Analysis of Murine Ia Antigen Glycosylation by Lectin Affinity Chromatography

I-Aα α CHAIN SUBSPECIES AND β CHAINS ARE EACH GLYCOSYLATED DIFFERENTLY*

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Mature Ia antigens consist of two glycosylated polypeptide chains, the α chain and the β chain. We have used lectin affinity chromatography to confirm previous work in our laboratory that three distinct, differentially glycosylated I-Aα α chains (α1, α2, and α3) exist and to compare the carbohydrate of the α chain with that of the β chain. Glycopeptides derived from pronase digestion of [3H]mannose-labeled I-Aα α1, α2, and α3 and β chains were sequentially passed over columns of immobilized concanavalin A, Lens culinaris lectin, phytohemagglutinin-E, and phytohemagglutinin-L in a prescribed manner to generate a lectin affinity profile, which, in turn, allowed assignment of a minimal oligosaccharide structure for each glycopeptide studied.

The lectin affinity profile for each chain was unique. The α1, α2, and β chains each possess complex-type N-linked oligosaccharides, although the branching pattern and specific sugar residues found on each differ. The α3 chain, on the other hand, possesses predominantly high mannose or hybrid-type N-linked oligosaccharides. Lectin affinity analysis of glycopeptides derived from pronase digestion of high pressure liquid chromatography-isolated tryptic-chymotryptic fragments from α2 and α3 and tryptic fragments from β revealed that specific minimal oligosaccharide structures were associated with particular fragments. In addition, although tryptic-chymotryptic peptide maps of α2 and α3 were similar, α2 fragments bear predominantly complex-type N-linked oligosaccharides, whereas homologous α3 fragments bear high mannose or hybrid-type N-linked oligosaccharides.

Possible explanations of the oligosaccharide heterogeneity are discussed.

Interest in the structure of the N-linked oligosaccharides of integral membrane glycoproteins has been sparked by the suggestion that these oligosaccharides may play important roles in intercellular communication and recognition mechanisms. The oligosaccharide structures of only a few specific membrane glycoproteins have been studied, among which are those from human erythrocyte band 3 glycoprotein (1, 2) and glycophorin (3-5) and those from bovine rhodopsin and the human erythrocyte band 3 glycoprotein (1, 2) and glycophorin (3-5).

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of the chains is sialylated (23). It is known that at least part of the polymorphism of these glycoprotein molecules is a function of differences in peptide structure (25-29). However, the contribution of the oligosaccharide moieties to Ia glycoprotein polymorphism is not known.

Two recent reports have demonstrated that not all Ia glycoproteins are glycosylated in the same way. Cullen et al. (30) found that I-A\(^\alpha\) chains from splenic macrophages lacked cleavable sialic acid residues present on the corresponding chains isolated from B-cells. In this report, we have described the isolation of three distinct I-A\(^\alpha\) chain subpopulations (\(\alpha_1\), \(\alpha_2\), and \(\alpha_3\)) which possess the same polypeptide backbone but are differentially glycosylated (21). The \(\alpha_1\) and \(\alpha_2\) chains appear to bear complex-type N-linked oligosaccharides since (i) they radiolabel with \([^{14}C]\)mannose and \([^{3}H]\)mannose, and (ii) their electrophoretic mobility is altered by treatment with neuraminidase, suggesting that they bear sialic acid residues. The \(\alpha_3\) chain is thought to bear high mannose-type N-linked oligosaccharides because it cannot be labeled with \([^{14}C]\)mannose and because its electrophoretic behavior is unaffected by neuraminidase treatment.

In this study, we have employed the technique of lectin affinity analysis to extend our examination of the nature of the glycosylation differences between \(\alpha_1\), \(\alpha_2\), and \(\alpha_3\) and to investigate the glycosylation pattern of the \(\beta\) chain.

**MATERIALS AND METHODS**

Animals and monoclonal antibody

Female C3H/HeJ mice, 8-12 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Maine. The monoclonal antibody 10-2-16 (anti-Ia-17, used as an anti-I-A\(^\alpha\) reagent) was obtained from the K.12-16 cell line (32) supplied by the Salk Institute.

