to isolate plasma LCAT (Ridgway and Dolphin, 1985). In the present study, we have determined whether Cra lectin immobilized to Sepharose is able to recognize and adsorb glycoproteins from human plasma and whether it can aid in the purification of LCAT.

METHODS

Purification, gel diffusion and conjugation of Cra lectin

Cra lectin was purified from a 10% (w/v) seed extract of *C. mollis* in 0.15 M NaCl as described previously (Correia and Coelho, 1995). In brief, proteins were precipitated using 40–60% ammonium sulphate fractionation, followed by affinity chromatography on a Sephadex G-75 column (70.0×1.9 cm) using 0.3 M D-glucose in 0.15 M NaCl for elution. Crude seed extract or pure Cra lectin (10 μl per well) were allowed to diffuse in agarose gels (1% in 0.15 M NaCl) in a humid chamber at 4°C against individual serum samples in other wells (Ashford *et al.*, 1982). After 24 h, the gels were exhaustively washed and then stained with Coomassie Brilliant Blue (0.4% (w/v) in 25% (v/v) ethanol and 8% (v/v) acetic acid) for 15 min. Cra lectin (12 mg) was covalently attached to 4 mL of CNBr activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions.

Immunoaffinity isolation of plasma lecithin-cholesterol acyltransferase

Human blood samples were taken from healthy subjects, anticoagulated with EDTA (1 mg/ml) and the plasma separated by centrifugation at 4°C. An approximately 3000-fold enriched LCAT fraction was isolated from human plasma using an immunoaffinity column of rabbit anti-human LCAT immunoglobulin G coupled to Sepharose 4B. As described earlier (Lima *et al.*, 1987; Humphries *et al.*, 1988), aliquots of plasma (1 mL) were circulated through the column overnight and bound LCAT containing material eluted with 0.5 M NaCl. For comparative purposes, highly purified LCAT was isolated from 500 ml of human plasma using a multicolumn chromatography procedure as described earlier (Lima *et al.*, 1996).

Western immunoblotting was used to confirm LCAT protein in the bound fraction. Eluted proteins were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions according to Laemmli (1970). Separated proteins were transferred from the gel to nitrocellulose paper in 25 mmol/liter Tris, pH 8.3, containing 192 mmol/liter glycine and 20% (v/v) methanol (Towbin *et al.*, 1979). Nonspecific binding sites were blocked by 3% gelatin and the nitrocellulose strips then incubated overnight at room temperature either with rabbit antiserum against human LCAT (Humphries *et al.*, 1988) or with non-immune serum. Detection was by incubation with commercial goat anti-rabbit immunoglobulin conjugated to horseradish peroxidase (BioRad Laboratories).

Cra-Sepharose affinity chromatography

Cra-Sepharose and Con A-Sepharose (Pharmacia) columns (6.5 × 1.0 cm) were equilibrated with 0.05 M Tris-HCl buffer, pH 8.0, and 0.2 ml of human plasma diluted to 1 ml with the Tris-HCl buffer was applied to the columns. After removal of unbound proteins with buffer, the adsorbed material was biospecifically eluted with 0.5 M D-glucose. Similar separations were performed with immunoaffinity-isolated LCAT fractions (about 20 μg protein) except that smaller Cra and Con A columns (3.0 × 0.5 cm) were used and bound proteins were eluted with 0.25 M methyl α-D-mannoside and 0.25 M methyl α-D-glucoside. Proteins in the bound and unbound fractions were separated by SDS-PAGE and were detected with either Coomassie Brilliant Blue or a sensitive silver stain (BioRad Laboratories).

RESULTS

The crude seed extract of *C. mollis* was able to precipitate proteins from sera as shown in Fig. 1, indicating that it contained lectin recognizing serum glycoproteins. A similar result was obtained with pure Cra lectin, while addition of 1 M D-mannose or 1 M methyl α-D-mannoside to the gel prevented formation of the precipitate (data not shown).

The coupling of Cra lectin to Sepharose 4B was efficient; 95% of the lectin (11.4 mg) was estimated to be covalently attached to 4 mL of the hydrated CNBr activated Sepharose 4B beads as indicated by disappearance of protein from the supernatant. A column (6.5 × 1.0 cm) of this immobilized lectin removed about 10% of proteins (0.762 mg) from 0.2 ml of human plasma (7.2 mg protein) and these were all biospecifically eluted with 0.5 M glucose (Fig. 2). All proteins in the bound material stained with the Schiff reagent, as well as with Coomassie Brilliant Blue, confirming that they were glycoproteins. Although Con A is also of the mannose/glucose binding class of lectins, when the plasma proteins adsorbed by the Con A-Sepharose column were compared to those bound by Cra-Sepharose a very different pattern was found (Fig. 2).

