Carbohydrate Research, 137 (1985) 239–243
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A method for monitoring elution profiles during the chromatography of amino sugar-containing oligo- and poly-saccharides

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(Received January 16th, 1984; accepted for publication, August 24th, 1984)

Many biopolymers, such as mucopolysaccharides, peptidoglycans, and chitin, contain amino sugars as constituents. Representative methods for the colorimetric determination of amino sugars are the Morgan–Elson[1], Elson–Morgan[2], and indole–HCl[3] methods. The first two methods involve alkaline conditions after hydrolysis of the polysaccharides with acid and, since they require concentration or neutralisation of the hydrolysates before analysis, they are time-consuming when used to monitor elution profiles in gel filtration and ion-exchange chromatography.

On the other hand, the indole–HCl[3] and pyrrole–HCl[4] methods involve acidic conditions. We now describe a simple modification of the indole–HCl method (see Experimental) which involves the three steps (*1*) hydrolysis with 4M HCl at 100° for 15 min, (*2*) treatment with sodium nitrite at room temperature, and (*3*) treatment with indole at 100° for 5 min and measurement of the absorbance at 492 nm. The method is superior to the original method since it (*a*) requires neither concentration

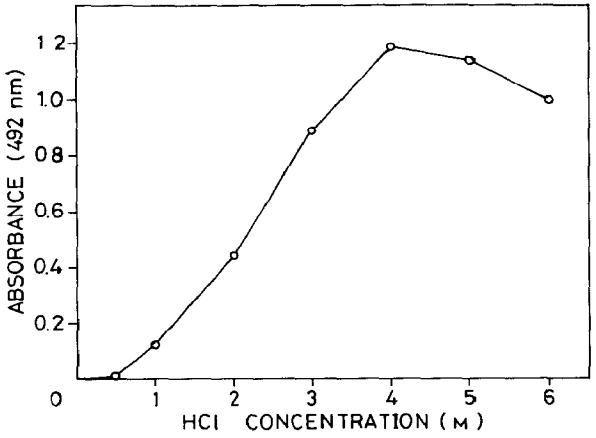

Fig. 1. Effect of the concentration of HCl on the development of colour from 2-amino-2-deoxyglucose (50 nmol) without the hydrolysis in step *1*.

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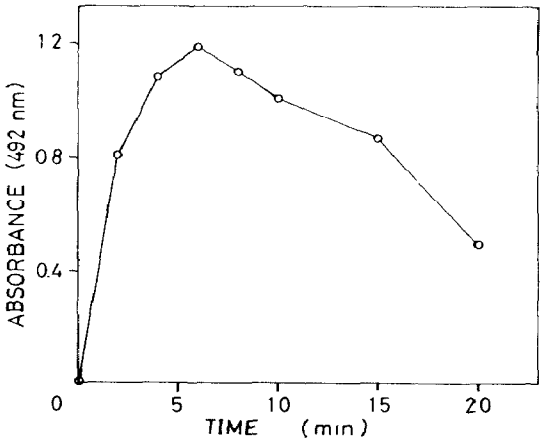

Fig. 2. Effect of time of heating in step *3* on the development of colour from 2-amino-2-deoxyglucose (50 nmol, 4M HCl) without the hydrolysis in step *1*

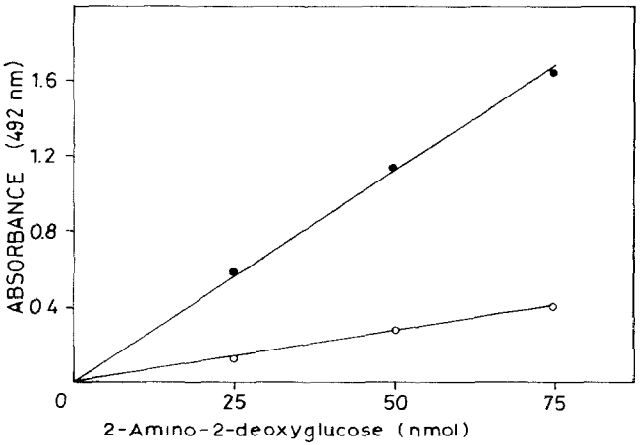

Fig. 3. Calibration curves for 2-amino-2-deoxyglucose, using the original (—○—) and modified (—●—) indole–HCl method but omitting the hydrolysis in step *1*.

nor neutralisation and involves a single tube per aliquot, (*b*) is about four times more sensitive, and (*c*) allows monitoring of the amino sugar in a polysaccharide within 2 h.

The optimum acid concentration and time of heating were determined from the results of Figs. 1 and 2. The calibration curves of the original and modified indole–HCl methods are shown in Fig. 3, which clearly indicate the higher sensitivity of the modified method. Calibration curves for 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose are shown in Fig. 4; the increases in absorbance were linear up to 75 nmol of amino sugar. The response from 2-amino-2-deoxymannose was low, reflecting the different course of reaction in the deamination stage. The molar responses for a variety of constituents found in materials obtained from