Isolation of radiolabeled I-A\(^\alpha\) molecules

\([^{14}C]\)Mannose (C-2,4-[\(^{14}C\)]mannose, 21 Ci/mmol) in 50K ethanol, New England Nuclear, Boston, MA) was dried under nitrogen at room temperature and dissolved in either glucose-free or glucose-deficient (100 mg) RPMI 1640 supplemented with 1.0 M sodium pyruvate, 15 mm HEPES, and 2 mm glutamine. Unfractionated sperm cells were suspended to 7x10\(^5\) cells in labeling medium and cultured for 6 hours at 37°C in a 5% CO\(_2\) atmosphere.

\(\alpha_1\) molecules were isolated from the biosynthetically radiolabeled cells as previously described (31). Briefly, the cells were detergent solubilized in NP-40 (Nonidet P-40, Particle Data Labs, Elmhurst, IL), centrifuged at 100,000 xg, and the supernatant cleared of endogenously labeled material by centrifugation. The tissue composites were assayed to SAC1, and following centrifugation the supernatant was saved (see below) and the pellets were washed three times in PBS (0.14 M NaCl, 2.7 M KCl, 1.5 M MgCl\(_2\), 8.0 mm Na\(_2\)PO\(_4\), pH 7.5) with 0.15% NaOAc, 0.02% deoxycholate, and 0.1% n-octyl glucoside (505, pH 7.4). The washed pellets were frozen. The immunoprecipitation procedure was then repeated on the supernatant that had been retained. Subsequently, immune composites were eluted from the pooled SAC1 pellets by boiling twice in 5x SDS, 62 mm tris, pH 6.8 in the presence of 2% w/v mercaptoethanol (elution buffer) for 2 minutes. Preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Dissolution of pronase 140 mmol/l sodium pyruvate and 140 mmol/l mannose was added to trypsin (56,300 Plg/ml, Calbiochem) in 50 ml of pronase buffer was added. Samples were incubated at 60°C in a bunsen atmosphere for 12 hours, after which another 2 ml of pronase in 50 ml of pronase buffer was added and the incubation continued for an additional 12 hours under nitrogen. Samples were then boiled for 10 minutes to denature remaining enzyme and centrifuged to remove insoluble material.

Exhaustive pronase digestion

Acrylamide gel slices or desalted NPC isolated chain fragments were subjected to exhaustive pronase digestion with 0.5 ml of 0.1 M Tris, 0.5 ml of 0.02% NaN\(_3\), and applied to a column containing 3 ml of Cdn-Sepharose (Pharmacia, Piscataway, NJ) in PBS. The material was eluted from the column at a flow rate of 1 ml/min, first with PBS at room temperature (ten-2 ml fractions collected - pool 1), then with 10 mm \(\alpha\)-methyl glucoside (SIGMA) in PBS at room temperature (twenty-2 ml fractions collected - pool 1), and finally with 100 mm \(\alpha\)-methyl mannose (grade III, SIGMA) in PBS warmed to 60°C (twenty-2 ml fractions collected - pool III).

Lectin affinity chromatography

Glycopeptides derived from pronase digests of isolated chains or fragments were assayed for their lectin affinity chromatography employing a series of columns described below. The radioactivity in aliquots from each lectin column fraction was determined using a Phosphor screen scintillation cocktail (Amersham), and appropriate fractions were pooled and dried on a a shaker-evaporator (Buchler Instruments, Fort Lee, N.J.). The dried samples were dissolved in 0.5-1.0 ml of 7% PEG (Burdick-Jackson Laboratories) and were assayed by gel filtration in the same solvent in a 1x 10.0 ml column of Sephadex G-25 (SIGMA Chemical Co., St. Louis, MO). The appropriate fractions were pooled and dried. The desalted glycopeptide was then reconstituted in appropriate buffer and analyzed for its affinity with the subsequent lectin column.

a. Con A affinity column

The bovine pronase-digested sample was diluted 3.4 volumes of PBS (0.05 M NaCl, 0.01 M Tris, 1 mm CaCl\(_2\), 1 mm MgCl\(_2\), 0.02% NaN\(_3\), pH 8.0) and applied to a 0.7 x 14 cm column containing 3 ml of Con A-Sepharose (Pharmacia, Piscataway, NJ) in PBS. The material was eluted from the column at a flow rate of 1 ml/min, first with PBS at room temperature (ten-2 ml fractions collected - pool 1), then with 10 mm \(\alpha\)-methyl glucoside (SIGMA) in PBS at room temperature (twenty-2 ml fractions collected - pool 1), and finally with 100 mm \(\alpha\)-methyl mannose (grade III, SIGMA) in PBS warmed to 60°C (twenty-2 ml fractions collected - pool III).

