Biochem. J. (1983) **211**, 55–63
Printed in Great Britain

Comparative study of the carbohydrate moieties of normal and pathological human immunoglobulins M

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(*Received 2 August 1982/Accepted 10 December 1982*)

The well-known heterogeneity of normal and pathological immunoglobulins M was investigated in a study involving the characterization of their carbohydrate moieties. Oligosaccharide units were released from the native molecule by hydrazinolysis, and they were fractionated by affinity chromatography on a concanavalin A–Sepharose column to yield separate *N*-acetyl-lactosaminic-type and oligomannosidic-type structures. Further identification of these oligosaccharides was attempted by t.l.c. on silica gel and by determination of their monosaccharide compositions. A comparative study of the oligosaccharide units belonging to each population of immunoglobulin M was possible. Similarities were found in the occurrence of both types of oligosaccharide structures, and, in addition, a common double heterogeneity could be demonstrated for *N*-acetyl-lactosaminic-type structures: they could be resolved by affinity chromatography into bi-, tri- and tetra-antennary structures, and they also showed differences in *N*-acetylneuraminic acid content. Though some variations were observed in the exact composition of the oligosaccharide units within each population, it was possible to consider a representative oligosaccharide-unit composition of normal immunoglobulin M as a standard for comparison. On this basis a predominance of multi-antennary structures was observed in the more glycosylated pathological immunoglobulins M (10% carbohydrate content), whereas oligomannosidic structures were increased in pathological immunoglobulins M with a lower content of carbohydrates (7%). These variations are thought to reflect differences in the biosynthetic processing pathway of the carbohydrate units of the pathological immunoglobulins M or the enhanced expression of a molecular clone.

Human IgM molecules are glycoproteins with large amounts of carbohydrate (7–15%) (Clamp & Johnson, 1972). In a recent investigation (Cahour *et al.*, 1981) a sampling of 18 monoclonal IgM species was resolved into two populations on the basis of their carbohydrate content: 12 IgM species contained 7.3% carbohydrate and six IgM species contained 10%. An average value of 7.2% carbohydrate content has been found for normal IgM. It is well known that these plasma proteins (Jouanneau *et al.*, 1970; Shimizu *et al.*, 1971; Hickman *et al.*, 1972), as do many glycoproteins (for a review see Montreuil, 1982), contain both-*N*-acetyl-lactosaminic-type and oligomannosidic-type asparagine-bound carbohydrate chains.

Abbreviation used: IgM, immunoglobulin M.

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Commonly, the techniques used for structural investigations of the carbohydrate moieties of immunoglobulins include a digestion with Pronase and subsequent fractionation of the resultant glycopeptides by gel filtration and ion-exchange chromatography.

In the present investigation, by using the combination of hydrazinolysis, affinity chromatography and g.l.c., the oligosaccharide units obtained directly from the native IgM were studied. Their resolution and their characterization in each IgM population were monitored by t.l.c. on silica gel.

The IgM oligosaccharide units could play a biological role either by interaction with membrane receptors or by acting directly on the conformation of the molecules, and we were interested in making a comparison of these structures both in normal and pathological IgM species. Some preliminary results

relevant to this aspect are reported in the present paper.

Experimental

Materials

Silica gel 60 thin-layer plates (0.2mm layer), solvents and chemicals of analytical grade were products of Merck (Darmstadt, Germany). Concanavalin A–Sepharose 4B was supplied by Pharmacia Fine Chemicals (Uppsala, Sweden), and methyl α-D-glucoside by Sigma Chemical Co. (St. Louis, MO, U.S.A.). NaB^3H_4 (36Ci/mmol) was purchased from the Commissariat à l'Energie Atomique (Saclay, France), and the scintillation mixture (Aqualyte™) was a product from J. T. Baker Chemicals BV (Deventer, The Netherlands). Bio-Gel P-2 (200–400 mesh) was from Bio-Rad Laboratories (Richmond, CA, U.S.A.), and hydrazine was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.).

Reference oligosaccharides

The following standard compounds (Table 1) were used for a provisional identification of carbohydrate units assayed by t.l.c. Oligosaccharides S_0, S_1 and S_2 were bi-antennary structures, respectively desialylated, monosialylated and disialylated, obtained from human serum transferrin by the procedure of Spik *et al.* (1975). Oligosaccharides O_3 and O_4 were tri- and tetra-antennary asialoglycans respectively obtained from human plasma α_1-acid glycoprotein (orosomucoid) (Fournet *et al.*, 1978). Oligomannosides with four to nine mannose residues (oligosaccharides M'_4–M'_9) were isolated from urine of patients with mannosidosis (Strecker *et al.*, 1976).