When 1 ml of plasma was circulated through the anti-LCAT column about half of the LCAT was

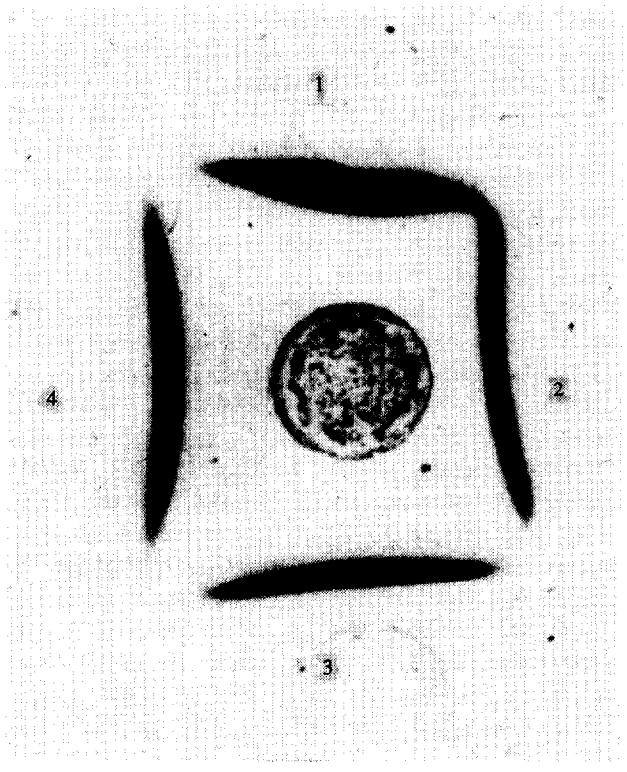

Fig. 1. Double diffusion of an extract from *C. mollis* seed against serum. A crude seed extract from *C. mollis* with specific hemagglutinating activity (19) of 2484 in the central well was allowed to diffuse against sera from four healthy individuals in the peripheral wells (1–4). Precipitated proteins were stained with Coomassie Brilliant Blue.

retained, as judged by assaying LCAT activity in the unbound fraction (Lima *et al.*, 1987; Gillett and Owen, 1992). Bound LCAT could be eluted entirely with 0.5 M NaCl and typically yielded about 20 μg protein, of which about 3 μg was LCAT. The presence of LCAT protein in this fraction was confirmed by Western immunoblotting; a single band was seen which had the same mobility as highly purified LCAT isolated by our conventional multistep procedure (Lima *et al.*, 1996) (Fig. 3 (A)). Immunoblotting did not detect, however, the residual LCAT in the unbound fraction, presumably because of the very high concentration of contaminating proteins. Indeed, the smudges of stained material evident in this fraction, including proteins in the 15–30 kDa range, were not simple cross-reactions of the antisera; rather the very heavy overloading of the gel was considered the likely reason since comparable staining was noted with non-immune sera (data not shown). Attempts to improve the purity of the LCAT by stepwise pre-elution with lower concentrations of NaCl were largely unsuccessful (Fig. 3 (B)) and thus were not investigated further. When the semi-pure LCAT eluate was circulated through the Cra-Sepharose column, a relatively small proportion of LCAT was bound with most appearing in the unbound fraction (Fig. 4). Similarly, most LCAT was not

