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SPECIFIC CONVERSION OF D-GALACTOSE INTO D-GALACTURONIC ACID RESIDUES IN GLYCOPROTEINS A FACILE METHOD FOR CARBOHYDRATE LINKAGE-ANALYSIS*

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ABSTRACT

The terminal D-galactopyranosyl residues of asialoglycopeptides isolated from human α_1-acid glycoprotein were oxidized in nearly quantitative yield to the corresponding uronic acid residues by a two-step sequence employing D-galactose oxidase followed by treatment with Tollens reagent, $Ag(NH_3)_2^+$ Mild acid hydrolysis of the oxidized glycopeptides led to the isolation of the corresponding aldobiuronic acid(s) Structural and colorimetric analysis revealed that only one aldobiuronic acid, 2-amino-2-deoxy-4-*O*-(β-D-galactopyranosyluronic acid)-D-glucose, was isolated from the oxidized glycopeptides of α_1-acid glycoprotein Thus method can readily distinguish between the (1→3), (1→4), and (1→6) isomers of the corresponding aldobiuronic acids

INTRODUCTION

A frequently occurring structural unit in serum glycoproteins is a heteropolysaccharide consisting of oligosaccharide chains with the sequence sialic acid (or L-fucose)→D-galactose→2-acetamido-2-deoxy-D-glucose, linked to a core region of D-mannose and 2-acetamido-2-deoxy-D-glucose residues[1] However, for a large number of glycoproteins, the linkage(s) of the penultimate D-galactose to the adjoining 2-acetamido-2-deoxy-D-glucose residue has not been unambiguously established

For example, the well-studied human α_1-acid glycoprotein was reported to contain some β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucose linkages[2], although the β-D-(1→4)-linkages appear to be the major type of linkage[3] Similarly for human transferrin, the major linkage between the D-galactose and 2-acetamido-2-

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deoxy-D-glucose residues seems to be[4,5] β-D-(1→4), but the occurrence of other linkages between these two sugar residues has not been rigorously excluded

We wish to present a simple method, applicable to a variety of glycoproteins containing the sequence D-galactose→2-acetamido-2-deoxy-D-glucose, to establish the linkage between the two sugar residues as either (1→3), (1→4), or (1→6), or a mixture of these linkages This method is based on the well known observation that the presence of a D-glucuronic or D-galacturonic acid residue stabilizes the glycosidic linkage to the adjoining sugar residue[6,7]

The terminal sialic acid (or L-fucose) residue of a glycopeptide was removed by mild acid hydrolysis which resulted in the exposure of the penultimate D-galactose residue as the nonreducing end of the oligosaccharide chain Selective oxidation of this D-galactose to a D-galacturonic acid residue was carried out as a two-step process, and was followed by acid hydrolysis The conditions of acid hydrolysis were chosen in such a way as to yield disaccharide(s) consisting of a D-galacturonic acid residue linked to the neighboring sugar residue Unambiguous identification of the isolated disaccharide(s) established the type of linkage between the D-galactose and 2-acetamido-2-deoxy-D-glucose residues in the glycopeptide

EXPERIMENTAL

Materials — Human α_1-acid glycoprotein was provided by the American Red Cross Fractionation Center with the partial support of NIH Grant No HL 13881 Purified β-D-galactosidase (EC 3 2 1 23) from *Diplococcus pneumoniae* was kindly provided by Dr Gilbert Ashwell National Institutes of Health Authentic samples of 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucose and of 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose were generous gifts of Dr Elvin A Kabat Columbia University D-Galactose oxidase (D-galactose oxygen 6-oxidoreductase EC 1 1 3 9) from *Polyporus circinatus* was obtained from Sigma Chemical Co , St Louis, MO 63178 and Worthington Biochemical Corp Freehold NJ 07728 and was used directly or after partial purification by DEAE-cellulose chromatography and ammonium sulfate precipitation[8] Beef liver catalase (EC 1 11 1 6) was obtained from Worthington Biochemical Corp and β-D-galactose dehydrogenase (D-galactose NAD^+ 1-oxidoreductase, EC 1 1 1 48) from Sigma Chemical Co

Preparation of asialoglycopeptides — Glycopeptides of human α_1-acid glycoprotein were prepared by the method of Jamieson[9], from 250 mg of glycoprotein (~20 μmol), 15 2 μmol of glycopeptides were isolated representing a 77% yield (based on sialic acid determination[10], and assuming 4 sialic acid residues per mol of glycopeptide) Removal of terminal sialic acid and L-fucose residues was accomplished by treatment with 0 05M H_2SO_4 for 1 h at 80° The amount of sialic acid removed was determined by the method of Warren[10], as well as by determination of the equivalent amount of D-galactose residues exposed D-Galactose was quantitatively determined by treatment with β-D-galactosidase, followed by measurement of free D-galactose with β-D-galactose dehydrogenase