Preparation of immunoglobulins

IgM species were prepared and checked for homogeneity as described previously (Cahour *et al.*, 1981). Monoclonal IgM species were obtained from plasmaphaereses of patients with Waldenström's macroglobulinaemia, and polyclonal IgM species were isolated from individual normal sera.

Hydrazinolysis

Specific cleavage of *N*-acetylglucosamine–asparagine *N*-glycosylaminic linkages was performed as described previously (Bayard & Montreuil, 1974) by the action of anhydrous hydrazine on native protein (1mg) for 20h at 85°C. *N*-Deacetylated glycans were obtained after drying of the hydrazinolysate under N_2 and elimination of traces of the reaction products under vacuum in the presence of conc. H_2SO_4, and after a further desalting on a Bio-Gel P-2 column (1.5cm × 32cm) they were eluted with aq. 1% (v/v) acetic acid. *N*-Reacetylation was performed with acetic anhydride as described by Reading *et al.* (1978), and the oligosaccharide fractions were purified on Bio-Gel P-2.

Radioisotopic labelling of oligosaccharides

Reduction with NaB^3H_4 was performed as described by Takasaki & Kobata (1978). About 100nmol of oligosaccharide was reduced with 8nmol (0.30mCi) of NaB^3H_4 in 300μl of 10mM-NaOH for 4h at 25°C. Then 10mg of $NaBH_4$ was added and the sample was kept for a further 2h at 25°C. The reaction was stopped by adding 1 drop of acetic acid, and after the mixture had been passed through a small column of Dowex 50W X8 (H^+ form) the boric acid was removed by co-distillation under vacuum three times with methanol. Next, descending paper chromatography for 16h on Whatman no. 1 paper with the solvent system butan-1-ol/ethanol/water (16:1:4, by vol.) removed radioactive contaminants derived from the NaB^3H_4. Reduced oligosaccharides were eluted with water and purified on Bio-Gel P-2.

Affinity chromatography of oligosaccharides on concanavalin A–Sepharose

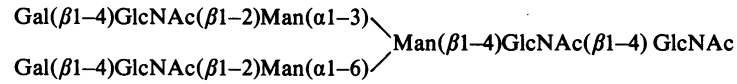
NaB^3H_4-reduced oligosaccharides were applied to a concanavalin A–Sepharose column (1.5cm × 9.5cm) equilibrated with 10mM-sodium acetate buffer, pH 5.0, containing 1mM-$CaCl_2$, 1mM-$MgCl_2$, 1mM-$MnCl_2$ and 0.1M-NaCl. Fractionation was performed by stepwise elution with methyl α-D-glucoside, with reference to previous studies of the binding capacities of concanavalin A for carbohydrate structures (Debray & Montreuil, 1978; Narasimhan *et al.*, 1979). For this purpose a previous calibration of the concanavalin A–Sepharose column was monitored with oligosaccharides of known structures. The tri- and tetra-antennary glycans oligosaccharides O_3 and O_4 (see Table 1), with no affinity for the column, were eluted with starting buffer. Bi-antennary oligosaccharides (i.e. reference oligosaccharides of serum transferrin) were bound with low affinity by the lectin and were eluted with 0.01M-methyl α-glucoside, whereas oligomannosidic oligosaccharides (see Table 1) were strongly bound by concanavalin A and needed 0.3M-methyl α-glucoside for their elution. ^3H radioactivity was counted in 3ml of scintillation solution in a Beckman liquid-scintillation spectrometer.

T.l.c.

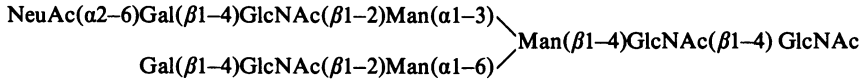
Oligosaccharides were resolved on silica gel 60 thin-layer plates with the following solvent systems: F, a fast one (Bayard *et al.*, 1979), ethanol / butan-1-ol / pyridine / water / acetic acid (100:10:10:30:3, by vol.), for *N*-acetyl-lactosaminic oligosaccharides, and S, a slow one (Palo & Savolainen, 1972), butan-1-ol / acetic acid / water

