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Note

Studies of the reactivity of methyl glycosides, oligosaccharides, and polysaccharides towards trifluoroacetolysis

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In studies of methods for the isolation of the carbohydrate chains of glycoproteins, we have investigated the possibility of stabilizing the glycosidic bonds in order to cleave the peptide bonds under acid conditions. We now demonstrate how glycosidic bonds are stabilized under trifluoroacetolysis conditions that will cleave amide linkages[1] (peptide bonds[2]).

Methyl glycosides, oligosaccharides, and polysaccharides were treated with mixtures of trifluoroacetic anhydride (TFAA) and trifluoroacetic acid (TFA) at 100° for 48 h. The proportions of TFAA and TFA were varied from 1:1 to 50:1, under which conditions methyl 2-acetamido-2-deoxy-α-D-glucopyranoside is quantitatively converted into methyl 2-deoxy-2-trifluoroacetamido-3,4,6-tri-*O*-trifluoroacetyl-α-D-glucopyranoside[1]. Each compound was subsequently *O*-detrifluoroacetylated, and characterized and quantitated by g.l.c.–m.s. using the appropriate derivatives.

TABLE I

TREATMENT OF GLYCOSIDES WITH TFAA/TFA AT 100° FOR 48 h

Compound	*Recovery (%)*[a]	
	1:1 TFAA/TFA	*50:1 TFAA/TFA*
Me-α-D-Glc*p*	>98	>98
Me-β-D-Glc*p*	>98	>98
Me-α-D-Man*p*	>98	>98
Me-α-D-Gal*p*	>98	>98
Me-β-D-Gal*p*	>98	>98
Me-α-D-Xyl*p*	90	95
Me-α-L-Fuc*p*	65	95
Me-β-L-Fuc*f*	70	97

[a]Determined by g.l.c. after *O*-detrifluoroacetylation, reduction, and acetylation.

TABLE II

TREATMENT OF OLIGOSACCHARIDES WITH TFAA/TFA AT 100° FOR 48 h

Compound	*Recovery (%)*[a]	
	1:1 TFAA/TFA	*50:1 TFAA/TFA*
β-D-Glc*p*-(1→4)-D-Glc	>98	>98
α-D-Glc*p*-(1→6)-D-Glc	>98	>98
β-D-Glc*p*-(1→6)-D-Glc	>98	>98
β-D-Gal*p*-(1→4)-D-Glc	>98	>98
α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc	>98	>98
α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc	>98	>98
α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-D-Gal	43	>98

[a]Determined by g.l.c.–m.s. after *O*-detrifluoroacetylation, reduction, and permethylation.

The methyl glycosides tested were all stable in 50:1 TFAA/TFA, being converted into the corresponding pertrifluoroacetates, and could be quantitatively recovered after *O*-detrifluoroacetylation (Table I). In 1:1 TFAA/TFA, the more acid-labile 6-deoxyhexosides and pentoside were partially hydrolyzed (Table I).

