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J. Lipid Mediators Cell Signalling 17 (1997) 191–205

LIPID MEDIATORS AND CELL SIGNALLING

Characterization and representative structures of *N*-oligosaccharides bound to apolipoprotein H

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Received 7 August 1997; received in revised form 3 October 1997; accepted 3 October 1997

Abstract

We studied the structure of *N*-linked carbohydrates bound to apolipoprotein H by a combination of two methods which make use of lectins. Digoxigenin-labelled lectins are used for the structural characterization of carbohydrate chains of glycoproteins. Concanavalin A lectin affinity chromatography was used to analyse apolipoprotein H according to the characteristics of its carbohydrate chain inner to sialic acid residues. Our results from digoxigenin-labelled lectins analysis showed that apolipoprotein H gave positive bands to SNA, DSA, GNA, PNA and AAA lectins. Apolipoprotein H gave a negative band when reacted with MAA lectin. When we applied apolipoprotein H onto the Concanavalin A lectin column no detectable amounts of protein were eluted with Concanavalin A buffer. After adding a buffer with low sugar concentration (10 mM glucoside) a large amount of apolipoprotein H was recovered. These molecules of apolipoprotein H weakly bound to the lectin. When a higher sugar concentration (500 mM mannoside) was added most of the sample applied was eluted. These molecules of apolipoprotein H firmly bound to the column having high affinity for the lectin. These results combined with those coming from the

Abbreviations: apo H, apolipoprotein H; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IEF, isoelectrofocussing; TBS, tris-buffer saline; EDTA, ethylenediaminetetraacetic acid; GNA, *Galanthus nivalis* agglutinin; SNA, *Sambucus nigra* agglutinin; MAA, *Maackia amurensis* agglutinin; PNA, peanut agglutinin; DSA, *Datura stramonium* agglutinin; ACA, *Amaranthus caudatus* agglutinin; AAA, *Aleuria aurantia* agglutinin; PHA, *Phaseolus vulgaris* agglutinin; Asn, asparagine; ASN, asparagine-peptide.

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PII S0929-7855(97)00029-1

digoxigen-labeled lectins method enable us to understand the inner structure of carbohydrate chains with their outer branches. Molecules of apolipoprotein H which weakly bind to Concanavalin A could bear complex *N*-glycans organized in biantennary or truncated hybrid structures. Firmly bound apolipoprotein H referred to molecules rich in *N*-glycan hybrid structures. They have an outer branch belonging to the high mannose carbohydrate chains which explain the ability to bind to the column and an other main branch bearing the sequence galactose β-(1-4)-*N*-acetylglucosamine β-(1-2) mannose. Galactose could be the terminal sugar or, alternatively, be masked with sialic acid α-(2-6) terminally linked. © 1997 Elsevier Science B.V.

Keywords: Affinity chromatography; Apolipoprotein H; Concanavalin A; Glycosylation; Lectin

1. Introduction

Apo H, also known as β_2-glycoprotein I, is a single, approximately 50 kDa chain glycoprotein (Lozier et al., 1984; David et al., 1994) present in the blood in a mature 326 amino acid form and associated with the plasma lipoproteins to the extent of 35% (Polz and Kostner, 1979). Apo H is composed of five repeating domains of about 60 amino acids highly glycosylated (Steinkasserer et al., 1991).

Its chemical and physical characteristics, particularly its affinity for negatively charged molecules, suggest that apo H is a coagulation inhibitor. It does, in fact, inhibit ADP-induced platelet aggregation (Nimpf et al., 1987), platelet prothrombinase activity (Nimpf et al., 1986), and contact activation of the coagulation pathway (Schousboe, 1985). Its role in lipid metabolism has been examined by several workers (Nakaya et al., 1980; Wurm et al., 1982; Eichner et al., 1989; Kamboh and Ferrell, 1991). The in vivo and in vitro data indicate that it is involved in triglyceride (Tg) metabolism (Nakaya et al., 1980; Wurm et al., 1982; Cassader et al., 1994). Many studies have shown that apo H is an obligate cofactor for binding some groups of antibodies to anionic phospholipids (McNeil et al., 1990; Jones et al., 1992; Gharavi et al., 1992; Hunt et al., 1993; Roubey, 1994; Kamboh et al., 1995).

Apo H displays a genetically determined structural polymorphism (Sepehrnia et al., 1989). Three alleles (APOH*1, APOH*2, APOH*3) in Caucasians (plus APOH*4 in blacks only) at a single locus on chromosome 17 code for isoforms identified by IEF and immunoblotting (Kamboh et al., 1988), revealing structurally different isoforms with different isoelectric points, representing a polypeptide chain with substituted amino acids (Kamboh et al., 1988). A postsynthetic polymorphism consisting of multiple glycoforms due to the number of terminal sialic acid moieties is also observed.