Table 1. *Reference oligosaccharides used for t.l.c.*

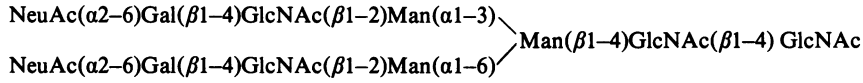
Type of structure	Bi-antennary *N*-acetyl-lactosaminic type
Origin	Human serum transferrin
Structures	



Oligosaccharide S_0

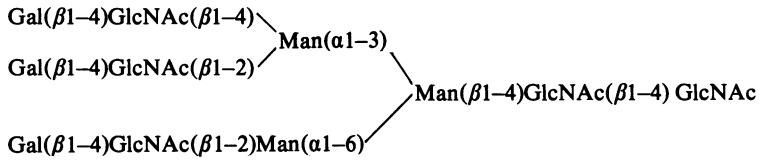


Oligosaccharide S_1

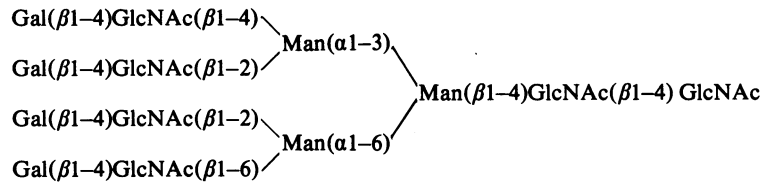


Oligosaccharide S_2

Type of structure	Tri- and tetra-antennary *N*-acetyl-lactosaminic type
Origin	Human plasma α_1-acid glycoprotein
Structures	

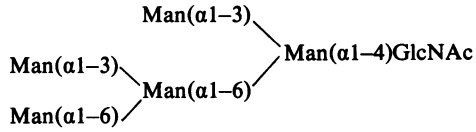


Oligosaccharide O_3*

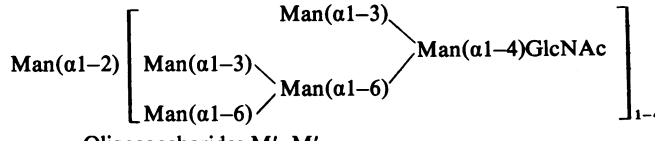


Oligosaccharide O_4

Type of structure	Oligomannosidic type
Origin	Urines of oligomannosidosis patients
Structures	



Oligosaccharide M'_5



Oligosaccharides M'_6–M'_9

* Asialo tri-antennary glycans or orosomucoid may possess an additional fucose residue α-1,3-linked to the *N*-acetyl-glucosamine residue 7 (oligosaccharide O_3F).

(2:1:1, by vol.), for oligomannosidic glycans. Migrating bands were detected by using orcinol (200mg of orcinol in 100ml of aq. 20% H_2SO_4) spray reagent with heating at 110°C for 10min.

G.l.c.

Conditions for g.l.c. were those described by Zanetta *et al.* (1972). *O*-Methylated glycosides were analysed as trifluoroacetate compounds on a glass column packed with 3% OV 210, with a temperature gradient of 2°C/min from 100 to 220°C. *meso*-Inositol was used as internal standard.

Desialylation

Desialylation was performed by mild acid hydrolysis (0.1 M-trifluoroacetic acid for 30min at 80°C) and was checked by t.l.c. with solvent F. Untreated oligosaccharide served as the control.

Results

Comparative t.l.c. of the whole oligosaccharide chains

Hydrazinolysis was performed on normal IgM species and on pathological IgM species with increasing contents of carbohydrates, i.e. 7% (IgM-7), 8% (IgM-8), 9% (IgM-9) and 10% (IgM-10). The released oligosaccharides were analysed by t.l.c. on silica gel in the solvent F (Fig. 1). All preparations of normal IgM exhibited the same pattern, an example of which is given (see Fig. 1, slot 1). For both polyclonal and monoclonal preparations of pathological IgM, patterns essentially consisted of the same bands, numbered I to VIII. A classification within three groups could be made: the first one included IgM-8 and IgM-9, t.l.c. profiles of which resembled that obtained with normal IgM; the

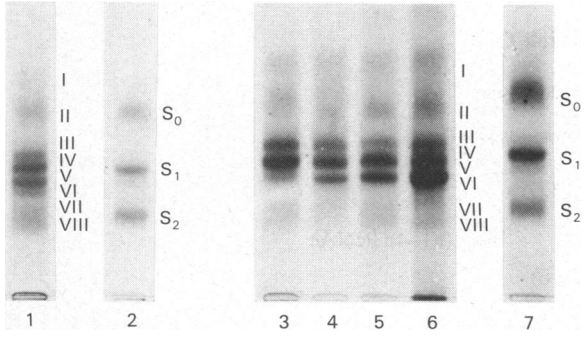


Fig. 1. *Comparative t.l.c. of the whole glycans liberated by hydrazinolysis from normal and pathological IgM species*

For full experimental details see the text. Solvent system F was used. Slot 1, normal IgM; slots 2 and 7, standards of asialo (S_0), monosialo (S_1) and disialo (S_2) bi-antennary glycans from serum transferrin; slots 3–6, pathological IgM species containing 7%, 8%, 9% and 10% carbohydrate respectively.