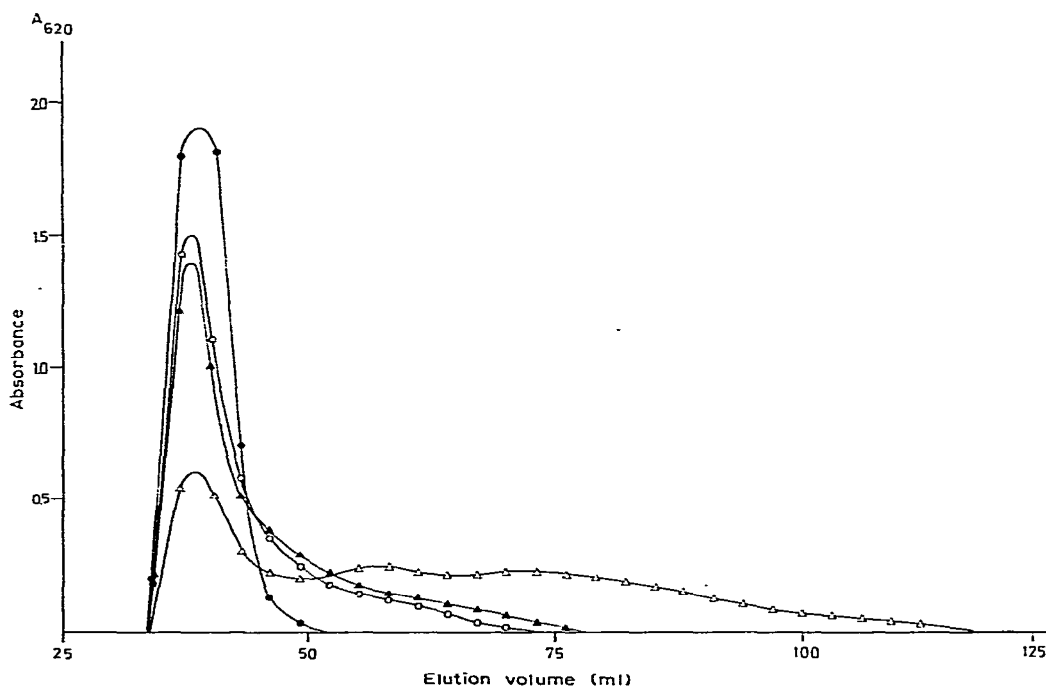

Fig. 1. Gel chromatography [on a column (1.5 × 55 cm) of Sephadex G-200] of dextran (mol. wt. 158 000) trifluoroacetylated under various conditions; elution with water at 5 ml/h: —●—, original dextran; —△—, treated with 1:1 TFAA/TFA; —▲—, treated with 20:1 TFAA/TFA; —○—, treated with 50:1 TFAA/TFA. Fractions were assayed with the anthrone reagent[7].

The oligosaccharides were treated with TFAA/TFA, and analyzed by g.l.c.–m.s. as their permethylated derivatives after *O*-detrifluoroacetylation and reduction. The results are shown in Table II.

On treatment with TFAA/TFA, the polysaccharides (dextran, guaran, and glycogen) dissolved and were converted into their trifluoroacetates. The trifluoroacetylated polysaccharides were detrifluoroacetylated with aqueous, methanolic ammonia, and recovered from the reaction mixture by gel chromatography on Sephadex G-25. The polysaccharides were then analyzed by sugar and methylation analysis. Since some depolymerisation of the polysaccharides could be expected, dextran (mol. wt. 158 000) was trifluoroacetylated in mixtures of TFAA/TFA varying from 1:1 to 50:1, *O*-detrifluoroacetylated, and subjected to gel chromatography on Sephadex G-200. The results are shown in Fig. 1.

The reagent TFAA/TFA should contain, besides TFAA and TFA, the ions $[CF_3CO]^+$ and CF_3COO^-. The reagent is a potent acylating agent under the conditions used, and all hydroxyl groups are rapidly trifluoroacetylated. The *O*-trifluoroacetyl groups are strongly electron-attracting and thus render the glycosidic acetal-group electron deficient. The *O*-trifluoroacetyl groups close to the glycosidic bond should be particularly efficient.

The methyl hexopyranosides were all stable towards trifluoroacetolysis and could be quantitatively recovered after *O*-detrifluoroacetylation. No anomerisation was evident. This finding shows that the *O*-trifluoroacetyl groups at positions 2, 3, 4, and 6 stabilise the glycosidic bond and that further stabilization from the aglycon is not needed. The pentopyranoside (Me-α-Xyl*p*) and the 6-deoxyhexopyranoside (Me-α-Fuc*p*) were stable in 50:1 TFAA/TFA, demonstrating that an *O*-trifluoroacetyl group is not needed at position 6, even though these glycosides are more sensitive towards acid-catalyzed hydrolysis than the hexopyranosides. From these results, it is not surprising that the very acid-labile furanoside Me-β-Fuc*f* is also stable in 50:1 TFAA/TFA, as it has a 2- and a 5-*O*-trifluoroacetyl group adjacent to the acetal oxygens of the glycosidic bond. The acid-labile Me-α-Fuc*p*, Me-β-Fuc*f*, and Me-α-Xyl*p* were, however, partially solvolyzed in 1:1 TFAA/TFA.

All linear oligosaccharides tested were stable (Table II) as expected, because the glycosidic bond(s) are also stabilized by *O*-trifluoroacetyl groups in the aglycon part of the molecule. The branched trisaccharide α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-D-Gal was recovered in near-quantitative yield after trifluoroacetolysis with 50:1 TFAA/TFA, but, as expected, it could be recovered in only ~40% yield after trifluoroacetolysis with 1:1 TFAA/TFA, because of hydrolysis of the L-fucopyranosyl linkage and formation of α-D-Gal*p*-(1→3)-D-Gal (40%) and L-Fuc (12%).