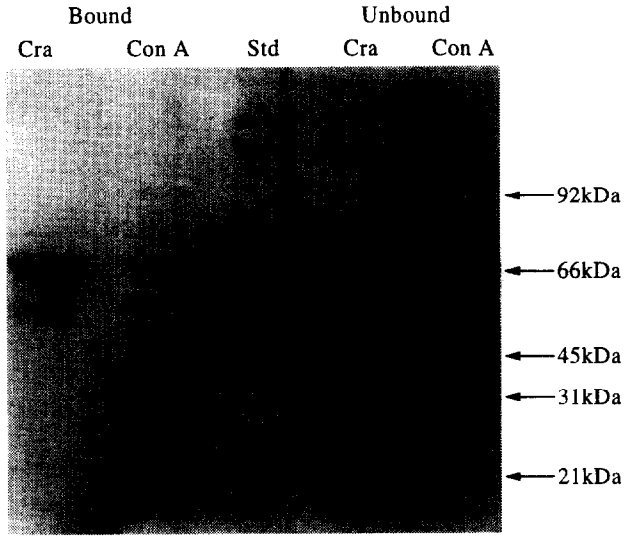

Fig. 2. Electrophoresis of human plasma proteins isolated by Cra-Sepharose chromatography. Human plasma (0.2 ml), diluted to 1 mL with Tris/HCl 0.05 M, pH 8, was applied to a Cra-Sepharose or a Con A-Sepharose column. The unbound fractions were eluted with the same buffer and then the bound material was released by elution with 0.5 M D-glucose in buffer. Approximately 50 μg of proteins from each of the four fractions were separated by 10% SDS-PAGE and stained with Coomassie Brillant Blue; the centre lane (Std) shows molecular weight markers.

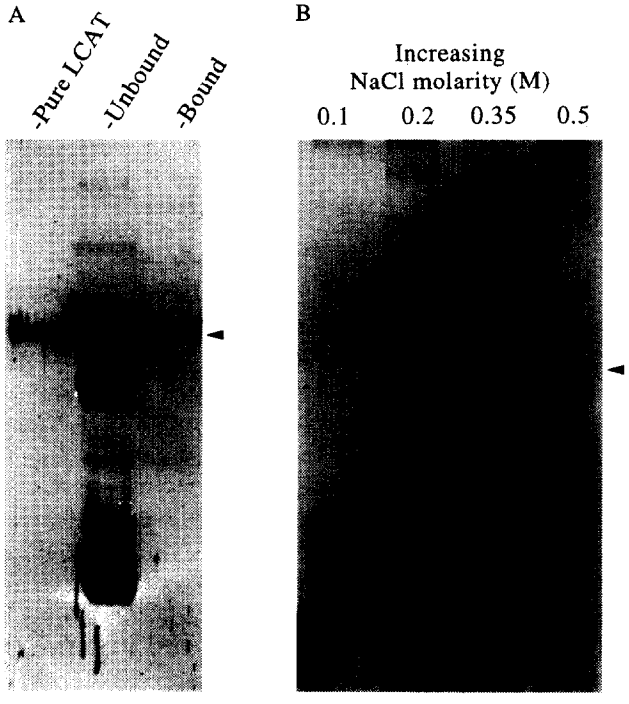

Fig. 3. Immunoaffinity-isolation of plasma LCAT. Plasma was circulated through an anti-human LCAT immunoaffinity column and bound material eluted with 0.5 M NaCl (A) or with stepwise increments of NaCl (0.1–0.5 M) as shown in (B). Fractions were separated by SDS-PAGE and either electrotransferred to a nitrocellulose membrane for immunoblotting with LCAT antisera (A) or directly stained with silver (B). The position of LCAT is indicated by the arrows on the right.

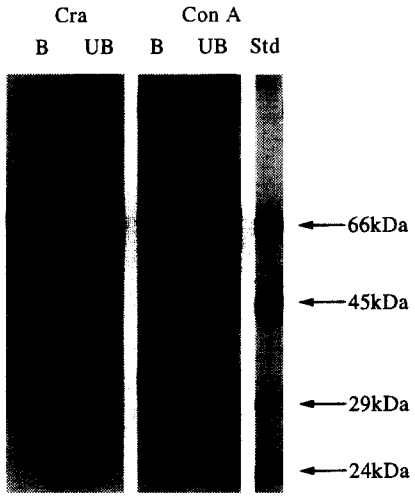

Fig. 4. Cra-Sepharose and Con A-Sepharose column chromatography of immunoaffinity-isolated plasma LCAT. Semi-pure LCAT preparations were equilibrated with a Con A-Sepharose or Cra-Sepharose column. Bound fractions were eluted with 0.25 M methyl α-D-mannoside plus 0.25 M methyl α-D-glucoside and portions (50%) of the unbound and bound material were separated by 10% SDS-PAGE and silver stained. The position of molecular weight standards is given on the right.

retained on passage of the immunoaffinity isolated fraction through the Con A-Sepharose column and the ability of immobilized Con A and Cra to bind plasma LCAT appeared equal (Fig. 4). However, the two columns did differ in their ability to bind contaminating (glyco)proteins; the LCAT bound by Cra-Sepharose was much purer than that bound by the Con A column.