The quantitative determination of D-galactose residues exposed was used to measure the amount of glycopeptide present, assuming four D-galactose residues per glycopeptide[11] Quantitative determination of glycopeptides by this method agreed well with the asparagine content of the samples[12]

Oxidation of glycopeptides — Asialoglycopeptides (1 0–6 5 μmol terminal D-galactose residues) were incubated in 0 5 ml of 20mM KH_2PO_4 (pH 7 0) containing 1800 units of catalase and 15–100 units of D-galactose oxidase for 15–24 h at room temperature Formation of the 6-aldehydo-D-galactoside was measured by determination of the reducing-sugar content according to Nelson[13] with 2-deoxy-D-*lyxo*-hexose as a standard and a boiling time of 80 min[8] Tollens reagent was prepared by mixing equal volumes of 10% (w/v) aqueous $AgNO_3$ and 10% (w/v) aqueous NaOH and then adding dilute ammonia dropwise until the precipitate was just dissolved[14] The freshly prepared Tollens reagent (1 5 ml) was added directly to the glycopeptide incubation-mixture, and the solution was kept at room temperature for ~8 h A silver mirror or blackening of the solution resulted The solution was then centrifuged to remove any particulate matter, and excess Ag^+ was removed by dropwise addition of M HCl After centrifugation the supernatant solution was collected, concentrated to a small volume (0 4–0 5 ml) and deposited onto a Sephadex G-15 column (1 1 × 52 cm) The column was eluted with water and the phenol–H_2SO_4-positive material was pooled, concentrated and found to be free of salts The quantity of D-galacturonic acid formed was measured by the carbazole–H_2SO_4 reaction[15] D-galacturonic acid being the standard The amount of terminal D-galactose remaining was measured by treatment with β-D-galactosidase, followed by quantitative determination of D-galactose released by β-D-galactose dehydrogenase

Isolation of aldobiuronic acids — The D-galactosyluronoglycopeptides (usually ~2 5 μmol) were hydrolyzed *in vacuo* in a sealed tube for 2 h in 0 25M HCl at 100° these conditions were the best to retain a maximal yield of aldobiuronic acids and to N-deacetylate the acetamido group of the 2-acetamido-2-deoxyhexose residues The solution was evaporated to dryness after several additions of water to remove HCl and then placed onto a column (0 5 × 9 cm) of Dowex AG-50 (H^+) cation-exchange resin All neutral components as well as free D-galacturonic acid were eluted with water (6 ml) The disaccharide, as well as other compounds having amino groups, was eluted with 0 5M HCl (5 ml) The sample was taken to dryness and stored overnight in a desiccator over NaOH The residue was dissolved in a small volume of water, the pH adjusted to 8 with ammonia and then the solution deposited onto a column (0 5 × 9 cm) of Dowex 1 (Cl^-) anion-exchange resin After a water wash to elute free 2-amino-2-deoxy-D-glucose (6 ml), the disaccharide was eluted with 0 05M HCl (5 ml)

Characterization of the disaccharide component derived from α_1-acid glycopeptide – The purified disaccharide obtained from the α_1-acid glycopeptide was subjected to high-voltage electrophoresis in 0 05M citrate buffer (pH 4 0) for 30 min at 4000 V, and localized by treatment with $AgNO_3$ reagent[16] In addition, a sample was hydrolyzed with 3M HCl in an evacuated sealed tube for 3 h at 100° The uronic acid

and hexosamine contents of the hydrolyzate were determined by the Dische[15] and the Morgan–Elson[17] method, respectively The hydrolyzate was subjected to descending paper chromatography in 6 4 3 (v/v) 1-butanol–pyridine–water and 10 3 7 (v/v) 1-butanol–acetic acid–water Another disaccharide sample was reduced with $NaBH_4$ prior to acid hydrolysis. and again the uronic acid and hexosamine contents were quantitatively determined