Although its physical and chemical characteristics were well studied, its physiological functions remain unknown. Nakaya et al. (1980) proposed that it acts as an activator of lipoprotein lipase. Schousboe (1985) has presented evidence that apo H binds to platelet membranes, interacts with negatively charged macromolecular

structures including heparin, DNA, phospholipids and modulates the activity of adenylate (Nimpf et al., 1986).

The carbohydrate content of apo H is approximately 19% of the molecular weight (Day et al., 1989). The reported five glucosamine-attached oligosaccharide side chains are composed of galactose, mannose, *N*-acetylglucosamine, fucose, and *N*-acetylneuraminic acid (Kamboh et al., 1988).

The aim of our work was to investigate on the carbohydrate inner structures of apo H, being that glycosylation is a very important process which is able to regulate the structure and the biological functions of proteins. Blocking the *N*-linked glycosylation often results in a damaged protein lacking functional activity (Riederer and Hinnen, 1991; Copeland et al., 1988). In particular asparagine glycosylation is very important for the appropriate folding and assembly of intact proteins (Imperiali and Rickert, 1995).

The specific binding of lectins to carbohydrate moieties is used to identify these structures. The lectins applied are conjugated with the steroid hapten digoxigenin which enables immunological detection of the bound lectins. When differentiating between carbohydrate structures, lectins which selectively recognize the terminal sugars are used, thus allowing the carbohydrate chain to be identified. Moreover, affinity chromatography of glycoproteins on immobilized lectins, such as Concanavalin A (Con A), has been proven to be a powerful method for oligosaccharide fractionation (Baenziger and Fiete, 1979; Cummings and Kornfeld, 1982). *N*-linked oligosaccharide structures were shown to interact with Con A according to their branching properties as follows: triantennary, tetraantennary, and bisecting oligosaccharides do not bind to Con A, biantennary and truncated hybrids weakly bind to Con A, and high mannose and hybrid oligosaccharides firmly bind to Con A (Gambino et al., 1997). The combination of the two methods allowed us to clarify the organization of *N*-linked glycans bound to apo H.

2. Materials and methods

2.1. Materials

Tris, urea, Tween 20, acrylamide and *N*,*N*′-methylene-bis-acrylamide, analytical grade, were purchased from Bio-Rad, Richmond, CA. Gel Bond and ampholytes (pH = 4–6.5 and pH = 6.5–9) were obtained from LKB, Bromma, Sweden; nitrocellulose sheets from Bio-Rad. Rabbit polyclonal antibody to apo H was kindly supplied by Behring, Scoppito, Italy. Goat anti-rabbit IgG (alkaline phosphatase conjugated) and the alkaline phosphatase conjugate substrate kit were purchased from Sigma. Molecular weight standards (low MW) were from Bio-Rad. DIG Glycan Differentiation Kit and lectins digoxigenin-labelled were purchased from Boehringer Mannheim Biochemica. Con A-Sepharose was purchased from Sigma, Milan, Italy.

2.2. Isolation and purification of apo H

Apo H was isolated from human plasma and purified through a combination of affinity chromatography and continuous elution electrophoresis as previously described (Gambino et al., 1996).

2.3. SDS–PAGE

In order to analyze the type of glycosylation, apo H was subjected to 12% SDS–PAGE electrophoresis in a Bio-Rad Mini Protean II apparatus under non-reducing conditions. Electrophoresis buffer was 25 mM Tris, 0.192 M glycine, 0.1% SDS pH 8.3.

2.4. Western blot

Apo H was then blotted after SDS–PAGE electrophoresis in 25 mM Tris, 0.192 M glycine, and 20% methanol, pH 8.3, at 295 mA for 100 min. With this method carbohydrate moieties of glycoproteins, bound to nitrocellulose can be characterized.