DISCUSSION

This study represents the first report of the use of immobilized Cra lectin to isolate glycoproteins from serum. Moreover, the Cra-Sepharose column used was found to have a very different specificity to that of commercial Con A-Sepharose even though both lectins recognize glycoproteins of the mannose/glucose binding class. Such differences in binding activity are not unusual; indeed mannose/glucose binding lectins are known to have very variable effects on macrophage stimulation (Rodriguez *et al.*, 1992) and in detecting multimolecular forms of enzymes (Fiddler *et al.*, 1979; Kerckaert and Bayard, 1980). Clearly, the use of oligosaccharides and glycoconjugates of well defined primary structure will be needed to delineate the binding specificity of Cra lectin. Whether this specificity can be exploited for purification or characterization purposes of certain serum glycoproteins must await further investigations although, as discussed below, our preliminary studies on plasma LCAT suggest that immobilized Cra lectin will be of value.

The ability of Cra- and Con A-Sepharose columns to adsorb LCAT only partially from a small amount of total protein (20 μg) when the column capacities were at least a hundredfold greater suggests that plasma LCAT is heterogeneous, even though the pure enzyme migrated as a single, relatively narrow 68 kDa protein by SDS-PAGE and immunoblotting. Although the four *N*-linked glycan chains present on native plasma LCAT are known to have important roles for full enzymic activity (Doi and Nishida, 1983; Francone *et al.*, 1993; O *et al.*, 1993; Qu *et al.*, 1993), they remain poorly characterized. Nevertheless, LCAT microheterogeneity has been demonstrated by isoelectric focusing (Doi and Nishida, 1983; Holmquist and Bjellqvist, 1988), while digestion with endoglycosidase F is reported to produce a molecular mass doublet by SDS-PAGE indicating considerable heterogeneity (Collet and Fielding, 1991).

Our findings support the heterogeneous nature of plasma LCAT; one form being recognized by the mannose/glucose binding lectins Cra and Con A, and presumably containing high mannose or possibly hybrid-type oligosaccharides, while the other more abundant form fails to interact and probably has only complex-type glycans. Indeed, the high sialic content of LCAT (Chung *et al.*, 1979; Doi and Nishida, 1983; Collet and Fielding, 1991) is consistent with the presence of predominantly complex-type glycans rather than oligomannose chains. By contrast, an early study found that almost all of a relatively pure preparation of plasma LCAT was bound by a Con A column and this procedure was suggested as a final purification step (Ridgway and Dolphin, 1985). This observation is now surprising, given the recent cumulative evidence for plasma LCAT as a complex-type glycoprotein (Doi and Nishida, 1983; Collet and Fielding, 1991; Francone *et al.*, 1993; O *et al.*, 1993; Qu *et al.*, 1993; Hill *et al.*, 1993) as well as our present findings. However, these workers had used screened plasma from a blood bank for LCAT isolation and had carried out lengthy purification procedures before the Con A chromatography step; conceivably, these factors may have altered the lectin specificity of LCAT, possibly because of copurification with plasma sialidase (Kato *et al.*, 1989).

Although the LCAT bound by Cra lectin was of higher purity than that eluted from Con A-Sepharose, such affinity chromatography would seem of limited use for bulk LCAT purification as yields would be too low. However, a Cra lectin column could be of value in detecting abnormal glycosylation patterns in diseases, including cancer, inflammation and hepatic cirrhosis which are known to cause subtle changes to the glycan chains of serum glycoproteins (reviewed in Turner (1992)). A study of LCAT in liver disease would seem of particular interest since defective glycosylation may contribute to the low plasma enzymic activity characteristically found in these patients (McIntyre and Owen, 1990). Indeed, this secondary LCAT deficiency of liver disease generates abnormal lipoprotein particles,

which have a number of adverse effects on cellular functions and hence are considered to have important pathophysiological consequences (Owen *et al.*, 1992).

ACKNOWLEDGEMENTS

This research was supported by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; contract 416.282/89) and Fundacçaõ de Apoio a Ciência e Tecnologia do Estado de Pernambuco (FACEPE; contract 060-2.08/90).