Preparation of disaccharide standards — 2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, the corresponding (1→3)-linked disaccharide, and 2-acetamido-2-deoxy-D-glucose were reduced by treatment with a 10-fold excess of sodium borotritide (Schwarz/Mann Div , Becton, Dickinson and Co , Orangeburg, NY 10962 5 Ci/mmol) in dilute $NaHCO_3$ All three samples were processed at the same time, using three individual aliquots of the same solution of $NaBT_4$ After keeping the samples for 2 h at room temperature, the excess of $NaBT_4$ was destroyed by addition of a 50% suspension of washed Dowex AG-50 (H^+) cation-exchange resin in water The resin was removed by centrifugation and washed several times with water The sample containing 2-amino-2-deoxy-D-glucitol was eluted with 0 5M HCl from the Dowex resin All supernatants were combined and evaporated to dryness Boric acid was removed by repeated additions and evaporations of methanol The quantity of 2-amino-2-deoxy-D-glucitol present was determined with ninhydrin[18], 2-amino-2-deoxy-D-glucose being the standard The specific activity of the reduced disaccharides, based on 2-amino-2-deoxy-D-[1-^{3}H]glucitol, was determined to be $3\,6 \times 10^7$ d p m /μmol

Oxidation of the D-galactose residue of the reduced disaccharides was performed in the same way as described for the glycopeptides The resulting aldobiuronic acids were then desalted by passage through a Bio-Gel P-2 column (1 × 76 cm) Final purification of the disaccharides was accomplished by depositing the solution onto a column (0 5 × 8 cm) of Dowex 1 (Cl^-) anion-exchange resin and eluting with 0 05M HCl

Periodate (Smith) degradation — The disaccharides derived from α_1-acid glycopeptides were reduced with $NaBT_4$ as just described In addition, the disaccharides were *N*-acetylated by treatment with saturated $NaHCO_3$ (0 1 vol) and 2% acetic anhydride in acetone (0 1 ml) After 30 min at room temperature, the sample was passed through a column (0 5 × 8 cm) of Dowex AG-50 (H^+) cation-exchange resin, and the effluent was collected and concentrated to dryness The residue was dissolved in 40mM sodium acetate buffer (pH 5 0) and oxidized by addition of a 10-fold molar excess of $NaIO_4$ The reaction was allowed to proceed for 8 h at 4° in the dark, and was terminated by addition of an 8-fold molar excess of 1,2-ethanediol The sample was then reduced by the addition of a 30-fold excess of $NaBH_4$ over the $NaIO_4$ used, and the reaction mixture was kept for an additional 20 h at 4° The reduced sample was acidified with acetic acid, passed through a column (0 7 × 10 cm) of Dowex AG-50 (H^+) cation-exchange resin, and the effluent and water wash were combined (25 ml) and evaporated to dryness under diminished pressure After removal of boric acid by repeated additions and evaporations of methanol, the

residue was dissolved in 4M HCl (0 6 ml) and hydrolyzed in an evacuated, sealed tube for 4 h at 100° The samples were then lyophilized, the residue dissolved in water, and *N*-acetylated as just described As measured by radioactivity, ~40% of the original radioactivity was recovered after the final step of the Smith degradation

Authentic 2-acetamido-2-deoxy-L-threitol, 2-acetamido-2-deoxy-D-xylitol, and 2-acetamido-2-deoxyglycerol were prepared by performing the Smith degradation just described on 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyluronic acid)-D-[1-^{3}H]glucitol, 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyluronic acid)-D-[1-^{3}H]-glucitol, and 2-acetamido-2-deoxy-D-[1-^{3}H]glucitol, respectively

Products of the Smith degradation were subjected to descending paper chromatography in 4 1 5 (v/v) 1-butanol–ethanol–water, organic phase, and radioactive areas were located by scanning with a Packard 7201 Radiochromatogram Scanner Radioactive spots were eluted and subjected to further analysis

Preparation of O-acetylated Smith-degradation products — A portion of the treated products from the Smith-degradation procedure obtained after paper chromatography were evaporated to dryness dissolved in anhydrous pyridine and the liquid removed by evaporation The residue was dissolved in 2 3 (v/v) anhydrous pyridine–acetic anhydride (0 5 ml), and the solution heated in a boiling-water bath for 40 min The reaction mixture was cooled, a small quantity of crushed ice added, and the aqueous mixture was extracted three times with 1 ml of chloroform The organic phase was re-extracted with water, followed by saturated $NaHCO_3$ and water The organic phase was dried ($MgSO_4$) and centrifuged after a period of several h The chloroform was evaporated and the residue taken up in anhydrous ethyl acetate Tritiated products were identified by t l c on precoated EM silica gel F-254 plates in 9 1 (v/v) chloroform–1-butanol