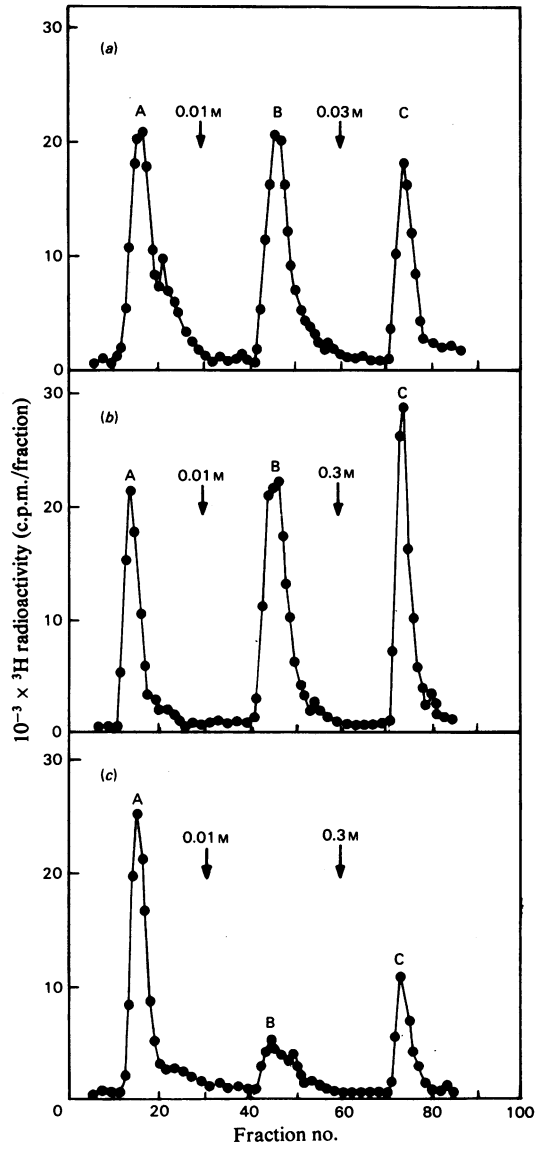


Fig. 2. *Fractionation by affinity chromatography on a concanavalin A–Sepharose 4B column (1.5cm × 9.5cm) of the oligosaccharides liberated by hydrazinolysis of IgM species*

For full experimental details see the text. The flow rate was 9ml/h. Fractions of volume 1ml, numbered in order of their emergence from the column, were collected and counted for radioactivity. Arrows indicate the starting of the elution of fractions B and C with 0.01 M- and 0.3 M-methyl α-D-glucopyranoside respectively. Profiles obtained: (*a*) for normal IgM; (*b*) for pathological IgM-7; (*c*) for pathological IgM-10.

second and the third groups were represented by monoclonal IgM-7 and IgM-10, with characteristic patterns different from those of the first group. Enrichments of band IV for IgM-7 and of band VI for IgM-10 were observed. These variations were not accompanied by significant differences in the molar monosaccharide composition (Table 2).

Fractionation of oligosaccharides by affinity chromatography on concanavalin A–Sepharose

Because of the small quantity of IgM-8 and IgM-9 at our disposal, further studies were limited to normal IgM and to the pathological IgM-7 and IgM-10. Fractionation on concanavalin A–Sepharose of glycans released by hydrazinolysis, *N*-reacetylated and then reduced gave three elution patterns corresponding to the three families of IgM (Fig. 2). Each profile possessed the three fractions A, B and C eluted with starting buffer, 0.01 M-methyl α-glucoside and 0.3 M-α-methyl glucoside respectively.