REFERENCES

Ashford, D., Allen, A.K. and Neuberger, A. (1982) The production and properties of an antiserum to potato (*Solanum tuberosum*) lectin. *Biochem. J.* **201,** 641–645.

Barondes, S.H. (1988) Bifunctional properties of lectins: lectins redefined. *Trends Biochem. Sci.* **13**, 480–482.

Chung, J., Abano, D.A., Fless, G.M. and Scanu, A.M. (1979) Isolation, properties and mechanism of *in vitro* action of lecithin: cholesterol acyltransferase from human plasma. *J. Biol. Chem.* **254**, 7456–7464.

Collet, X. and Fielding, C.J. (1991) Effects of inhibitors of *N*-linked oligosaccharide processing on the secretion, stability, and activity of lecithin: cholesterol acyltransferase. *Biochemistry* **30**, 3228–3234.

Correia, M.T.S. and Coelho, L.C.B.B. (1995) Purification of a glucose/mannose specific lectin, isoform 1, from seeds of *Cratylia mollis* Mart. (Camaratu bean). *Appl. Biochem. Biotech.* **55**, 261–273.

Delanghe, J.R., De Buyzere, M.L., De Scheerder, I.K., Faust, U. and Wieme, R.J. (1989) Activation energy and lectin affinity chromatography of gamma-glutamyltransferase as a marker for enzyme heterogeneity. *Clin. Biochem.* **22**, 115–199.

Doi, Y. and Nishida, T. (1983) Microheterogeneity and physical properties of human lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **258**, 5840–5846.

Dwek, R.A. (1995) Glycobiology: 'towards understanding the function of sugars'. *Biochem. Soc. Trans.* **23**, 1–25.

Fiddler, M.B., Ben-Yoseph, Y. and Nadler, H.L. (1979) Binding of human liver hydrolases by immobilized lectins. *Biochem. J.* **177**, 175–180.

Francone, O.L., Evangelista, L. and Fielding, C.J. (1993) Lecithin-cholesterol acyltransferase: effects of mutagenesis at *N*-linked oligosaccharide attachment sites on acyl acceptor activity. *Biochim. Biophys. Acta* **1166**, 301–304.

Gillett, M.P.T. and Owen, J.S. (1992) Cholesterol esterifying enzymes—lecithin:cholesterol acyltransferase (LCAT) and acylcoenzyme A:cholesterol acyltransferase (ACAT). In *Lipoprotein Analysis. A Practical Approach*, ed. C.A. Converse and R.E. Skinner. IRL Press, Oxford, pp. 187–201.

Gioannini, T., Foucaud, B., Hiller, J.M., Hatten, M.E. and Simon, E.J. (1982) Lectin binding of solubilized opiate receptors: evidence for their glycoprotein nature. *Biochem. Biophys. Res. Commun.* **105**, 1128–1134.

Glomset, J.A. (1968) The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9**, 155–167.

Goldstein, I.J..,Hughes, R.C., Monsigny, M., Osawa,T. and Sharon, N. (1980) What should be called a lectin? *Nature* **285**, 66.

Hill, J.S.O., K., Wang, X., Paranjape, S., Dimitrijevich, D., Lacko, A.G. and Pritchard, P.H. (1993) Expression and characterization of recombinant human lecithin:cholesterol acyltransferase. *J. Lipid Res.* **34**, 1245–1251.

Hock, R.A., Nexo, E. and Hollenberg, M.D. (1980) Solubilization and isolation of human placenta receptor for epidermal growth factor urogastrone. *J. Biol. Chem.* **255**, 10737–10743.

Holmquist, L. and Bjellqvist, B. (1988) Microheterogeneity of human plasma lecithin:cholesterol acyltransferase examined by isoelectric focusing in immobilised pH gradients. *Electrophoresis* **9**, 580–582.

Humphries, S.E., Chaves, M.E., Tata, F., Lima, V.L., Owen, J.S., Borysiewicz, L.K., Catapano, A., Vergani, C., Gjone, E., Clemens, M.R., Williamson, R. and McIntyre, N. (1988) A study of the structure of the gene for lecithin:cholesterol acyltransferase in four unrelated individuals with familial lecithin:cholesterol acyltransferase deficiency. *Clin. Sci.* **74**, 91–96.