In experiments involving *O*-acetylation with [1-^{14}C]acetic anhydride (9 3 Ci/mol, New England Nuclear, Boston, MA 02118) a procedure identical to that just described was used with the exception that only a 20-fold molar excess of [1-^{14}C]-acetic anhydride was employed After developing the thin-layer chromatogram, the area corresponding to the *O*-acetylated derivative was scraped off and eluted with chloroform The chloroform was evaporated, the residue dissolved in anhydrous ethyl acetate, and the radioactivity determined for both ^{3}H and ^{14}C To determine the specific activity of the [1-^{14}C]acetic anhydride employed, glycerol was *O*-acetylated concomitantly with the samples After t l c in 7 3 (v/v) benzene–ethyl acetate, the radioactive area containing tri-*O*-acetylglycerol was eluted with chloroform and deacetylated with sodium methoxide, and the glycerol formed quantitatively determined by periodate degradation, followed by formic acid determination with chromotropic acid[19]

RESULTS AND DISCUSSION

The specificity of the present method, to convert only terminal D-galactose to D-galacturonic acid residues, is based on the initiation of the oxidation process by

D-galactose oxidase, which leads to a 6-aldehydo-D-galactopyranoside intermediate The 6-aldehydo derivative is then almost quantitatively converted into D-galacturonic acid with Tollens reagent, a relatively mild oxidizing agent Table I shows typical results obtained by oxidation of terminal D-galactose residues when asialo-glyco-

TABLE I

OXIDATION BY D-GALACTOSE OXIDASE AND TOLLENS REAGENT OF GLYCOPEPTIDES OBTAINED FROM α_1-ACID GLYCOPROTEIN[a]

Stage	*Residues of*		
	Terminal Gal	*GalUA*	*Gal+GalUA*
Before oxidation	2 88 (100)	0 (0)	2 88 (100)
After oxidation	0 20 (7)	2 40 (83)	2 60 (90)

[a]Molar content in percent in parentheses

peptides obtained from human α_1-acid glycoprotein were subjected to this procedure, and emphasizes the high yields (80–95%) obtainable For human α_1-acid glycopeptide, the sequence of individual sugars is well established and only four penultimate D-galactose residues are present per glycopeptide[2 3] Prior to the application of this method to other glycoproteins a qualitative and quantitative analysis of the content for D-galactose and 2-acetamido-2-deoxy-D-galactose is required Both sugars are substrates for D-galactose oxidase, and the enzyme is capable of acting on non-terminal residues as well As the extent and the kinetics of the oxidation of these sugar residues is not readily predictable[20], possible complications can arise with polymers containing a number of internal D-galactosyl or 2-acetamido-2-deoxy-D-galactosyl residues

Following oxidation, the isolation of aldobiuronic acids from the α_1-acid glycopeptide was facilitated by the change of a D-galactopyranosyl into a D-galacto-pyranosyluronic acid residue, which is known to stabilize the adjoining glycosidic linkage[6 7] Acid hydrolysis conditions were selected (0 25M HCl for 2 h at 100°) to obtain the maximum yield of aldobiuronic acids These hydrolysis conditions are sufficiently strong to cleave quantitatively the *N*-acetyl group of 2-acetamido-2-deoxyhexose residues In many glycoproteins, the sugar residue vicinal to a D-galactose residue is 2-acetamido-2-deoxy-D-glucose Therefore, oxidation by D-galactose oxidase–Tollens reagent followed by acid hydrolysis should result in the formation of 2-amino-2-deoxy-(D-galactopyranosyluronic acid)-D-glucose Isolation of the expected aldobiuronic acid derivatives was based on the functional groups both amino group and carboxyl group on the same molecule permitted retention on both cation- and anion-exchange resins, while upon high-voltage electrophoresis, the disaccharide behaved like an amphoteric compound

Identification of the disaccharide included hydrolysis with 3M HCl, followed by paper chromatography in two solvent systems, which revealed only two components

having R_I values identical with those of authentic D-galacturonic acid and D-glucosamine No mannose was found In addition, analysis of the 3M HCl hydrolyzate showed that it contained equimolar amounts of hexosamine and uronic acid, but no mannose Reduction with $NaBH_4$ prior to 3M HCl hydrolysis resulted in complete removal of the hexosamine component, while the uronic acid component was recovered without loss (Table II) Although it is known that *N*-deacetylation of 2-acetamido-2-deoxy-D-glucopyranoside residues prior to scission of the glycosidic

TABLE II

PARTIAL ANALYSIS OF DISACCHARIDE OBTAINED FROM α_1-ACID GLYCOPEPTIDES OXIDIZED WITH D-GALACTOSE OXIDASE AND TOLLENS REAGENT[a]