2.5. Glycoprotein detection

All filters are incubated in blocking solution at 4°C overnight. They are then washed once in 0.01 M Tris–HCl, 0.015 M NaCl, 0.01% Thimerosal pH 7.6 (TBS) and twice in lectin buffer, 1 mmol/l $MgCl_2$, 1 mmol/l $MnCl_2$, 1 mmol/l $CaCl_2$ in TBS. Digoxigen-labelled lectins are diluted in lectin buffer and the filters are incubated at room temperature for 1.5 h by gentle agitation. When this incubation is completed nitrocellulose membranes are washed three times in TBS at room temperature by gentle agitation to remove unbound lectins. Lectins used are: GNA (*Galanthus nivalis* agglutinin), SNA (*Sambucus nigra* agglutinin), MAA (*Maackia amurensis* agglutinin), PNA (Peanut agglutinin), DSA (*Datura stramonium* agglutinin), ACA (*Amaranthus caudatus* agglutinin), AAA (*Aleuria aurantia* agglutinin), PHA (*Phaseolus vulgaris* agglutinin). GNA recognizes terminal mannose, α-(1-3), α-(1-6) or α-(1-2) linked to mannose. SNA recognizes sialic acid linked α-(2-6) to galactose. MAA recognizes sialic acid linked α-(2-3) to galactose. PNA recognizes the core disaccharide galactose β-(1-3)-*N*-acetylgalactosamine. DSA recognizes Gal-β-(1-4)-*N*-acetylglucosamine in complex and hybrid *N*-glycans, in *O*-glycans and *N*-acetylglucosamine in *O*-glycans (Crowley et al., 1984). ACA shows a high specificity for the α-anomer of the disaccharide unit Gal-β-(1-3)-*N*-acetylgalactosamine–Ser/Thr. AAA binds specifically to α-(1-6) linked fucose residues in complex *N*-glycan structures. PHA binds preferentially to the β-(1-6) linked lactosamine branch of complex *N*-glycans. A positive reaction indicates higher branched complex chains. Nitrocellulose membranes are, then, incubated with polyclonal sheep anti-digoxigenin *Fab* fragments, conjugated with alkaline phosphatase for 1.5 h at room temperature by gentle agitation and washed three times in TBS. Bands are visualized by the immunoblot assay kit of Bio-Rad.

2.6. Deglycosylation of apo H

Four micrograms of apo H in 100 μl were denatured in 0.2% SDS (w/v) by boiling for 2 min. One hundred microliters for *N*-glycosidase F incubation buffer (0.05 M Phosphate, 0.05 M EDTA, Nonidet-P 40 1%) were added. These mixtures were again boiled for 2 min, cooled down to 37°C, the enzyme added (4 units *N*-Glycosidase F in 200 μl) and incubated for 18 h at 37°C. Fifty microliters (about 1 μg protein) were loaded onto one lane of an SDS-gel. Detection of glycations was performed as described above. In order to exclude any aspecific proteolitic digestions due to a long incubation at 37°C, apo H was also incubated without *N*-glycosidase F.

2.7. Concanavalin A lectin affinity chromatography

Concanavalin A lectin affinity chromatography was performed as previously described (Gambino et al., 1997). Briefly, lectin column was equilibrated with a buffer containing 10 mM Tris–HCl, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 0.01% Thimerosal pH 8.0 (Con A buffer). After apo H was applied to the column unbound sample was washed away with Con A buffer. Weakly bound apo H was eluted from the Con A column with 10 mM α-D-methyl-glucopyranoside (Sigma). Firmly bound apo H was subsequently eluted with 500 mM α-D-methylmannopyranoside (Sigma). Eluates containing unbound, weakly bound and firmly bound were collected into 0.6 ml fractions and subjected to lectin analysis.

3. Results

Undigested apo H resulted very positive for SNA and DSA as shown from different pictures. SNA interacts with glycoproteins by recognizing sialic acid residues, terminally linked (2-6) to galactose or *N*-acetylgalactosamine (Fig. 1 lane 4).

DSA binds to oligosaccharides with the special sequence galactose-β-(1-4)-*N*-acetylglucosamine (Fig. 2 lane 4).

The reaction with PNA is less positive than the previous ones but it is still well appreciable (Fig. 3 lane 4). PNA is specific for galactose-β-(1-3)-*N*-acetylgalactosamine and it does not recognize the disaccharide if it carries a substitution either on the galactose or on the *N*-acetylgalactosamine residue and, therefore, it will not bind sialylated structures. PNA lectin has been used to reveal *O*-linked saccharides of glycoproteins.

A positive reaction with GNA recognizes terminal mannose α-(1-6), or α-(1-3) or α-(1-2) linked to mannose (Fig. 4 lane 4).

The reaction with MAA was absolutely negative indicating no sialic acid is terminally linked α-(2-3) to galactose.