Kato, H., Nakanishi, T., Arai, H., Nishida, H.I. and Nishida, T. (1989) Purification, microheterogeneity, and stability of human lipid transfer protein. *J. Biol. Chem.* **264**, 4082–4087.

Kerckaert, J.-P. and Bayard, B. (1980) Affinity differences between some commercially available immobilized Con A with rat alpha-fetoprotein. *Biochem. Biophys. Res. Commun.* **92**, 95–101.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lima, V.L.M., Harry, D.S., Owen, J.S. and McIntyre, N. (1987) Polyclonal antibodies to human plasma lecithin-cholesterol acyltransferase. *Biochem. Soc. Trans.* **15**, 477–478.

Lima, V.L.M., Harry, D.S., McIntyre, N., Owen, J.S. and Chaves, M.E.C. (1996) Characterization and potential uses of rabbit polyclonal antibodies against human plasma lecithin-cholesterol acyltransferase. *Braz. J. Med. Biol. Res.* **29**, 957–968.

McIntyre, N. and Owen, J.S. (1990) Plasma lipids and lipoproteins in liver disease. In *The Metabolic and Molecular Basis of Acquired Disease*, ed. R.D. Cohen, B. Lewis, K.G.M.M. Alberti and A.M. Denman, pp. 1176–1192. Baillière Tindall, London.

Montreuil, J., Bouquelet, S., Debray, H., Lemoine, J., Michalski, J.-C., Spik, G. and Strecker, G. (1994) In *Carbohydrate Analysis—A Practical Approach*, ed. M.F. Chaplin and J.F. Kennedy, 2nd edn, pp. 143–204. IRL Press, Oxford.

O, K., Hill, J.S., Wang, X., McLeod, R. and Pritchard, P.H. (1993) Lecithin:cholesterol acyltransferase: role of *N*-linked glycosylation in enzyme function. *Biochem. J.* **294**, 879–884.

Owen, J.S., Rafique, S., Osman, E. and Burroughs, A.K. (1992) Ability of *S*-adenosyl-L-methionine to ameliorate lipoprotein-induced membrane lipid abnormalities and cellular dysfunctions in human liver disease. *Drug Invest.* **4**(Suppl. 4), 22–40.

Qu, S.-J., Fan, H.-Z., Blanco-Vaca, F. and Pownall, H.J. (1993) Effects of site-directed mutagenesis on the *N*-glycosylation sites of human lecithin:cholesterol acyltransferase. *Biochemistry* **32**, 8732–8736.

Ridgway, N.D. and Dolphin, P.J. (1985) Serum activity and hepatic secretion of lecithin:cholesterol acyltransferase in experimental hypothyroidism and hypercholesterolemia. *J. Lipid Res.* **26**, 1300–1313.

Rodriguez, D., Cavada, B.S., Oliveira, J.T.A., Moreira, R.A. and Russo, M. (1992) Differences in macrophage stimulation and leukocyte accumulation in response to intraper-

itoneal administration of glucose/mannose-binding plant lectins. Braz. J. Med. Biol. Res., **25**, 823–826.

Ryder, S.D., Smith, J.A. and Rhodes, J.M. (1992) Peanut lectin: a mitogen for normal human colonic epithelium and human HT29 colorectal cancer cells. *J. Natl. Cancer Inst.* **84**, 1410–1416.

Sarkar, M., Majender, G. and Chatterjee, T. (1991) Goat sperm membrane: lectin-binding sites of sperm surface and lectin affinity chromatography of the mature sperm membrane antigens. *Biochim. Biophys. Acta* **1070**, 198–204.

Sharon, N. and Lis, H. (1990) Legume lectins—a large family of homologous proteins. *FASEB J.* **4**, 3198–3208.

Torres, B.V. and Smith, D.F. (1988) Purification of Forsmann and human blood group A glycolipids by affinity chromatography on immobilized Helix pomatia lectin. *Anal. Biochem.* **170**, 209–219.

Towbin, H., Staehilin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Nat. Acad. Sci. USA* **76**, 4350.

Tsuji, T., Tsunehisa, S., Watanabe, Y., Yamamoto, H. and Osawa, T. (1983) The carbohydrate moiety of human platelet glycocalicin. *J. Biol. Chem.* **258**, 6335–6339.

Turner, G.A. (1992) *N*-Glycosylation of serum proteins in disease and its investigation using lectins. *Clin. Chim. Acta* **208**, 149–171.