Treatment	*Uronic acid*	*Hexosamine*
3M HCl hydrolysis	0 23	0 24
$NaBH_4$ reduction prior to 3M HCl hydrolysis	0 19	0

[a]Molar content

bond results in a 2-amino-2-deoxy-D-glucosyl linkage more resistant to hydrolysis[21] apparently no such stabilization occurred under our conditions since no compound having a mol wt larger than that of a disaccharide was isolated 2-Amino-2-deoxy-(D-galactopyranosyluronic acid)-D-glucose was isolated in good yield as shown in Table III The occurrence of free D-galacturonic acid (19%), as indicated in this Table, is an expected degradation product of the hydrolysis, and comparable amounts were reported previously[22] It is important to note that hydrolysis of standard 2-amino-2-deoxy-3-*O*-(β-D-galactopyranosyluronic acid)-D-glucose showed no difference in the rate of hydrolysis This excludes the possibility that acid hydrolysis will cause preferential cleavage of a specific linkage

TABLE III

YIELD OF PRODUCTS AS D-GALACTURONIC ACID AFTER MILD ACID HYDROLYSIS OF OXIDIZED α_1-ACID GLYCOPEPTIDES

Sample	*Yield of D-galacturonic acid*	
	Molar proportion	%
Glycopeptide prior to acid hydrolysis	2 40	100
After 0 25M HCl hydrolysis and passage through cation- and anion-exchange columns		
Free D-galacturonic acid	0 45	19
D-Galacturonic acid as component of disaccharide	1 85	77
Total D-galacturonic acid	2 30	96

The aldobiuronic acid(s) isolated from α_1-acid glycopeptide was analyzed by colorimetry by the Morgan–Elson method[17] as *N*-acetylated derivative(s) Failure of

the aldobiuronic acid to yield a positive Morgan–Elson reaction (Table IV) suggests that the D-galactosyluronic acid residue was linked to OH-4 of the reducing 2-amino-2-deoxy-D-glucose residue This interpretation was possible because the color yield of various *O*-substituted 2-acetamido-2-deoxyhexoses in the Morgan–Elson reaction is indicative of the position of the hydroxyl group substitution[23] For example, the 3-*O*-, 4-*O*-, and 6-*O*-methyl derivatives of 2-acetamido-2-deoxy-D-glucose yield color intensities equal to 160, 3, and 100%, respectively, of that given by an equimolar amount of 2-acetamido-2-deoxy-D-glucose[24]

TABLE IV

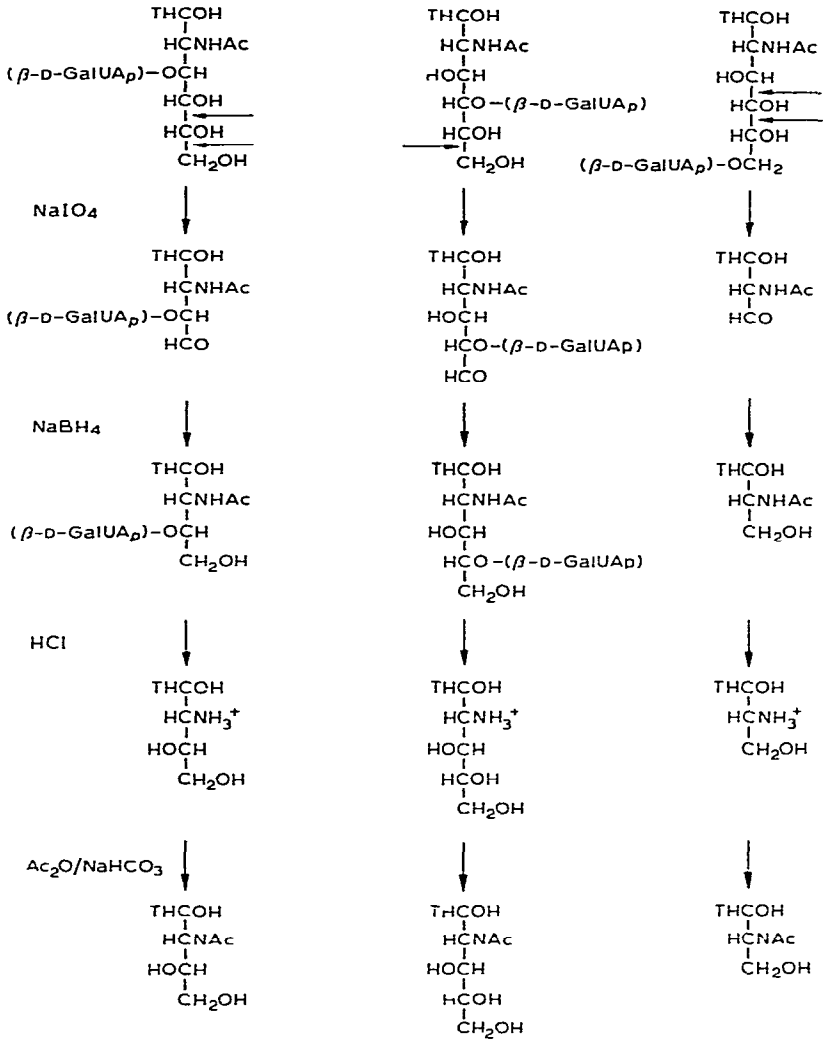
COLOR VALUE[a] OF THE MORGAN–ELSON REACTION APPLIED TO THE ALDOBIURONIC ACID ISOLATED FROM α_1-ACID GLYCOPEPTIDES