The reactions with ACA and PHA were negative. A band was observed with AAA (Fig. 5 lane 2). AAA is a specific lectin for *N*-linked oligosaccharides with the fucose α-(1-6) residue at reducing terminal *N*-acetylglucosamine. The interaction is not altered by the presence of a bisecting *N*-acetylglucosamine or by the structures of outer chain moieties except for the presence of α-fucose residues. This enables us to separate *N*-linked oligosaccharides with and without the fucosyl residue linked to the trimannosyl core. For differentiating between *N*- and *O*-linked chains the carbohydrate residues in the asparagine-linked chains was removed with the enzyme *N*-glycosidase F and after the enzymatic digestion apo H was probed with the same lectins as described above. *N*-glycosidase F cleaves all types of asparagine (Asn) bound *N*-glycans provided that the amino group as well as the carboxyl group are present in a peptide linkage and that the oligosaccharide has the minimum length of the chitobiose core unit (Chu, 1986). After the enzymatic deglycation the mass of apo H is substantially reduced to 32 500 Da as proved by the increased electrophoretic mobility (data not shown). As assessed by the positive SNA band, deglycosylated apo H has sialic acid, terminally linked α-(2-6) to galactose or *N*-acetylgalactosamine in *O*-glycan structures too. On a digested apo H DSA also recognizes galactose-β-(1-4)-*N*-acetylglucosamine structures in *O*-glycans, such as

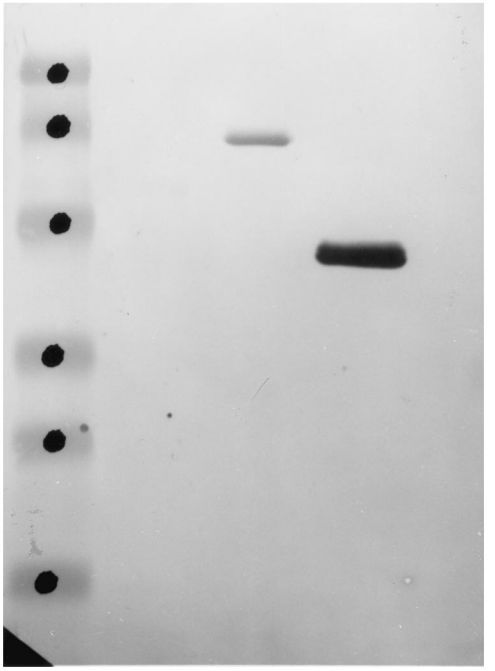

Fig. 1. Western blot of β_2-glycoprotein I probed with SNA lectin. (from left to right). Lane 1: prestained molecular mass markers marked with black points are (from top to bottom): 106.0, 80.0, 49.5, 32.5, 27.5, 18.5 kDa. Lane 2: carboxypeptidase Y, negative control glycoprotein. Lane 3: transferrin, positive control glycoprotein. Lane 4: native β_2-glycoprotein I.

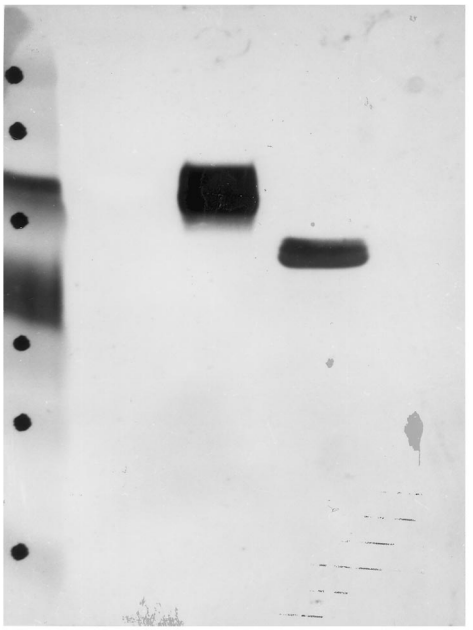

Fig. 2. Western blot of β_2-glycoprotein I probed with DSA lectin (from left to right). Lane 1: prestained molecular mass markers marked with black points are (from top to bottom): 106.0, 80.0, 49.5, 32.5, 27.5, 18.5 kDa. Lane 2: carboxypeptidase Y, negative control glycoprotein. Lane 3: fetuin, positive control glycoprotein. Lane 4: native β_2-glycoprotein I.

those found in certain mucin for example. Furthermore, DSA is suitable for identifying individual *N*-acetylglucosamine residues with *O*-glycosidic links to serine or threonine. When deglycosylated apo H was probed with PNA, galactose-β-(1-3)-*N*-acetylgalactosamine structures in *O*-glycans were shown. GNA-lectin probed on digested apo H gave a bare band.