Qualitatively the pattern obtained for polyclonal IgM resembled that of pathological IgM-7, but quantitatively marked differences were observed in the percentage distribution of fractions (see Table 2). On the one hand *N*-acetyl-lactosaminic units (fractions A and B) were predominant in normal IgM and pathological IgM-10, with 80% of the label. On the other hand, pathological IgM-7 was enriched in high-mannose oligosaccharides (32% against 20% for the two other populations). In addition, for *N*-acetyl-lactosaminic glycans variations were observed in the proportions of tri- and tetra-antennary structures (fraction A) and bi-antennary structures (fraction B). The proportions were equivalent for polyclonal IgM, but the degree of branching was increased for monoclonal IgM-10 whereas it was decreased for monoclonal IgM-7.

Characterization of the carbohydrate chains

To investigate the extent of the heterogeneity exhibited by the carbohydrate moieties of IgM, oligosaccharidic fractions were submitted to g.l.c. after methanolysis (Table 2). The molar proportions obtained were in good agreement with those expected after fractionation of the hydrazinolysates on concanavalin A–Sepharose, and were characteristic of chiefly tri-antennary structures for fraction A, bi-antennary structures for fraction B and oligomannosidic structures for fraction C.

In addition, the usefulness of the t.l.c. method in the further qualitative resolution of IgM oligosaccharide is demonstrated in Fig. 3. In fraction A (Fig. 3*a*), for the three samples essayed, four migrating oligosaccharides were identified (bands IV, V_1, VI and VIII), with a predominance of band VI. A double band in the position for this band VI was noted with polyclonal IgM. This double band might represent oligosaccharides with similar structure but with a possible additional fucose residue on the component forming the slower-migrating band.

Table 2. *Carbohydrate compositions of native IgM glycans and of oligosaccharidic fractions produced by affinity chromatography on immobilized concanavalin A after hydrazinolysis of IgM*

For full experimental details see the text. Results for native IgM glycans obtained after g.l.c. were the same for both native IgM glycans and hydrazinolysates; data for normal IgM and pathological IgM-7 and IgM-10 are corrected and expressed in terms of the sum of the percentage distribution of the fractions A, B and C. Results for the concanavalin A—Sepharose fractions are normalized to 3.0 mannose residues for fractions A and B and of 2.0 *N*-acetylglucosamine residues for fraction C. L refers to structures of the *N*-acetyl-lactosaminic type, and M to structures of the oligomannosidic type.

	Carbohydrate composition (molar proportions)													
		Normal IgM			Pathological IgM									
					IgM-7				IgM-8	IgM-9	IgM-10			
		Concanavalin A fractions				Concanavalin A fractions						Concanavalin A fractions		
	Native IgM	A	B	C	Native IgM	A	B	C	Native IgM	Native IgM	Native IgM	A	B	C
% of radioactivity	...	40	40	20	—	27	41	32	—	—	—	58	24	18
% of L and M structures	...	80		20	—	68		32	—	—	—	82		18
Fucose	0.67	0.77	0.4	—	0.52	0.80	0.70	—	0.76	0.73	0.79	0.98	0.78	—
Mannose	4.1	3.0	3.0	6.2	4.1	3.0	3.0	7.2	4.5	4.4	4.1	3.0	3.0	4.5
Galactose	1.6	2.2	1.8	—	1.2	2.1	1.6	—	1.2	1.2	1.2	2.0	1.4	—
N-Acetylglucosamine	3.1	4.7	3.0	2.0	3.5	4.8	3.6	2.0	3.1	3.1	3.1	4.5	3.7	2.0
N-Acetylneuraminic acid	0.78	1.3	1.0	—	0.47	1.0	0.5	—	0.51	0.61	0.64	0.85	0.50	—