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b. Lens culinaris affinity column
Strategies of lectin affinity analysis

Lectin affinity profiles were generated as previously described (7) by first passing glycopeptides derived from pronase-digested [3H]mannose-labeled I-A<sup>k</sup> α and β chains over Con A columns. Pool I glycopeptides were then analyzed on lentil lectin, L-PHA, and E-PHA. Overall profiles for each chain were then examined for qualitative differences, with slow initial definition of the nature of the oligosaccharide(s) attached to the I-α and I-β chains.

RESULTS

Lectin Affinity Profiles of I-A<sup>k</sup> α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub> and β Chain Glycopeptides—[3H]mannose-labeled I-A<sup>k</sup> α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, and β chains were isolated as previously described and lectin affinity profiles were generated for glycopeptides derived from each. These profiles are shown in Figs. 1 and 2.

The ConA profiles of the glycopeptides from each α chain subspecies revealed that the distribution of glycopeptides in ConA pools I, II, and III differed from one subspecies to the next (Fig. 1). Most striking was the observation that, while significant proportions of glycopeptides from α<sub>1</sub> (Fig. 1a) and α<sub>2</sub> (Fig. 1f) were found in pools I and II (complex-type oligosaccharides), over 90% of the glycopeptides from α<sub>3</sub> (Fig. 1f) was found in pool III (high mannose/hybrid-type oligosaccharides).

The ConA profiles also suggested oligosaccharide differences between α<sub>1</sub> and α<sub>2</sub> (Fig. 1, a versus f). Although the relative amount of material appearing in pool I was similar for each chain, α<sub>2</sub> had significantly more material eluting in pool II than did α<sub>1</sub> while α<sub>1</sub> had significantly more material eluting in pool III than did α<sub>2</sub>. In addition, a small but reproducible shoulder on the pool I peak (pool I') was evident in the α<sub>2</sub> profile but was missing from the α<sub>1</sub> profile. This shoulder contains glycopeptides which interact weakly with ConA such that their flow through the column is retarded, in contrast to pool I glycopeptides, which freely flow through the column. None of the pool I' glycopeptides interacted with any of the other lectin columns (data not shown). These glycopeptides are classified as pool I'T.

Additional differences between the α<sub>1</sub> and α<sub>2</sub> glycopeptides were found by analysis on other lectins. Lentil lectin bound only a small percentage of α<sub>1</sub> pool II glycopeptides, but half of the α<sub>2</sub> pool II glycopeptides (Fig. 1d versus i). Pool I glycopeptides from α<sub>1</sub> were not retarded by E-PHA (Fig. 1b) and did not bind significantly to lentil lectin (Fig. 1c). However, some of the glycopeptides from α<sub>1</sub> pool I were retarded by L-PHA (Fig. 1e). The α<sub>2</sub> pool I glycopeptides were not retarded by E-PHA (Fig. 1g), but a significant fraction was retained by lentil lectin (Fig. 1h), and approximately half of those glycopeptides which bound to lentil lectin were retarded by L-PHA (Fig. 1l). Pool II'A glycopeptides were not retarded by L-PHA (Fig. 1j). Although 92% of the α<sub>3</sub> glycopeptides was found in pool III, 6% was found in pool II (Fig. 1f) and, of this, 71% bound to lentil lectin (Fig. 1m).

Glycopeptides derived from the I-A<sup>k</sup> β chain (Fig. 2) were found predominantly in ConA pool II (Fig. 2a). When pool II was applied to lentil lectin, 100% was bound (Fig. 2d). ConA pool I material did not interact with any of the other lectins (Fig. 2b, c, and e).

Based on the distribution of radiolabeled glycopeptides in the point at which elution of glycopeptides with competing sugar was begun. Vertical arrows in the L-PHA profiles indicate where unretarded and retarded glycopeptides would appear (see text). Radioactivity is expressed as disintegrations/min/fraction.
**Lectin Affinity Analysis of I-A^k α and β Chains**

**β PROFILE**

Fig. 2. Lectin affinity profile of [3H]mannose-labeled I-A^k β chain glycopeptides generated as described under "Materials and Methods." Percentages in parentheses indicate the relative proportion of each glycopeptide pool in that particular chromatographic run. See legend to Fig. 1 for explanation of arrows. Radioactivity is expressed as disintegrations/min/fraction.