Apo H interacted with the Con A lectin. Detectable amounts of protein were not found in the first chromatographic fractions suggesting that apo H is not eluted with Con A buffer. After adding a buffer with low sugar concentration (10 mmol glucoside) a large amount of apo H was recovered. These molecules of apo H weakly bound to the lectin. When a higher sugar concentration (500 mmol mannoside) was added most of the sample applied was eluted. These molecules of apo H firmly bound to the column having high affinity for the lectin.

4. Discussion

We studied the structure of *N*-linked carbohydrates bound to apo H by a combination of two methods which make use of lectins. Lectins bind specifically to carbohydrate moieties allowing these structures to be identified. Digoxigenin-la-

belled lectins are used for the structural characterization of carbohydrate chains of glycoproteins bound to nitrocellulose which have been separated on a SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Con A lectin affinity chromatography was used to analyse apo H according to the characteristics of its carbohydrate chain inner to sialic acid residues. Con A has the advantage of sialyl residues not being recognized.

N-linked glycans are distinguished by the presence of the Asn-*N*-acetylglucosamine (GlcNAc) linkage (Lennarz, 1980). There are three major classes of asparagine-linked oligosaccharides: complex, hybrid, and high-mannose. Each type shares a common pentasaccharide, but they differ in their outer branches. The presence of the common pentasaccharide is explained by the fact that all three classes share an initial common mechanism of biosynthesis. The oligosaccharide branches are referred to as antennae, so that bi-, tri-, tetra-, and penta-antennary may all be found (Lennarz, 1980).

Our results from digoxigenin-labelled lectins analysis showed that apo H resulted very positive to SNA. SNA recognized sialic acid α-(2-6) to galactose. It is suitable for identifying complex, sialylated *N*-glycan chains. After hydrolysis with *N*-glycosidase F the reaction was still positive, indicating sialic acid is β-(2-6) linked to galactose also in *O*-glycan structure.

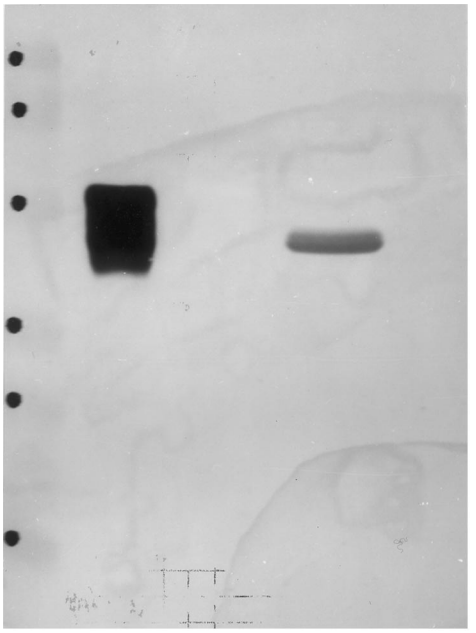

Fig. 3. Western blot of β_2-glycoprotein I probed with PNA lectin (from left to right). Lane 1: prestained molecular mass markers marked with black points are (from top to bottom): 106.0, 80.0, 49.5, 32.5, 27.5, 18.5 kDa. Lane 2: asialofetuin, positive control glycoprotein. Lane 3: fetuin, negative control glycoprotein. Lane 4: native β_2-glycoprotein I.

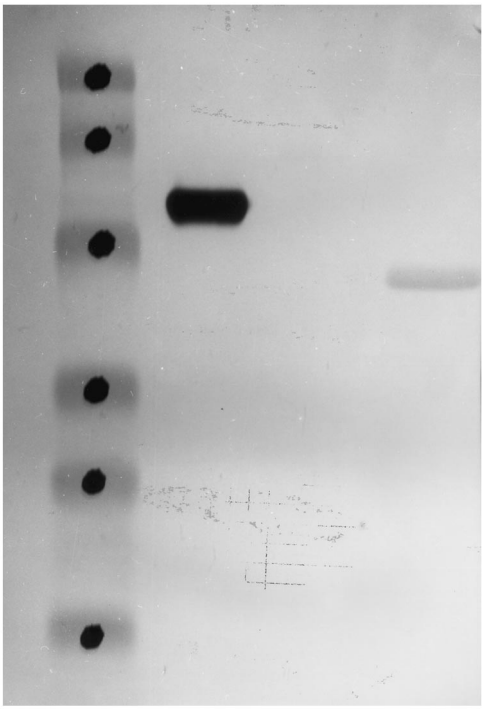

Fig. 4. Western blot of β_2-glycoprotein I probed with GNA lectin. (from left to right). Lane 1: prestained molecular mass markers marked with black points are (from top to bottom): 106.0, 80.0, 49.5, 32.5, 27.5, 18.5 kDa. Lane 2: carboxypeptidase Y, positive control glycoprotein. Lane 3: transferrin, negative control glycoprotein. Lane 4: native β_2-glycoprotein I.