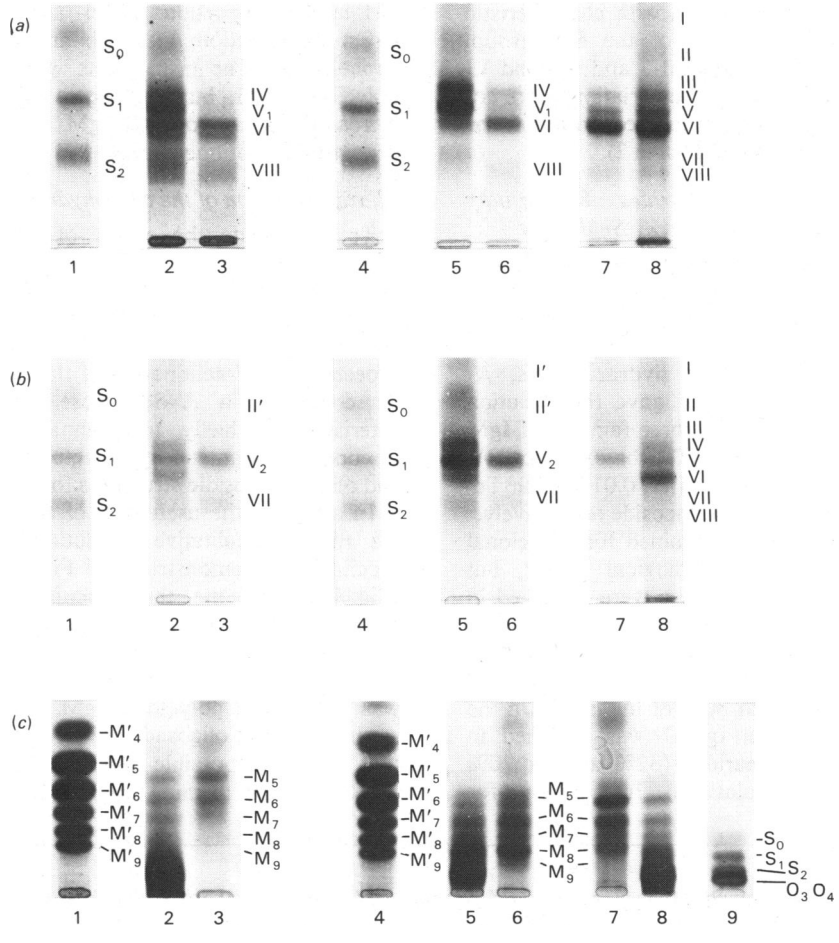


Fig. 3. *T.l.c. of fractions A, B and C obtained by affinity chromatography on immobilized concanavalin A of oligosaccharides liberated by hydrazinolysis of normal and pathological IgM species*

For full experimental details see the text. (*a*) T.l.c. of fraction A in solvent system F. Slots 1 and 4, standards of asialo (S_0), monosialo (S_1) and disialo (S_2) bi-antennary glycans from serum transferrin; slots 2, 5 and 8, oligosaccharides released by hydrazinolysis from normal IgM (slot 2), from pathological IgM-7 (slot 5) and from pathological IgM-10 (slot 8); slots 3, 6 and 7, oligosaccharides of fraction A obtained from normal IgM (slot 3), from pathological IgM-7 (slot 6) and from pathological IgM-10 (slot 7). (*b*) T.l.c. of fraction B in solvent system F. Slots 1 and 4, standards of asialo (S_0), monosialo (S_1) and disialo (S_2) bi-antennary glycans from serum transferrin; slots 2, 5 and 8, oligosaccharides released by hydrazinolysis from normal IgM (slot 2), from pathological IgM-7 (slot 5) and from pathological IgM-10 (slot 8); slots 3, 6 and 7, oligosaccharides of fraction B obtained from normal IgM (slot 3), from pathological IgM-7 (slot 6) and from pathological IgM-10 (slot 7). (*c*) T.l.c. of fraction C in solvent system S. Slots 1 and 4, standards of urinary oligomannosides (M'_4–M'_9); slots 2, 5 and 8, oligosaccharides released by hydrazinolysis (M_5–M_9) from normal IgM (slot 2), from pathological IgM-7 (slot 5) and from pathological IgM-10 (slot 8); slots 3, 6 and 7, oligosaccharides of fraction C obtained from normal IgM (slot 3), from pathological IgM-7 (slot 6) and from pathological IgM-10 (slot 7) (the bands that migrate above M_5 have been identified as heavy contaminants of methyl α-D-glucoside); slot 9, reference oligosaccharides having a slight migration in this solvent system, i.e. asialo (S_0), monosialo (S_1) and disialo (S_2) bi-antennary glycans from serum transferrin, and asialo tri-antennary (O_3) and asialo tetra-antennary (O_4) glycans from α_1-acid glycoprotein. N.B. Bands V_1 and V_2 migrated as compound V but exhibited different affinities for concanavalin A; bands I and II contained a mixture of both *N*-acetyl-lactosaminic and oligomannosidic structures because the components were not fractionated on concanavalin A, whereas bands I′ and II′ were exclusively of the bi-antennary type. With regard to the oligomannosidic glycans, M'_4–M'_9 designated pathological urinary oligosaccharides lacking one *N*-acetylglucosamine residue and M_5–M_9 designated hydrazinolysate complete oligosaccharides.

