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FURTHER FRACTIONATION OF THE ASPARAGINYL–CARBOHYDRATE FROM OVALBUMIN*†

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ABSTRACT

The asparaginyl–carbohydrate from ovalbumin has been further separated to give a total of seven fractions. Additional heterogeneity was found in this study with the subfractionation of the original AC-C and AC-D components to give AC-C_1 and AC-C_2, and AC-D_1 and AC-D_2, respectively. The compositions of the principal components were found to be the following: AC-C_1 ($AsnGlcNAc_5Man_3$), AC-C_2 ($AsnGlcNAc_4Man_6$), AC-D_1 ($AsnGlcNAc_4Man_4$), and AC-D_2 ($AsnGlcNAc_2Man_6$). The extent of the digestion of these AC fractions with exo-hydrolases is discussed in relation to the use of such asparaginyl–carbohydrates in studies of glycosyltransferase enzymes.

INTRODUCTION

The carbohydrates of ovalbumin have been isolated as the β-L-asparaginyl derivatives, which were separated by ion-exchange chromatography into five fractions identified[1,2] as AC-A through AC-E. These fractions were homogeneous by several criteria[3]. Nevertheless, suggestions of heterogeneity persisted; fraction AC-B contained 0.5 residue per mol of galactose, all of which was hydrolyzed by β-D-galactosidase[3], and fractional amounts of 2-acetamido-2-deoxy-D-glucose were cleaved from AC-D by 2-acetamido-2-deoxy-β-D-glucosidase[2]. We report the further fractionation of AC-C and AC-D by anion-exchange chromatography of the borate complexes.**

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**The mixture of L-β-aspartamido–carbohydrate components, free from peptide contaminants, isolated from native ovalbumin will be abbreviated AC, with each of the original five fractions being denoted AC-A through AC-E. Subfractions of these fractions are identified by subscripts, such as AC-C_1. Derivatives obtained by removing α-D-mannopyranosyl residues with α-D-mannosidase carry the superscript Man, for example AC^{Man}. Similarly, derivatives resulting from treatment with 2-acetamido-2-deoxy-β-D-glucosidase carry the superscript GlcNAc thus AC-C_2^{GlcNAc}.

EXPERIMENTAL

Materials. — Ovalbumin was prepared according to the method of Kekwick and Cannan[4]. Asparaginyl–carbohydrate fractions AC-A, -B, -C, -D, and -E were prepared according to the method of Huang, *et al.*[2].

α-D-Mannosidase was coupled to Sepharose and used for hydrolysis of AC as described previously[5]. α-D-Mannosidase (jack bean)[6], β-D-mannosidase (*Polyporus sulfureus*)[7], and 2-acetamido-2-deoxy-β-D-hexosidase (jack bean)[8], used for digestions of the AC subfractions, were gifts from Dr. Y.-T. Li.

Analytical methods. — The hexose content of the asparaginyl–carbohydrate fractions was quantitated by the following methods: (*a*) without acid hydrolysis, by the phenol–sulfuric acid method[9]; (*b*) without acid hydrolysis, by using an automated carbohydrate analyzer as described by Lee *et al.*[10]; (*c*) after hydrolysis in 2M trifluoracetic acid for 3 h at 110° and subsequent analysis on the carbohydrate analyzer[10]. The values for mannose by these methods were essentially the same.

L-Aspartic acid and 2-amino-2-deoxy-D-glucose in the AC fractions were determined with a Beckman 120C amino acid analyzer following their release by hydrolysis with 4M hydrochloric acid for 5 h at 110°.

AC, from four digestions of ovalbumin with pronase[2], was further purified to yield an unfractionated AC mixture, containing only the AC-A through AC-E components as follows: the desalted, pronase-digested material containing 2.5 mg of mannose equivalents was applied to a Dowex-50 × 8 column (2.5 × 30 cm) equilibrated with mM sodium acetate, pH 2.6. Under these conditions, fractions AC-A through AC-E were eluted with 60–150 ml of buffer as a single, asymmetric, broad peak. This peak (AC) was subsequently desalted on Bio-gel P-2 and freeze-dried.