DSA reacted positively with apo H before and after treatment with *N*-glycosidase F suggesting that galactose was β-(1-4) linked to *N*-acetylglucosamine in complex *N*-glycans and in *O*-glycans structures too. Furthermore, in *O*-glycans structures apo H has galactose β-(1-3) linked to *N*-acetylgalactosamine, as proved by PNA reaction. However, galactose was not covered with sialic acid, otherwise this reaction would have been negative.

GNA gave a positive band in native apo H. This lectin recognized terminally linked mannose which is always present in the common pentasaccharide of *N*-linked glycoproteins. The *O*-glycosidically linked mannoses reacting also with GNA are common among the yeast glycoproteins. As a matter of fact, after enzymatic digestion with *N*-glycosidase F the reaction faded away. The very faint band could be due to an incomplete digestion or an aspecific reaction.

MAA gave a negative band before and after treatment with *N*-glycosidase F. This reaction suggested that sialic acid is not terminally linked α-(2-3) to galactose in complex *N*-glycan or in *O*-glycan structure in apo H.

PNA recognized the disaccharide galactose β-(1-3)-*N*-acetylgalactosamine in apo H. This disaccharides usually forms the core unit of *O*-glycans. That is why the

reaction was still positive in *N*-deglycosylated protein. PNA recognized only *O*-glycan structure because galactose could not be linked β-(1-3) in complex *N*-glycans.

Fucose is α-(1-6) linked to *N*-acetylgalactosamine in complex *N*-glycan structures.

Lectins are 'sugar-binding glycoproteins of non-immune origin' bearing at least two sugar binding sites, the presence of which explains the ability to bind glycoproteins (Lennarz, 1980). The lectins are able to bind laterally to glycoprotein

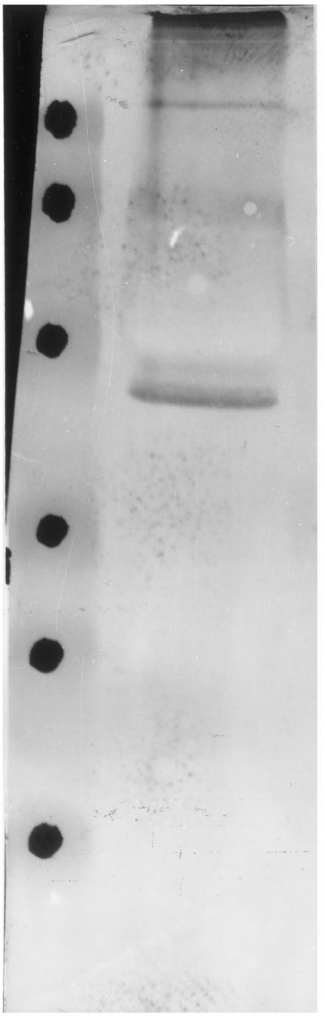

Fig. 5. Western blot of β_2-glycoprotein I probed with AAA lectin. (from left to right). Lane 1: prestained molecular mass markers marked with black points are (from top to bottom): 106.0, 80.0, 49.5, 32.5, 27.5, 18.5 kDa. Lane 2: native β_2-glycoprotein I.

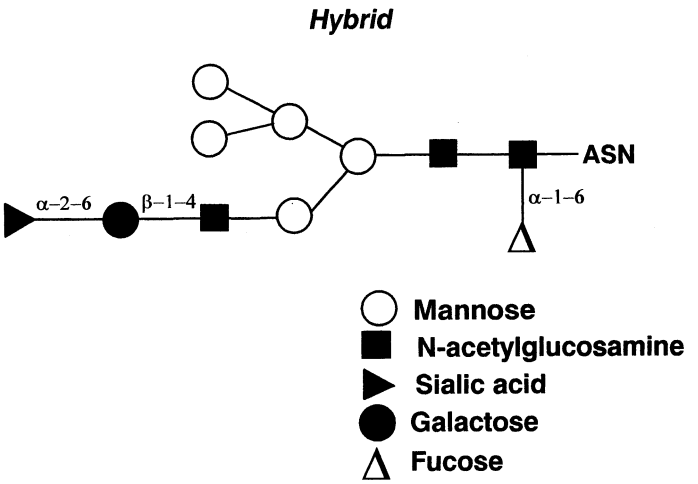

Fig. 6. Representative structures of theoretical β_2-glycoprotein I oligosaccharides based on Con A affinity chromatography and lectin analysis. β_2-Glycoprotein I with hybrid structure oligosaccharides firmly bound to Con A Sepharose column. It was eluted with 500 mM α-methylmannopyranoside. Fucose residue could be α-(1-6) linked to *N*-acetylglucosamine. Expose terminal mannose residues were necessary for binding. ASN is the abbreviation for asparagine-peptide.