Compound	*Color values*[a]	
	Expected	*Found*
Standards		
2-Acetamido-2-deoxy-D-glucose	100	100
β-D-GalUA-(1→3)-GlcNAc	160	130
β-D-GalUA-(1→4)-GlcNAc	<3	2 4
Isolated disaccharide		<2[b]

[a]Expressed in % of the chromogen formed by an equivalent amount of 2-acetamido-2-deoxy-D-glucose [b]Sufficient material (0 24 μmol) was assayed that 5% of the (1→3) isomer could be detected

Further confirmation of the result of the colorimetric linkage-analysis was obtaining by a Smith degradation of the disaccharide and characterization of the degradation products by several methods (Scheme 1) To facilitate this analysis, the aldobiuronic acid was first *N*-acetylated and the product reduced with $NaBT_4$ Subsequently, this compound and standards of reduced disaccharides were subjected to the Smith degradation The tritiated fragments isolated after the degradation were subjected to descending paper chromatography Fig 1 shows the radiochromatogram scans, which contained enough radioactive sample such that 10% of contamination could have easily been noticed As is readily apparent, only one radioactive peak was obtained from the disaccharide derived from the α_1-acid glycoprotein, and this peak migrated exactly like authentic 2-acetamido-2-deoxy-D-[1-^{3}H]xylitol, the compound which was also obtained from the Smith degradation of the β-D-(1→4)-linked standard

Additional experimental evidence for the identity of the radioactive Smith-degradation fragments was obtained by *O*-acetylation with acetic anhydride Separation of the *O*-acetyl derivatives by t l c on silica gel is shown in Fig 2 Only one radioactive component was found for the sample derived from the α_1-acid glycoprotein. and its migration was identical with that of authentic, fully acetylated 2-amino-2-deoxy-D-xylitol The 2-amino-2-deoxy-D-xylitol fragment derived from α_1-acid glycoprotein was further characterized by *O*-acetylating another aliquot with



Scheme I

TABLE V

RATIO OF ^{14}C TO ^{3}H IN THE FULLY ACETYLATED COMPOUND RESULTING FROM THE SMITH DEGRADATION OF THE ALDOBIURONIC ACID DERIVED FROM α_1-ACID GLYCOPEPTIDE

Compound	*Ratio ^{14}C to ^{3}H*	
	Expected	*Found*
Standards		
2-Acetamido-2-deoxy-L-threitol	3 0	3 12
2-Acetamido-2-deoxy-D-xylitol	4 0	4 19
Compound derived from α_1-acid glycopeptide		4 26