The pools indicated in Figs. 1 and 2, various minimal oligosaccharide structures, shown in Fig. 3, can be assigned to the α1, α2, α3, and β chains. The α1 chains seem to carry at least three different minimal structures, IIA, II'A2, and III, and an additional unknown structure(s) found in pool II'Al. Structure II'A2 is unique to α1. It should not be inferred that all these structures are present on every α1 chain. α2 chains bear a more diverse series of oligosaccharides with structures IIA, IIB, II'B1, II'B2, III, and additional unknown structures associated with pools II'Al and I'I'Al. Structures I'I'B1 and II'B2 are present in pools II'Al and II'I'Al. Structures are cias-

**Lectin Affinity Profiles of I-A^k α, α2, and β Glycopeptides Isolated by Preparative HPLC—Tryptic-chymotryptic peptide mapping of [3H]mannose-labeled I-A^k α2 and α3 chains by reverse-phase HPLC resolves three well defined fragments (Ref. 31 and Fig. 4, a and b). The first (TC-1) and second (TC-2) fragments from the α2 and α3 chains have identical retention times on a C8 column. The third fragment (TC-3) elutes as a relatively sharp peak in the α3 map (Fig. 4b), but as a broader and later peak in the α2 map (Fig. 4a). This difference appears to be attributable to sialic acid residues on the α3 fragment since neuraminidase digestion converts the α2 TC-3 peak to a peak similar to α3 TC-3 (31). The patterns generated by the preparative HPLC separation of the tryptic-chymotryptic fragments of I-A^k α2 and α3 are identical with those seen in analytical studies (31).

In order to localize each of the different oligosaccharide structures from intact chains to a particular tryptic-chymotryptic fragment, we examined the lectin affinity profiles of glycopeptides derived from pronase digests of each of the α2 and α3 HPLC-isolated tryptic-chymotryptic fragments. The α1 chain could not be recovered in sufficient quantity to permit this type of analysis. The results of these studies are shown in Figs. 5 for α2 and 6 for α3 and are summarized in Table I. α2 fragment TC-1 contains oligosaccharide which is found predominantly in glycopeptide fraction IIB (Fig. 5 and Table I). In addition, small proportions of glycopeptides were found in fractions II'B1, II'B2, and III. The glycopeptide distributions of α3 TC-2 and TC-3 (Fig. 5 and Table I) are similar to
one another, but different from that of TC-1. Glycopeptide fraction IIA is the predominant structure associated with TC-2 and TC-3, along with fraction I'IA. Together, glycopeptide fractions IIA and I'IA accounted for 74–83% of the total TC-2 and TC-3 glycopeptide pool (Table I). The remaining glycopeptides were found in fractions I'IA and III.

The lectin affinity profile of the \( \alpha \) tryptic-chymotryptic fragments which did not interact with the HPLC column ("breakthrough," Fig. 4a, fractions 1-75) was similar to the profile of glycopeptides derived from the intact \( \alpha \) chain (data not shown). Therefore, it is likely that the breakthrough fragments are partial digestion products.

These results indicate that oligosaccharides with structures IIB, I'IB1, and I'IB2 (Fig. 3) are found associated exclusively with \( \alpha \) TC-1. On the other hand, structure IIA and pool I'IA are associated exclusively with TC-2 and TC-3. High mannose/hybrid-type oligosaccharides are common to TC-1, TC-2, and TC-3 in varying amounts.

The HPLC tryptic-chymotryptic profile of \( \alpha \) (Fig. 4b) resembles that of \( \alpha \) (Fig. 4a) with the exception of TC-3, as discussed above. However, the lectin affinity profiles of glycopeptides derived from the \( \alpha \) tryptic-chymotryptic fragments (Fig. 5 and Table I) are markedly different from their \( \alpha \) counterparts. \( \alpha \) TC-1, TC-2, and TC-3 all possess glycopeptides found predominantly in ConA pool III, whereas the \( \alpha \) chain bears complex-type oligosaccharides on the homologous tryptic-chymotryptic fragments. Glycopeptides derived from tryptic-chymotryptic fragments in the HPLC breakthrough (Fig. 4b) were found predominantly in ConA pool III, along with small proportions of glycopeptides I'IA, IIA, and IIB (data not shown).