glycans. For Con A the structures which are recognized are α-D-Mannose and α-D-glucose. *N*-linked oligosaccharide structures were shown to interact with Con A according to their branching properties as follows: triantennary, tetraantennary, and bisecting oligosaccharides do not bind to Con A, biantennary and truncated hybrids weakly bind to Con A, and high mannose and hybrid oligosaccharides firmly bind to Con A (Papandreou et al., 1993).

When we applied apo H onto the Con A lectin column detectable amounts of protein were not found in the first chromatographic fractions suggesting that apo H is not eluted with Con A buffer. After adding a buffer with low sugar concentration (10 mM glucoside) a large amount of apo H was recovered. These molecules of apo H weakly bound to the lectin. When a higher sugar concentration (500 mM mannoside) was added most of the sample applied was eluted. These molecules of apo H firmly bound to the column having high affinity for the lectin (data not shown).

These results combined with those coming from the digoxigen-labeled lectins method enable us to understand the inner structure of carbohydrate chains with their outer branches. Molecules of apo H which weakly bind to Con A could bear complex *N*-glycans organized in biantennary (Fig. 6) or truncated hybrid structures (Fig. 7). Biantennary structures (Fig. 6) have galactose β-(1-4) linked to *N*-acetyl-glucosamine as indicated by DSA reaction. This disaccharide could be also masked by terminal sialic acid which is α-(2-6) linked to galactose as proved by SNA reaction. Fucose could be α-(1-6) linked to a *N*-acetylglucosamine residue which is linked to Asn (Asparagine). Fucose was indicated by the AAA reaction. These

terminal oligosaccharides are all linked to mannose belonging to the common pentasaccharide. Truncated hybrid (Fig. 7) structures have a branch bearing two or three residues of mannose and the other branch bearing the same sugars illustrated for biantennary structures. Firmly bound apo H referred to molecules rich in *N*-glycan hybrid structures (Fig. 8). They have an outer branch belonging to the high mannose carbohydrate chains which explain the ability to bind to the column and an other main branch bearing the sequence galactose β-(1-4)-*N*-acetylglucosamine β-(1-2) mannose. Galactose could be the terminal sugar or alternatively be masked with sialic acid α-(2-6) terminally linked.

Carbohydrates are mainly linked to asparagine residues in the carbohydrate acceptor sequence Asn-X-Ser/Thr (Lennarz, 1980). In apo H the oligosaccharides are attached to asparagine residues at positions 143, 164, 169, 174, and 234 (Lozier et al., 1984). At asparagine-174 and asparagine-234 the acceptor sequence is Asn-Trp-Ser/Thr. This sequence is very rare among glycoproteins (Lozier et al., 1984).

When the Con A fractions were subsequently subjected to SDS-PAGE, they resolved into a band whose molecular weight was some 50 000 Da. Apparent molecular weights did not significantly differ from each other suggesting that lectin chromatography was able to isolate two classes of apo H molecules. The difference