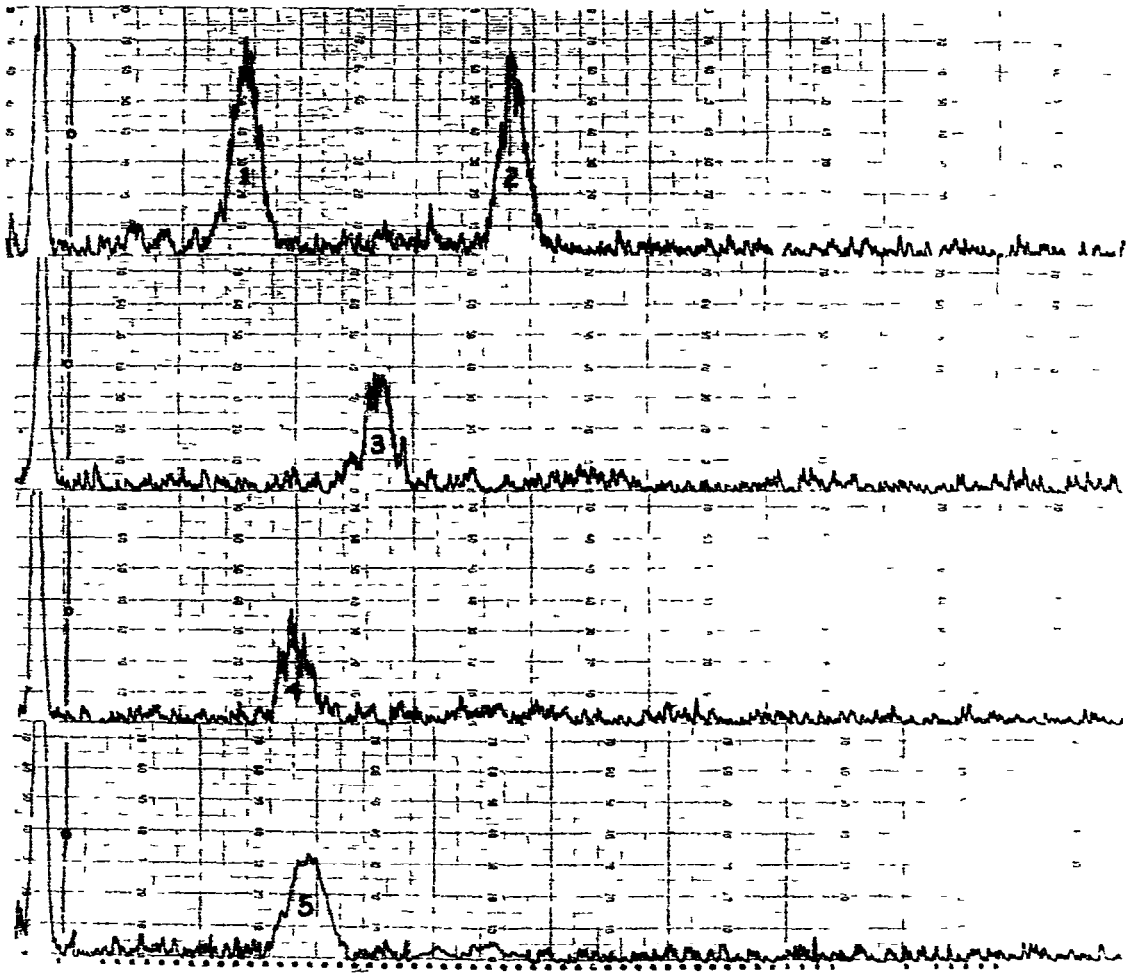


Fig. 1. Descending paper chromatography of tritiated fragments isolated from Smith degradation of the reduced aldobiuronic acid isolated from α_1-acid glycopeptide. The paper was developed in 4:1:5 (v/v/v organic phase) 1-butanol:ethanol:water for 20 h. The radioactive peaks were located with a Packard Model 7201 Radiochromatogram Scanner. (1) 2-Acetamido-2-deoxy-D-glucitol; (2) 2-acetamido-2-deoxy-glycerol; (3) 2-acetamido-2-deoxy-L-threitol; (4) 2-acetamido-2-deoxy-D-xylitol; and (5) Smith degradation product isolated from α_1-acid glycopeptide, as described in the text.

[^{14}C]acetic anhydride under conditions where the unlabeled acetyl group of the acetamido group would not be exchanged. The ^{14}C- and ^{3}H-labeled product was then isolated by t.l.c., the molar proportion of the two radioisotopes was determined, and the results are shown in Table V.

The sole compound isolated from human asialo-α_1-acid glycopeptide by the present degradation technique was 2-amino-2-deoxy-4-*O*-(*β*-D-galactopyranosyluronic acid)-D-glucose. This establishes the linkage between the terminal (formerly penultimate) D-galactose and the vicinal 2-acetamido-2-deoxy-D-glucose residues in