Fractionation of AC and AC-fractions by using anion-exchange chromatography in borate buffer. — AC and the AC-fractions were chromatographed by a modification of the method of Lee *et al.*[10], for the separation of neutral sugars. Durrum resin DA-4 was converted into the borate form and packed into a jacketed column (0.6 × 30 cm) to give a bed height of 25 cm. The temperature was maintained at 55°. The column was equilibrated with 0.4M sodium borate, pH 8.0, at a flow rate of 0.75 $ml.min^{-1}$. For quantitative analysis, the sample (containing the equivalent of approximately 10–100 μg of D-mannose in a volume of 100–200 μl) was eluted with a linear gradient formed from equal volumes (70 ml) of 0.4M sodium borate (pH 8.0) and 0.4M sodium borate (pH 10.0). The effluent was monitored by an autoanalyzer operating at 420 nm, using orcinol–sulfuric acid[10].

Individual AC fractions from Dowex-50 chromatography were further subfractionated in the foregoing system. For these preparative separations, samples of 5–10 mg by weight (2.5–5 mg of mannose equivalents) were fractionated on the same column. Fractions of 1.5 ml were collected and the absorbance at 200 nm was determined by using a Gilford 200 spectrophotometer. Fractions corresponding to separated, sharp peaks were combined, freeze-dried, and desalted on Bio-gel P-2,

400 mesh. The desalted fractions were homogenous by gel chromatography on a column (1.0 × 75 cm) of Bio-gel P-2, 400 mesh.

Fractionation of ovalbumin on Concanavalin A-Sepharose and isolation of the corresponding AC components. — A column of Con A–Sepharose (2.0 × 30 cm) was equilibrated with 0.01M sodium acetate buffer, pH 5.0, containing 0.15M sodium chloride, mM manganese chloride, and mM calcium chloride, at 4°. Ovalbumin (50 mg) was dissolved in 5 ml of buffer and applied to the column. Fractions of 5 ml were collected at a rate of 1 ml per min. Peak I (Oval I) was eluted with approximately 50 ml of equilibration buffer. Peak II (Oval II) was then eluted with 0.1M methyl-α-D-mannoside or 0.1M methyl α-D-glucoside in equilibration buffer (approximately 75 ml). Both fractions were extensively dialyzed against water and lyophilized. For large-scale preparations, a column (5 × 60 cm) was used whereby one g of ovalbumin could be separated into unbound (300–350 mg) and bound (600–650 mg) fractions. Unfractionated AC mixtures from Oval I and Oval II were obtained as described above for native ovalbumin.

Action of α-D-mannosidase on the AC fractions. — Aliquots (10–25 μl containing 30–50 μg of mannose) of standard solutions of the AC fractions in water were incubated with 5 μl of α-D-mannosidase containing 1.5 units. The total volume of the mixture was brought to 50 μl by the addition of 0.05M sodium acetate buffer, pH 4.0. The mixture was incubated for up to 24 h and the free mannose was determined at varying times on the carbohydrate analyzer.

Action of β-D-mannosidase on the AC fractions. — Aliquots (10–25 μl) of standard solutions of the AC fractions were incubated with 25 μl of β-D-mannosidase containing 0.4 units of activity. The total volume was made up to 50 μl with 0.05M glycine–HCl, pH 2.8. The mixture was incubated for up to 24 h and the free mannose was determined at various times on the carbohydrate analyzer.

Action of 2-acetamido-2-deoxy-β-D-hexosidase on the AC fractions. — Aliquots (10–25 μl) of the standard solutions of the AC fractions containing 0.05–0.01 μmol were incubated with 5 μl of 2-acetamido-2-deoxy-β-D-hexosidase containing 18 units of activity. The total volume was brought to 50 μl by the addition of 0.05M sodium acetate buffer, pH 4.0. Aliquots containing 0.005–0.04 μmoles were removed at various times up to 48 h and analyzed for free GlcNAc by the method of Reissig, Strominger, and Leloir[11]. GlcNAc standards were run concurrently with each assay.

Combined action of two enzymes on the AC fractions. — Combined enzyme hydrolyses were carried out as before, with two or more enzymes present, where the additional enzyme volume replaced the buffer.