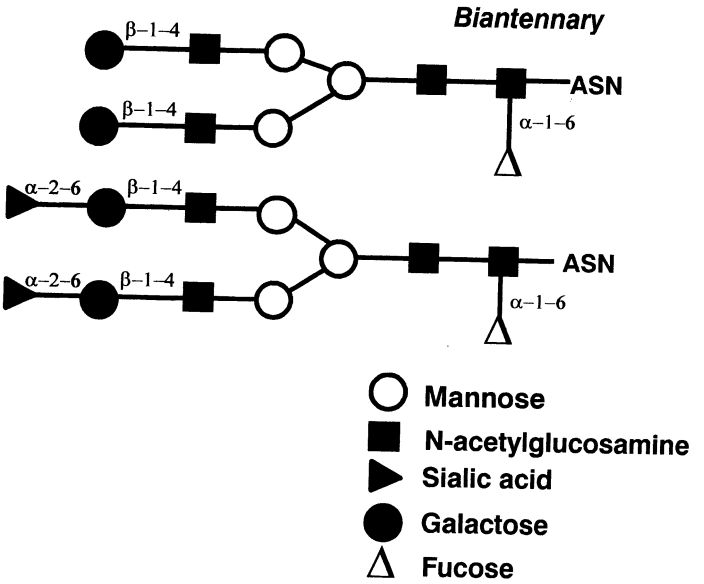

Fig. 7. Representative structures of theoretical β_2-glycoprotein I oligosaccharides based on Con A affinity chromatography and lectin analysis. β_2-Glycoprotein I with biantennaty structure oligosaccharides weakly bound to Con A Sepharose column. It was eluted with 10 mM α-methylglucopyranoside. Fucose residue could be α-(1-6) linked to *N*-acetylglucosamine. Galactose β(1-4) linked to *N*-acetylglucosamine could be a terminal residue or masked with α-(2-6) sialic acid. ASN is the abbreviation for asparagine-peptide.

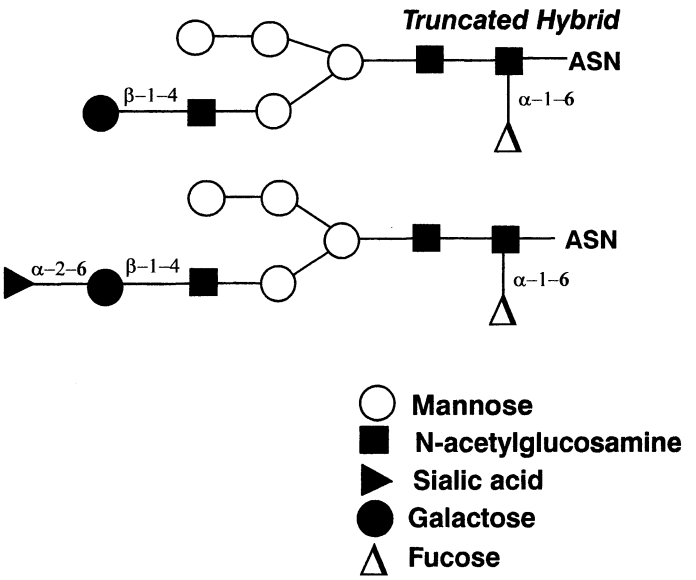

Fig. 8. Representative structures of theoretical β_2-glycoprotein I oligosaccharides based on Con A affinity chromatography and lectin analysis. β_2-Glycoprotein I with truncated hybrid structure oligosaccharides weakly bound to Con A Sepharose column. It was eluted with 10 mM α-methylglucopyranoside. Fucose residue could be α-(1-6) linked to *N*-acetylglucosamine. Galactose β-(1-4) linked to *N*-acetylglucosamine could be a terminal residue or masked with α-(2-6) sialic acid.

between weakly and firmly bound molecules lies in the different carbohydrate organization as investigated above.

The aim of our work was to give an idea about the carbohydrate structures of *N*-glycans in apo H molecules since the structural and functional integrity of many proteins relies on specific co- and posttranslational protein-modification reactions. Asparagine-linked protein glycosylation may serve many diverse roles. Some proteins require *N*-linked oligosaccharides to maintain proper function (Joao et al., 1992; Rudd et al., 1994) or to be correctly targeted (Pfeffer and Rothman, 1987). *N*-linked glycosylation occurs cotranslationally (Bergman and Kuehl, 1978; Kiely and Schimke, 1976) and has the potential to affect the course of protein folding. Glycosylation serves a vital role in the folding and assembly of viable proteins (Copeland et al., 1988; Marquardt and Helenius, 1992). Glycosylation can alter the conformational profile of a polypeptide and allow it to sample conformational space not originally accessible to it. Glycosylation event could serve to funnel the nascent polypeptide structure through a particular pathway for folding. In the absence of glycosylation, specific folded intermediates would be inaccessible, and the outcome would be a delinquent protein product (Imperiali and Rickert, 1995). Furthermore, the potential of *N*-linked oligosaccharides for structural variation is not confined to their chain-terminating sugars. Carbohydrate branching also affects the biological activity of glycoproteins by inducing variations in their tertiary

structure (Papandreou et al., 1993). Moreover, the high amount of sialic acid is known to regulate the blood circulation of glycoproteins by protecting them from hepatic galactose receptor (Ashwell and Harford, 1982; Morell et al., 1971). Thus, further experiments are necessary to understand whether apo H is involved into a similar physiological mechanism.

Acknowledgements

This work was supported in part by grants from Consiglio Nazionale delle Ricerche, Roma (Grant no. 95.010.15.PF40, Progetto Finalizzato "INVECCHIAMENTO") and "Ministero dell'Universita' e della Ricerca Scientifica e Tecnologica" (Quota 60%), Roma, Italy.

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