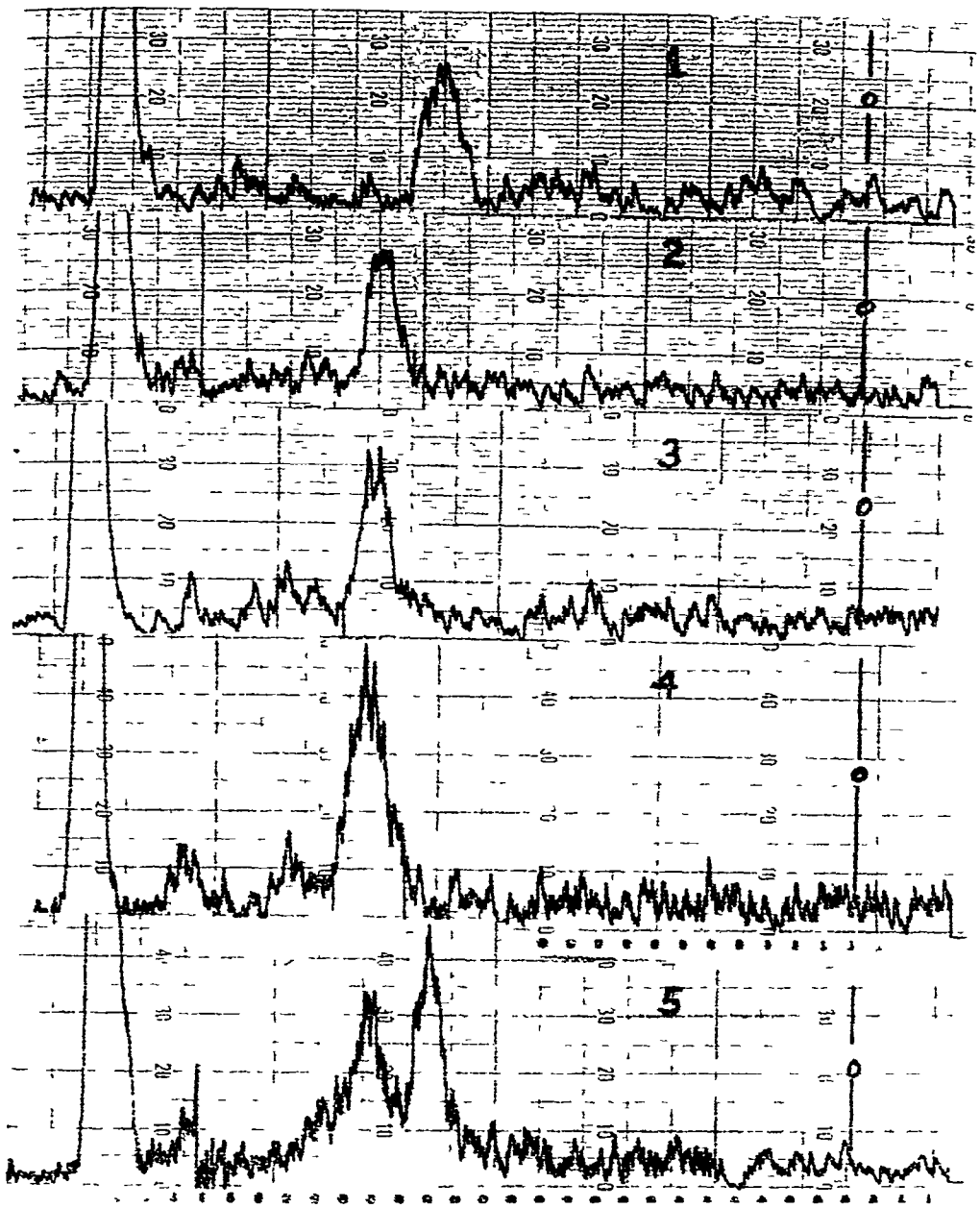


Fig. 2. Electrophoresis on silica gel of the fully acetylated fragments resulting from the Smith degradation as described in the text. The silica plates were developed three times with 9:1 (v/v) chloroform–butanol and scanned for radioactivity. (1) 2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-L-threitol. (2) 2-Acetamido-1,4,5-tetra-*O*-acetyl-2-deoxy-D-xylitol. (3) fully acetylated Smith degradation product isolated from acid glycopeptide. (4) mixture of fully acetylated Z_1-acid glycopeptide fragment and 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2-deoxy-D-xylitol. and (5) mixture of fully acetylated Z_1-acid glycopeptide fragment and 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-L-threitol.

the native glycoprotein to be (1→4) The oxidation of the terminal D-galactose to D-galacturonic acid residue by the sequence D-galactose oxidase and Tollens reagent, as described in this paper, should be applicable to various asialoglycoproteins Isolation of the corresponding aldobiuronic acid by mild acid hydrolysis should permit the rapid and unambiguous elucidation of the linkage(s) between the D-galactose and the adjoining carbohydrate residue present in the native glycoprotein Preliminary data indicate that this oxidation procedure may also be carried out on the intact asialoglycoprotein This leads to an asialoglycoprotein with terminal D-galacturonic acid residues It will be interesting to test these specifically modified glycopeptides and glycoproteins for their ability to be recognized by the hepatic-binding protein for asialoglycoproteins in the systems described by Ashwell and Morell[25]

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