RESULTS

Anion-exchange separation of glycosides in borate buffer reflects both the availability of vicinal *cis*-hydroxyl groups to form borate complexes and the stability of these and other sugar–borate complexes. Mannopyranosides form a very stable complex with the borate ion, unless the hydroxyl group at C-2 or C-3 is blocked, in

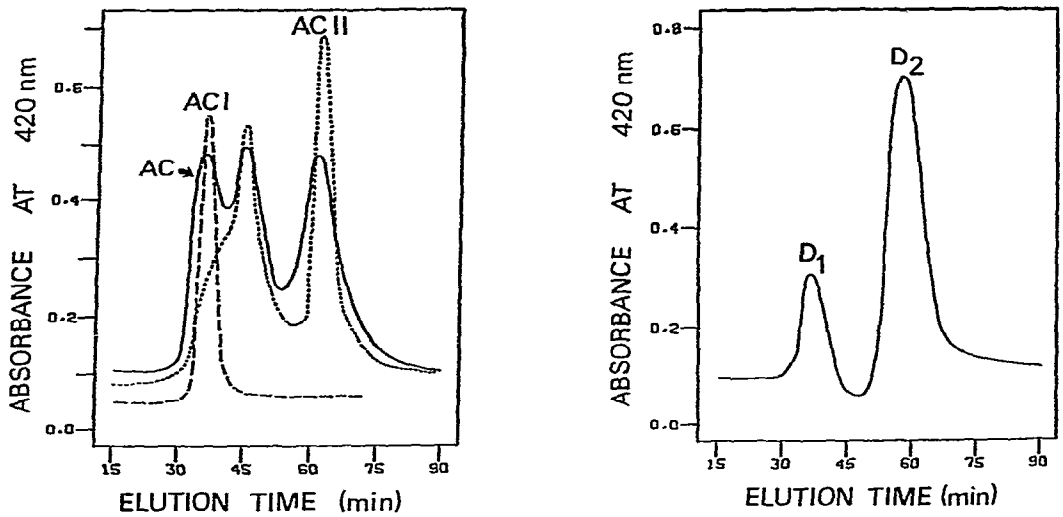


Fig. 1. Fractionation of AC, AC-I, and AC-II on Durrum DA-4 resin in borate buffer. AC, AC-I, and AC-II were each chromatographed on a column (0.6 × 35 cm) of Durrum DA-4 resin in borate buffer, at 55°. The sample was eluted with a linear gradient of 70 ml of 0.4M borate, pH 8.0, to 70 ml of 0.4M borate, pH 10.0. The effluent was monitored by an autoanalyzer at 420 nm using orcinol–sulfuric acid.

Fig. 2. Fractionation of AC-D in borate buffer. AC-D, isolated from Dowex-50 chromatography, was chromatographed on a column (0.6 × 25 cm) of Durrum DA-4 resin in borate buffer. For further experimental conditions, see the legend for Fig. 1.

which event the remaining hydroxyl groups form weaker complexes. In the case of GlcNAc, the C-4 and C-6 positions complex weakly with borate, and a substituent at either of these positions prevents formation of this complex. The separation of a mixture of oligosaccharides should therefore be possible if the stability of the borate complexes with each component differs sufficiently to give various mobilities on an anion-exchange resin. Those oligosaccharides having little or no ability to complex with borate will elute close to the front of the eluting solvent, and the more-stable borate complexes will migrate more slowly. The more-tightly bound complexes may require a change in pH of the eluting borate to weaken the complex enough to effect elution. These variations in eluting conditions were achieved in this study by using a linear gradient of 0.4M sodium borate between pH 8.0 and 10.0. Under these conditions, AC was separated into three heterogeneous fractions (Fig. 1), but when AC-C and AC-D were analyzed separately, each was subfractionated into a faster-migrating peak and a major, slower-moving peak, identified as AC-C_1 and AC-C_2, and AC-D_1 and AC-D_2, respectively. The separation of AC-D_1 and AC-D_2 (Fig. 2) showed the latter fraction to comprise approximately 75% of the AC-D. This is also true of AC-C_2 in its separation from AC-C_1. Under these conditions, AC-C_1 chromatographed slightly more slowly than AC-D_1, but neither of these components was separable from AC-A and AC-B in admixture. AC-D_2 and AC-E also chromatographed together, accounting for the three peaks in the chromatography of AC.

The middle peak is principally AC-C_2. By doubling the length of the anion-exchange borate column, a partial separation of AC-A and AC-B was obtained, with AC-B eluting first.