All these glycans were expected to contain more than two antennae. With fraction B (Fig. 3*b*), the chromatographic behaviour of its oligosaccharides, which were assumed to be bi-antennary, could be compared with that of serum transferrin glycans (oligosaccharides S_0, S_1 and S_2). Four bands, numbered I′, II′, V_2 and VII, were observed for pathological IgM species, band I′ being absent from the normal IgM pattern. Compound V_2 was predominant in all the IgM species and possessed the same t.l.c. mobility as monosialylated and bi-antennary standard oligosaccharide S_1. Bands I′ and II′ migrated in the same zone as oligosaccharide S_0, which is the bi-antennary oligosaccharide with the highest mobility because it is desialylated. Migration of band VII was compared with that of disialylated reference oligosaccharide S_2. Fraction C (Fig. 3*c*) gave rise to numerous oligosaccharides, which were predicted to be of the oligomannosidic type in view of their strong affinity for concanavalin A–Sepharose. These oligosaccharides were analysed by t.l.c. with solvent S. In this solvent, *N*-acetyl-lactosaminic glycans are known to migrate poorly and they stayed (slot 9) near to the origin. It could be verified for the three samples of fraction C studied that there were no such structures left after fractionation by affinity chromatography (slots 3, 6 and 7) by comparison with the corresponding whole range of oligosaccharides before fractionation (slots 2, 5 and 8). The chromatographic mobilities of these high-mannose units were checked with appropriate standards, i.e. urinary oligomannosides (see Table 1). It should be noted that, because of their origin, these reference compounds lack one *N*-acetylglucosamine residue at the reducing end, and consequently exhibited a slightly higher mobility than the corresponding oligosaccharides possessing the additional *N*-acetylglucosamine residue. We have estimated this difference in migration by comparing the behaviour of such pathological IgM oligosaccharides with that of bovine lactotransferrin oligomannosides obtained after hydrazinolysis (Van Halbeck *et al.*, 1981) in the same t.l.c. system. It was approximately equivalent to the effect of the loss of one mannose residue of the largest oligomannosidic structure, with a progressive enhancement in the upper region of the plate. Taking this rule into account, we were able to identify the bands numbered M_5–M_9 as oligosaccharides containing five to nine mannose residues respectively; there was a predominance of larger oligomannosides for pathological IgM-7 and a predominance of smaller oligomannosides for normal IgM and pathological IgM-10.

Further structural investigation was performed on *N*-acetyl-lactosaminic units by eliminating the heterogeneity due to the presence of *N*-acetyl-neuraminic acid residues and by then making a provisional identification of the structures of the residual oligosaccharides. Desialylation with trifluoroacetic acid was performed on fractions A and B for each IgM sample, and t.l.c. migration of their asialoglycans was compared with that of neutral reference oligosaccharides (Fig. 4). On such treatment of fraction A it was found that all the oligosaccharides obtained migrated more slowly than did the neutral bi-antennary standard oligosaccharide S_0. The major compound obtained (X_2) had the same chromatographic behaviour as the fucosylated asialo triantennary glycan of orosomucoid (oligosaccharide O_3F) and as oligosaccharide IV (Fig. 3*a*). As visualized for pathological IgM-10 (slot 3), the compound X_1, which migrated between oligosaccharides O_3 and S_0, was smaller than oligosaccharide O_3, and the oligosaccharide X_3, which migrated the same distance as did oligosaccharide O_4, was presumed to be a tetra-antennary unit. On desialylation of fraction B, as expected, all structures were converted into glycans that migrated in the same zone as oligosaccharide S_0 (zone X_4–X_5). From these results it can be concluded that fraction A is chiefly composed of tri-antennary oligosaccharides accompanied by a smaller amount of tetra-antennary ones, and that fraction B is essentially constituted of bi-antennary oligosaccharides. The oligosaccharides derived from pathological IgM-10 exhibited the greater heterogeneity of structure.

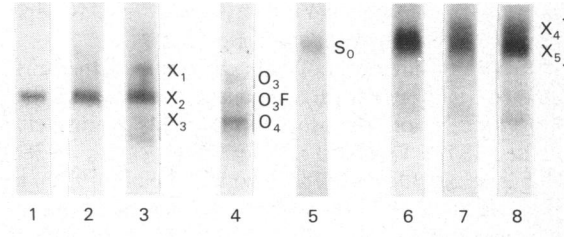


Fig. 4. *T.l.c. of oligosaccharides of desialylated fractions A and B*

For experimental details see the text. Solvent system F was used. Slots 1, 2 and 3, asialo-glycans from fraction A from normal IgM (slot 1), from pathological IgM-7 (slot 2) and from pathological IgM-10 (slot 3); slots 4 and 5, reference oligosaccharides from α_1-acid glycoprotein [asialo triantennary (O_3 and O_3F) and asialo tetra-antennary (O_4) glycans] (slot 4) and from serum transferrin [asialo bi-antennary glycan (S_0) (slot 5); slots 6–8, asialoglycans from fraction B from normal IgM (slot 6), from pathological IgM-7 (slot 7) and from pathological IgM-10 (slot 8).