The polysaccharides dextran, guaran, and glycogen were all stable in TFAA/TFA, and could easily be recovered, after *O*-detrifluoroacetylation, in the void volume of a Sephadex G-25 column. Sugar and methylation analyses of the recovered polysaccharide were identical to those of the original polysaccharides. When gel chromatography on Sephadex G-200 was used to detect degradation of trifluoroacetylated and *O*-detrifluoroacetylated dextran, it was found that substantial de-

gradation occurred when the trifluoroacetolysis was carried out in 1:1 TFAA/TFA; this degradation was diminished by lowering the proportion of TFA and was virtually absent when trifluoroacetolysis was performed in 20:1 TFAA/TFA (Fig. 1).

The stability of glycosides, oligosaccharides, and polysaccharides in TFAA/TFA under conditions that transamidate amide (peptide) bonds makes it possible to use the reagent for isolation of carbohydrate chains from glycoproteins[2]. Since most oligosaccharides and polysaccharides are brought into solution by the reagent, it should be possible to use trifluoroacetolysis to extract carbohydrates from biological material. Such compounds as proteins and fats are broken down by the reagent and can easily be removed. Studies to evaluate the usefulness of the reagent TFAA/TFA in the isolation of carbohydrate structures are in progress.

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at bath temperatures not exceeding 40°. G.l.c. was performed with a Perkin–Elmer 3920 instrument fitted with flame-ionization detectors. Separations were performed on (*a*) glass columns (2 m × 0.5 cm) containing 3% of ECNSS-M on Gas Chrom Q (80/100 mesh) at 200° (alditol acetates and peracetylated glycosides), and (*b*) glass-capillary columns (25 m × 0.25 mm) wall-coated with SE-30 (LKB, Stockholm, Sweden), at 180–330° (permethylated oligosaccharide alditols). For g.l.c.–m.s., the columns (*a* and *b*) were used in a Varian MAT 311A combined g.l.c.–m.s. instrument. Glass capillary columns were connected directly to the ion source of the instrument. The mass spectra were recorded at an ionization potential of 70 eV, an ionization current of 3 mA, and an ion-source temperature of 120°. All data were processed by an on-line computer system (Spectrosystem 100, Varian MAT).

Treatment of methyl glycosides with TFAA/TFA. — The methyl glycoside (5 mg) and *myo*-inositol (internal standard) were treated with TFAA/TFA (1:1 or 50:1, v/v; 2 ml) at 100° in a serum flask (10 ml) sealed with a rubber cap. After 48 h, the reaction mixture was cooled and evaporated to dryness. The residue was dissolved in M ammonia in aqueous methanol (2 ml), and the solution was evaporated to dryness. The residue was conventionally reduced with borohydride, and the products were acetylated with acetic anhydride–pyridine before analysis by g.l.c.–m.s.[3,4] (Table I).

Treatment of oligosaccharides with TFAA/TFA. — The oligosaccharides (5 ml) and maltose (inert, internal standard) were treated with TFAA/TFA as described above. After evaporation of the reaction mixture, the residue was reduced with sodium borohydride (10 mg) in 90% aqueous ethanol (5 ml). After processing as described above, the residue was permethylated, and analyzed by g.l.c.–m.s.[5] (Table II).

Treatment of polysaccharides with TFAA/TFA. — The polysaccharide (10 mg) was treated with TFAA/TFA (1:1, v/v; 5 ml) as described above. After *O*-detrifluoroacetylation with ammonia in aqueous methanol, the residue was purified by gel chromatography on Sephadex G-25 (2 × 45 cm) (elution with water).

All polysaccharides were eluted with the void volume, free of reagents. After lyophilization, the polysaccharides were analyzed by g.l.c.–m.s. after sugar[3,4] and methylation[6] analysis. Dextran (10 mg) was also treated with TFAA/TFA (20:1 and 50:1, v/v) and subjected to gel chromatography on Sephadex G-200 (1.5 × 55 cm) after *O*-detrifluoroacetylation (Fig. 1).

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