The order of elution of the AC fractions indicates that AC-D_2 and AC-E form the most-stable borate complexes, and the interaction of AC-B and AC-C_1 with the borate ion is minimal. The major difference between AC-C_1 or AC-B and AC-D_2 as far as borate complexation is concerned is the absence of terminal mannose residues in AC-B and AC-C_1, as reflected by the resistance of these fractions to hydrolysis by α-D-mannosidase (Table III). This result suggests that the terminal GlcNAc and inner mannosyl residues complex only weakly with the borate ion, and that the most-stable complexes involve the *cis*-diol configuration in the terminal mannose residues. Further support for this conclusion derives from the observation that, following exhaustive hydrolysis of the mixture of oligosaccharides in AC with α-D-mannosidase, the unhydrolyzed core-structures were eluted as a single peak in a manner similar to AC-B or AC-C_1 (Fig. 3).

The capacity of the analytical borate column was such that up to 10 mg of AC-C or -D were each separated into their corresponding subfractions, as shown by using absorbance at 200 nm to monitor the column effluent for the elution pattern. Each subfraction was desalted by chromatography on a column of Bio-gel P-2, from which it eluted as a chromatographically homogeneous peak.

The compositions of the new subfractions of AC are summarized in Table II. The fractional values for GlcNAc residues in AC-C_1, AC-C_2, and AC-D_1 suggests that these materials may still be heterogeneous, but in general, it was noted that the

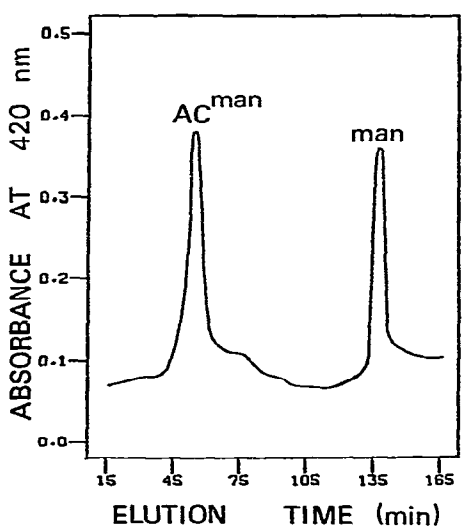


Fig. 3. Fractionation of AC in borate buffer following hydrolysis with α-D-mannosidase. AC was incubated at 37° with α-D-mannosidase–Sepharose for 140 h. An aliquot was removed from the mixture and chromatographed on a column (0.6 × 50 cm) of Durrum DA-4 resin in borate buffer. Other experimental details are given in the legend to Fig. 1.

weighted averages of the corresponding subfractions gave values that were similar to those previously reported for AC-C and AC-D. The principal components in each subfraction are thought to be represented by $AsnGlcNAc_5Man_3$ for AC-C_1, $AsnGlcNAc_4Man_6$ for AC-C_2, $AsnGlcNAc_4Man_4$ for AC-D_1, and $AsnGlcNAc_2Man_6$ for AC-D_2. The most-striking difference between the subfractions is the greater number of GlcNAc residues in AC-C_1 and AC-D_1, which apparently prevent any significant interaction with the borate ion.

Previous studies of the AC fractions had included the extent of their digestion with glycosidases, and it was noted that the rate of hydrolysis by 2-acetamido-2-deoxy-β-D-glucosidase was low[2]. Similar results have been found in digestions of lipid-linked oligosaccharides by glycosidases[20,21]. However, a fractional (0.4 mol per mol of aspartic acid) release of GlcNAc from AC-D is now explained by the isolation of AC-D_1 with peripheral GlcNAc residues[2]. Our earlier studies involved the stepwise addition of enzymes at points when the rate of sugar release decreased. The present studies used a greater initial enzyme–substrate ratio, with a consequent shorter hydrolysis-time, to a point of no further measurable increase in sugar released. The results are summarized in Table III, where it may be seen that AC-E and AC-D_2, having the two hexosamine residues in the core carbohydrate as proposed earlier[23], are resistant to hydrolysis by 2-acetamido-2-deoxy-β-D-glucosidase. Both AC-D_2 and AC-E are hydrolyzed extensively by an α-D-mannosidase, but surprisingly were also partially hydrolyzed by the β-D-mannosidase from *Polyporus sulfureus*.