The lectin affinity profiles of HPLC-isolated \(^{3}H\)mannose-labeled I-A\(^{b}\) \( \beta \) chain fragments were also examined (Fig. 7 and Table I). Only trypsin digestion was used in this case because digestion with trypsin produced fragments which were resolvable, whereas digestion with both trypsin and chymotrypsin produced fragments which were not resolvable. The tryptic peptide map of \(^{3}H\)mannose-labeled I-A\(^{b}\) \( \beta \) chain revealed three peaks (Fig. 4c). Glycopeptides derived from each tryptic fragment (T-1, T-2, and T-3) were found predominantly in glycopeptide fraction IIB (Fig. 7 and Table I). The only difference in affinity profiles among the T-1, T-2, and T-3 fragments was that glycopeptides derived from T-1 material appeared to contain relatively more I'IA glycopeptides than did the material derived from T-2 and T-3. Thus, all of the glycopeptides fractionated from pronase digests of intact \( \beta \) chains could also be identified among the glycopeptides derived from the tryptic fragments. The lectin affinity profile of the tryptic fragments in the HPLC breakthrough (Fig. 4c) was similar to that of glycopeptides derived from the intact \( \beta \) chain (data not shown). Thus, the \( \beta \) chain bears predomi-

**Table I**

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<th>Glycopeptide fraction</th>
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* Values indicate the distribution of glycopeptides fractionated by lectin affinity chromatography as described under "Results." These values were calculated using the percentages in Figs. 4, 5, and 7-9 which indicated the relative proportion of each glycopeptide in a particular chromatographic run.

* Glycopeptide fractions are defined in Figs. 1 and 2.

*—, no significant amount detected.
Lectin Affinity Analysis of I-A<sup>+</sup> α and β Chains

**Fig. 5.** Lectin affinity profiles of [³H]mannose-labeled I-A<sup>+</sup> α<sub>2</sub> TC-1 (a-f), TC-2 (g-k), and TC-3 (l-p) glycopeptides generated as described under "Materials and Methods." Percentages in parentheses indicate the relative proportion of each glycopeptide pool in that particular chromatographic run. See legend to Fig. 1 for explanation of arrows. Radioactivity is expressed as disintegrations/min/fraction.

**Fig. 6.** ConA profiles of [³H]mannose-labeled I-A<sup>+</sup> α<sub>3</sub> TC-1 (a), TC-2 (b), and TC-3 (c) glycopeptides. Percentages in parentheses indicate the relative proportion of each glycopeptide pool in that particular chromatographic run. Radioactivity is expressed as disintegrations/min/fraction.

Lectin affinity analysis of I-A<sup>+</sup> α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, and β chains in this study (summarized in Table I) has revealed that each of these chains is glycosylated in a different manner. In addition, based on the specificity of each of the lectins used, the structural basis for those differences was determined (Table I and Fig. 3). It should be emphasized that the structures associated with each chain are minimal and that additional sugar residues may be present which do not influence binding to a given lectin.

Since complex-type N-linked oligosaccharides are known to be synthesized through a high mannose-type intermediate (37-39), it was possible that α<sub>3</sub> is a precursor form of α<sub>1</sub> and/or α<sub>2</sub>. However, a preliminary pulse-chase study<sup>3</sup> indicates that there is no simple kinetic relationship between these three species of α. Further analysis of α<sub>3</sub> and α<sub>2</sub> pool III glycopeptides may be helpful in resolving this issue. If the

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<sup>3</sup> E. P. Cowan, B. D. Schwartz, and S. E. Cullen, unpublished observations.
Lectin Affinity Analysis of I-A<sup>α</sup> and β Chains

Fig. 7. Lectin affinity profiles of [3H]mannose-labeled I-A<sup>α</sup> β T-1 (a–e), T-2 (f–g), and T-3 (h–i) glycopeptides generated as described under "Materials and Methods." Percentages in parentheses indicate the relative proportion of each glycopeptide pool in that particular chromatographic run. See legend to Fig. 1 for explanation of arrows. Radioactivity is expressed as disintegrations/min/fraction.

oligosaccharide in α<sub>1</sub> is a hybrid-type structure that is not characteristic of a processing intermediate, then it is unlikely that α<sub>0</sub> would be a precursor to α<sub>2</sub>. A more likely possibility is that, since we are examining molecules isolated from biosynthetically radiolabeled unseparated spleen cells, α<sub>1</sub>, α<sub>2</sub>, and α<sub>3</sub> are expressed on different spleen cell subsets. Support for this idea comes from our observations (30) that I-A<sup>α</sup> chains from splenic macrophages may express I-A molecules predominantly bearing sialic acid-poor α<sub>3</sub> chains. Analysis of [3H]mannose-labeled I-A molecules from macrophages is in progress.