Discussion

In the present study, by using reproducible and sensitive methodology, a comparison between the numerous glycans present in various IgM species was possible in spite of their well-known heterogeneity.

The application of these rapid procedures to small quantities of native immunoglobulins indicates the coexistence of both *N*-acetyl-lactosaminic type and oligomannosidic type of structures for each IgM population studied, suggesting that oligomannosides are not a typical feature of pathological IgM molecules, as has been suggested (Jouanneau *et al.*, 1981). T.l.c. allows the identification of at least 13 migrating oligosaccharides for each IgM treated, in agreement with the structural heterogeneity previously reported by Chapman & Kornfeld (1979*a*,*b*). According to those authors, all high-mannose intermediate oligosaccharides seem to be present as revealed by t.l.c. in solvent S (see Fig. 3*c*). However, further structural investigations are needed to verify the identity of such structures.

In the present work affinity chromatography on concanavalin A–Sepharose was introduced as an extremely useful tool for the fractionation of glycans from the complex mixtures and for the determination of their respective distribution in IgM families (Table 2). Qualitatively and quantitatively polyclonal IgM species show an intermediate behaviour, presumably reflecting a statistical cellular production of a balanced range of oligosaccharide units. This approach was used as the basis for interpretation of the distribution patterns of oligosaccharides obtained from pathological IgM families. In this regard, monoclonal IgM-7 exhibits a predominance of smaller oligosaccharides, whereas IgM-10 appears to be enriched in complex glycans.

From consideration of both the origin of the IgM studied and previously published data on the biosynthesis of oligosaccharide units (Kornfeld *et al.*, 1978; Li *et al.*, 1978; Tabas *et al.*, 1978; Tabas & Kornfeld, 1978; Parodi & Leloir, 1979), a possible interpretation of the differences observed can be proposed. Normal IgM species reflect their polyclonal origin and can be considered as a statistical population, whereas a monoclonal IgM appears as the expression of one single immunoglobulin-producing cell activity among the multipotentiality due to the diversity of cells. Since it is firmly established that oligomannosidic units serve as intermediates in the biosynthesis of *N*-acetyl-lactosaminic glycans, it can be postulated that the extent of processing of high-mannose oligosaccharides along the pathway towards the formation of complex oligosaccharides is different within each IgM population. On taking the polyclonal IgM glycans as a standard for comparison, results for monoclonal IgM-10 suggest an 'over-processing' leading to a higher production of more complex units, whereas a smaller degree of elaboration of the oligosaccharide chains seems to occur for monoclonal IgM-7. This might be due to altered activity either of the *N*-acetylglucosaminyltransferase, responsible for the substitution of one mannose residue on the core pentasaccharide, or of the α-mannosidase.

The most probable interpretation of the differences observed between normal and pathological IgM species is that regulating factors are involved in the sequence of the metabolic events (i.e. mannose removal and addition of the outer residues) leading to the biosynthesis of glycans. One could imagine that in pathological cases (e.g. in the present case of Waldenström's macroglobulinaemia) such control mechanisms are altered. The factors implicated remain to be determined.

In conclusion, in the field of investigations of structure–function relationships, the information available on the structures of glycans still does not allow an elucidation of their biological role; nevertheless, as in the present study, by comparing the structures of normal and pathological IgM species it is possible to make an approach towards an understanding of the mechanisms involved in their metabolism.

This work was supported in part by the Centre National de la Recherche Scientifique (C.N.R.S.) (Laboratoire Associé no. 217: Relations Structure–Fonction des Constituants Membranaires; Equipe de Recherche Associée no. 070696: Immunochimie et Biologie Moléculaire), by the Institut National de la Santé et de la Recherche Médicale (Unité de Recherches no. 202: Immunochimie et Biologie Moléculaire) and by the Fondation pour la Recherche Médicale. We are very much obliged to Professor G. Spik, Professor B. Fournet and Dr. G. Strecker for their generous supply of standards for t.l.c. We are also grateful to Y. Leroy and J. F. Massias for their skilful assistance.

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