An application of the new analytical procedure for the asparaginyl carbohydrates follows. It is well known that the binding of carbohydrates to concanavalin A is largely dependent upon the presence of unsubstituted α-D-mannopyranosyl residues[15], with binding also noted in structures where α-D-mannopyranosyl residues are (1 → 2)-linked[16]. By using this property in affinity chromatography, it was found that native ovalbumin could be separated into two fractions (I and II) on Con A–Sepharose[17,18]. One fraction, designated ovalbumin I, was not bound to the Con A,

TABLE I

FRACTIONATION OF AC-FRACTIONS ON BORATE COLUMNS

Fraction	R_m[a]
AC-A	0.54
AC-B	0.49
AC-C	0.49 (AC-C_1)
	0.66 (AC-C_2)
AC-D	0.54 (AC-D_1)
	0.88 (AC-D_2)
AC-E	0.88

[a]The elution times are expressed with respect to D-mannose. The fractionation conditions are described in the Experimental Section.

TABLE II

COMPOSITION OF THE AC-C AND AC-D SUBFRACTIONS

Fraction	*Asp*	*GlcNAc*[a]	*Man*[a]
AC-C_1	1	4.7 ±0.2 (4)	3.1 ±0.2 (2)
AC-C_2	1	3.6 ±0.3 (4)	6.0 ±0.3 (2)
AC-D_1	1	3.7 ±0.3 (4)	4.2 ±0.3 (3)
AC-D_2	1	1.9 ±0.3 (4)	5.9 ±0.2 (3)
AC-C[b]	1	4.1	5.9
AC-D[b]	1	2.2	5.9

[a]The number of analyses are noted in parentheses. [b]From Huang *et al.*[2]

TABLE III

ENZYMIC HYDROLYSES OF SOME AC-SUBFRACTIONS AND DERIVATIVES

Fraction	*% of Sugar hydrolyzed by*		
	α-D-Mannosidase	*β-D-Mannosidase*	*2-acetamido-2-deoxy-β-D-glucosidase*
AC-C_1	0	0	51
AC-C_2	14	0	50
AC-D_1	20	5	23
AC-D_2	72	16	3
AC-C_1^{GlcNAc}	40		
AC-C_2^{GlcNAc}	60		
AC-D_1^{GlcNAc}	51		
AC-E	(75)[a]	15	(0)[a]

[a]The values in parentheses are taken from Huang *et al.*[2].

whereas the second fraction, ovalbumin II, was bound and required 0.1M methyl α-D-glucopyranoside or -mannopyranoside for elution. Each ovalbumin fraction was digested with pronase as described for native ovalbumin, and the corresponding mixtures of asparaginyl carbohydrates, designated AC-I and AC-II, were analyzed on borate columns (Fig. 1). AC-I was eluted as a single peak, corresponding approximately to the first eluted peak from AC that contained principally AC-A, -B, -C_1, and -D_1, whereas AC-II gave two peaks that contained AC-C_2, -D_2, and -E. Digestion of AC with α-D-mannosidase gave a product in which all components behaved like AC-I, that is, either no borate complexes or weak ones were formed. The correlation that exists between the borate complexation of ovalbumin and its AC components is also noted by the observation that the binding of native ovalbumin with Con A is lost following treatment of the ligand with α-D-mannosidase[18], which is the subject of a subsequent publication.

DISCUSSION

Native chicken ovalbumin contains carbohydrate composed nearly exclusively of D-mannose and 2-acetamido-2-deoxy-D-glucose in the molar ratio of 5 to 3 per mol of protein[12]. It is also recognized that there is one oligosaccharide group per polypeptide chain, but a paucidispersity exists in the carbohydrate of samples of ovalbumin[2]. The difficulties in structural studies lie in the separation of pure carbohydrate components, which have usually been in the form of the β-L-aspartamido derivatives[2]. The availability of chicken ovalbumin and a suitable system for studying its biosynthesis has led to a number of investigators to use ovalbumin as a model and occasionally to single out the structure of one component as representative of the glycoprotein carbohydrate[13,14]. It was considered important therefore to study further the fractionation of the oligosaccharides of ovalbumin and to demonstrate the wide spectrum of structural types.