The lectin affinity profiles of α<sub>2</sub> and α<sub>3</sub> tryptic-chymotryptic fragments clearly demonstrated that, whereas α<sub>2</sub> fragments bear predominantly complex-type oligosaccharides, homologous α<sub>3</sub> fragments almost exclusively bear high mannose/hybrid-type oligosaccharides. This result is of note because it indicates that the TC-1 or TC-2 glycopeptide has a similar retention on the HPLC column whether it bears complex- or high mannose-type oligosaccharides, thus implying that the oligosaccharide has little influence on the retention of TC-1 or TC-2. In contrast, the retention time of TC-3 is clearly affected by degree of sialylation. Thus, application of reverse-phase HPLC for peptide mapping has the strength that, unlike ion exchange chromatography, it resolves glycopeptides, but it has the possible weakness that a peptide may or may not be influenced in mobility by heterogeneity in glycosylation (e.g. sialylation).

One of our initial aims in separating oligosaccharide-bearing fragments by HPLC was to determine the number of glycosylation sites on the intact glycoproteins. In theory, HPLC separation of fragments should even permit distinction of identical oligosaccharides attached to different parts of the molecule. Our HPLC maps show that digestion generates three oligosaccharide-bearing α chain fragments and three oligosaccharide-bearing β chain fragments. However, these results appeared to be in conflict with the data of Shackelford et al. (40) on HLA-DR oligosaccharides and from Sung and Jones (41) on murine I-A oligosaccharides which indicated that the α chain bears two oligosaccharide chains while the β chain bears only one.

When the α chain data are examined, it is clear that, while the oligosaccharide on TC-1 is quite different from that of TC-2/3, the oligosaccharides on TC-2 and TC-3 are quite similar to one another. It is possible that these two fragments may in fact bear the same glycosylation site, but may have small modifications in the oligosaccharide and/or protein portion which render them separable. If this postulate is true, then the finding of three fragments for two glycosylation sites would be explained. The HPLC maps of digested β chain show three peaks with apparent periodicity. The affinity
profiles from these peaks show that all three fragments bear quite similar oligosaccharide structures. We consider it possible that these three peaks are all fragments bearing the same glycosylation site and that they are separated because of differential dialylation, because of some aberrant or incomplete proteolytic cleavage, or because of some variable modification in the protein portion leading to differential retention.

We also found several distinct oligosaccharide structures associated with a given α or β tryptic-chymotryptic or tryptic fragment (Table I). This finding can be explained either by postulating multiple glycosylation sites on a given fragment or by postulating a single site which is glycosylated in more than one way. In the context of the data indicating that there are two glycosylation sites on the α chain and one glycosylation site on the β chain, the latter explanation seems more likely. This explanation is also favored by the fact that α2 TC-1 and β T-2 and T-3 each bear one predominant minimal oligosaccharide structure (IIB). Although the predominance of one minimal structure on α2 TC-2 and TC-3 (IIA) and on β T-1 (IIB) over other structures is not as clear cut, it is likely that each of the other three fragments contains a single glycosylation site.

One explanation for the heterogeneity of oligosaccharide structures at a given glycosylation site is that, in this case, we are detecting biosynthetic intermediates. As shown in Table I, every lectin affinity profile displays a small percentage of pool III glycopeptides which are probably the high mannose precursors of the complex-type oligosaccharide predominant at that glycosylation site. Incompletely processed complex-type oligosaccharides may be detected as well; for example, α2 TC-1 I1/B1 may be the pre-galactosylated form of I1/B2. An additional explanation for glycosylation heterogeneity might be that different splenocyte subpopulations bear I-A^k molecules which are glycosylated differently, as discussed above. Clearly, it will be necessary to examine molecules isolated from purified populations of spleen cells and to determine the kinetic relationships between different chains found to be synthesized by the same cell type.

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REFERENCES

36. Deleted in proof.