It has been known for several years that the asparaginyl-carbohydrate of ovalbumin could be separated into five components, each of which appeared homogeneous on ion-exchange chromatography, zone electrophoresis, ultracentrifugation, and countercurrent distribution of the 3,5-dinitrophenyl derivatives[3]. From studies of these AC-fractions using the Smith degradation procedure[3] and the action of α-D-mannosidase, it was clear that the carbohydrate groups differed in the number of non-reducing, terminal α-D-mannopyranosyl residues and also those mannose residues having vicinal hydroxyl groups. On the basis of these observations, a study of the chromatography of the AC-borate complexes led to a different fractionation of AC from that using ion-exchange chromatography of the protonated forms[2]. Ion-exchange chromatography of the borate complexes succeeded in separating each of two of the earlier fractions (AC-C and AC-D) into two subfractions having significantly differing compositions (Table II). The four new fractions behaved as chromatographically homogeneous components, as had the earlier five fractions.

It may be noted that, under the conditions studied, the chromatography of AC-A, AC-B, and AC-E on anion–borate columns did not yield further subfractions. This should not lead to the conclusion that these fractions contain single components as it has been shown that AC-B contains approximately 0.5 mol residues of D-galactose, all of which is hydrolyzed by β-D-galactosidase[3]. Also, a fraction from ovalbumin having a composition similar to that of AC-E was separated into three fractions following endoglycosidase cleavage of the β-L-aspartamido-carbohydrate and subsequent reduction[19] of the oligosaccharide with NaB^3H_4.

Most of the borate complexes involved the terminal α-D-mannopyranosyl residues as treatment of AC with α-D-mannosidase resulted in a mixture that moved on the borate column with little binding (Fig. 3). Further support for this hypothesis is found by comparing the elution times of the AC-fractions (Table I) with the extent of hydrolysis of the fractions with α-D-mannosidase (Table II). The slowest migration, R_m 0.49, is seen for AC-B and AC-C_1, which have no terminal D-mannosyl residues. Fractions AC-A and AC-D_1, with approximately one terminal D-mannosyl residue

hydrolyzed, display faster migration (R_m 0.54) on the column. The structures of AC-D_2 and AC-E as proposed by Tai *et al.*[19] show three, non-reducing, terminal α-D-mannopyranosyl residues, an equal extent of enzymic hydrolysis, and the same elution time, R_m 0.88. Fraction AC-C_2 contains one α-D-mannosyl residue that was hydrolyzed by the exo-hydrolase, as was AC-A and AC-D_1; the difference in R_m values is not yet explained, but may be due to complexation with a non-terminal mannosyl residue.

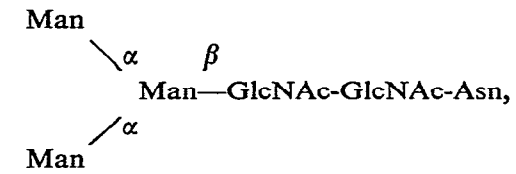
It was surprising to find that AC-D_2 and AC-E were partially hydrolyzed by a β-D-mannosidase from *P. sulfureus*. The possibility that the main portion of the α-D-mannose–oligosaccharide might be attached to one of the GlcNAc residues and not to the β-D-mannosyl residue in the core carbohydrate was excluded by our preliminary methylation studies[22] and by Tai *et al.*[19] who did not identify any disubstituted GlcNAc residues. It is suggested, therefore, that the β-D-mannosidase preparation contained some linkage-specific α-D-mannosidase, such as those described by Bahl and coworkers[24]. Ashwell and associates found endo-glycosidase activity in this enzyme preparation[7], but this would not account for the present results. In many respects, AC-D_2 and AC-E are similar, differing by the presence of an additional α-D-mannosyl residue in AC-D_2. It is not immediately obvious from the structures proposed by Tai *et al.*[19] for oligosaccharides that would correspond to AC-D_2 and AC-E which mannosyl residue is hydrolyzed by the "β-D-mannosidase". The non-reducing terminal linkages in these structures are linked 1 → 2, 1 → 3, and 1 → 6, and it is possible that the unknown mannosidase requires a specific oligosaccharide structure and not only a specific linkage. Such requirement was noted for the endo-2-acetamido-2-deoxy-β-D-glucosidase digestion of AC-D_2-, and -E-like oligosaccharides[19].

A similar linkage-specificity is suggested for the exo-2-acetamido-2-deoxy-β-D-glucosidase hydrolysis of AC-C_1, -C_2, and -D_1. Two of the four GlcNAc residues in AC-C_2 were hydrolyzed by the hexosaminidase, whereas in AC-D_1, only one mol of the expected two mol of GlcNAc was released. Also, in AC-C_1, 2.5 mol of GlcNAc were released instead of 3.0 mol. All but two of the GlcNAc residues in each component of AC are peripheral to the mannosyl oligosaccharide, as concluded from earlier studies in which two GlcNAc residues in the core carbohydrate were found resistant to prolonged hydrolysis with hexosaminidase[2]. Smith degradation of these same fractions caused a similar stepwise removal[3]. The partial hydrolysis of the peripheral GlcNAc residues in AC-C_1 and AC-D_1 may thus be due to a large difference in reactivities of a residue to enzyme hydrolysis or a further unresolved heterogeneity in these subfractions.

It is clear from these data and those of several other investigators that the action of glycosidases frequently results in the incomplete release of sugars[21,25–27], which adds to additional heterogeneity in the products from these hydrolyses. The problem is exacerbated when sequential glycosidase degradations are contemplated, and it is a matter of concern in studies of glycosyltransferases[25]. The stepwise degradation of the AC-subfractions (Table III) illustrates this point, where AC-C_1, after incomplete removal of the peripheral GlcNAc residues, afforded a product

AC-C_1^{GlcNAc} from which 40% instead of an expected 66% of mannose was hydrolyzed by α-D-mannosidase. Similarly, AC-D_1^{GlcNAc} released 51% instead of 75% of the mannose present.

The presence of three and four mannose residues in AC-C_1 and -D_1, respectively, is of importance when considering the intermediates in the biosynthesis of asparagine-linked oligosaccharides. Struck and Lennarz have shown that lipid-linked sugars participate in the assembly of the ovalbumin carbohydrate[28]. The core oligosaccharide they propose has the structure: (α-Man)$_n$-β-Man-(1 → 4)-β-GlcNAc(1 → 4)-GlcNAc-polypeptide, where the value[29,30] for n can be ⩾ 0. The AC fractions -C_1 and -D_1 contain only 3 and 4 mannose residues, respectively. Therefore, the largest structure common to all the AC fractions would be:



with GlcNAc or α-D-mannose residues being added to the two terminal, non-reducing mannose to give the larger oligosaccharides. This structure is common to several glycopeptides[20,31] studied recently, and might well represent the largest common unit transferred via the lipid-linked oligosaccharide system.

Two possibilities then exist for introduction of heterogeneity of the carbohydrate moieties as found in ovalbumin: (*i*) through glycosyltransferase addition to an oligosaccharide–polypeptide core produced via the lipid system, or (*ii*) through attachment of various lengths of (mannose)$_n$ core-lipid units to polypeptide acceptors, followed by specific glycosyltransferase addition. For example, AC-C_2 and -D_2 might have the same (Man)$_5$-Man-GlcNAc-GlcNAc-Asn core from lipid–oligosaccharide transfer, followed by 2-acetamido-2-deoxy-β-D-glucosyl transferase addition of two GlcNAc residues to the terminal non-reducing mannose residues to afford -C_2. Similarly, AC-D_1 and AC-C_1 could have the same, lipid-originated (Man)$_2$-Man β-GlcNAc-GlcNAc-Asn core, with one mannosyl residue and 2 GlcNAc residues added via transferases to give -D_1 and 3 GlcNAc residues added to produce -C_1. Mannosyl transferases have recently been described in a particulate fraction from calf thyroid[32], and 2-acetamido-2-deoxy-β-D-glucosyl transferases have been studied in Chinese hamster ovary cells[25], which will effect transfer of sugar residues to specific, exogenous acceptors.

The use of chicken ovalbumin as a model glycoprotein continues to stimulate structural and biosynthetic studies. Both need the preparation of pure glycopeptides, which will also serve as substrates for the delineation of the specificities of glycosidases and glycosyl transferases. The fractionation of oligosaccharides as their borate complexes is one additional tool, to which may be added those analytical techniques involving other complexes, such as the lectin-affinity columns. Such studies will be the subject of another paper.

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