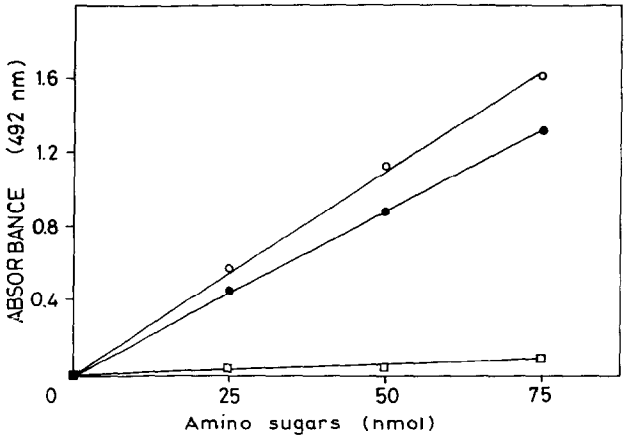

Fig. 4. Calibration curves of amino sugars obtained using the modified indole–HCl method but omitting the hydrolysis in step *1*: 2-amino-2-deoxyglucose (—○—), 2-amino-2-deoxygalactose (—●—), 2-amino-2-deoxymannose (—□—).

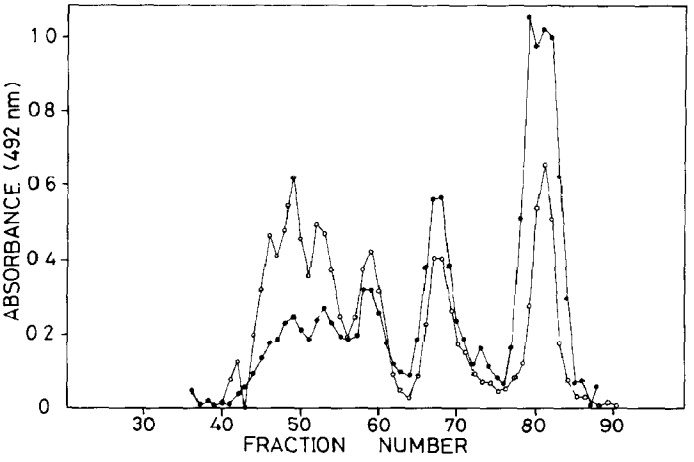

Fig. 5. Elution profile of chito-oligosaccharides on a column (1.5 × 150 cm) of Bio Gel P2 (3-mL fractions): —○—, modified indole–HCl method; —●—, Park–Johnson method[8].

biological origin are shown in Table I. Only neuraminic acid, galacturonic acid, and 2-deoxyribose gave responses that were other than negligible.

Most amino sugar constituents of mucopolysaccharides and glycoproteins are *N*-acetylated. Although quantification of the amino sugar content requires complete hydrolysis of the glycosidic linkages, this is not necessary in the modified indole–HCl method since residual glycosaminidic linkages are cleaved in Step *2*. *N*-Deacetylation of 2-acetamido-2-deoxyglucose in 4M HCl at 100° (conditions in step *1*) was essentially complete in ~30 min and >80% complete within 15 min.

An application of the modified indole–HCl method to determine the elution

TABLE I

RELATIVE MOLAR ABSORBANCE[a]

2-Amino-2-deoxyglucose	100	Rhamnose	2
2-Amino-2-deoxygalactose	74	Xylose	3
2-Amino-2-deoxymannose	2	Glucose	1
2-Acetamido-2-deoxyglucose	1	Glucose 6-phosphate	1
2-Acetamido-2-deoxygalactose	1	Galacturonic acid	14
2-Acetamido-2-deoxymannose	0	Ribose	3
Neuraminic acid	12	2-Deoxyribose	10
N-Acetylmuramic acid	1	Bovine serum albumin[b]	2
2-Amino-2-deoxyglucose 6-phosphate	86	Phenylalanine[c]	2
Mannitol	0	Hydroxyproline	3
Glucitol	0	Tryptophan	1

[a]Each value is expressed relative to that of 2-amino-2-deoxyglucose (100) and was obtained without the hydrolysis in step *1*. [b]For 1 mg of bovine serum albumin per mmol of 2-amino-2-deoxyglucose. [c]A zero response was obtained with Glu, Thr, Ser, His, Val, Ile, Gly, Asp, Arg, Met, Leu and Ala.

profile of chito-oligosaccharides on a column of Bio Gel P2 is shown in Fig. 5. When the profile was determined on the basis of reducing power, the higher oligosaccharides appeared to be only poorly resolved. However, sharp resolution was obtained by using the modified indole–HCl method, which, although similar to that obtained by the Elson–Morgan or ninhydrin method, required significantly less time.

Recently, automated analyses of amino sugar based on the ninhydrin and Elson–Morgan methods have been reported[5,6]. The most serious defect of these methods is the requirement for complete hydrolysis to monosaccharides; moreover, the ninhydrin method is not suitable for glycoproteins. The modified indole–HCl method described here should be advantageous in this context.

EXPERIMENTAL

Chitin-oligosaccharides were prepared by mild, acid hydrolysis of chitin[7]. Spectrophotometric measurements were performed on a Hitachi 100–10 instrument.

Standard procedure. — The original method was performed as described[3]. The optimised, modified method was as follows: (*1*) to an aqueous solution (400 μL) containing 0–75 nmol of hexosamine was added conc. HCl (200 μL), and the mixture was heated at 100° for 15 min and then cooled in ice–water; (*2*) aqueous 5% sodium nitrite (200 μL) was added and followed, after 10 min at room temperature, by aqueous 12.5% ammonium sulfamate (200 μL), and the mixture was stored at room temperature for 30 min with occasional shaking; (*3*) ethanolic 1% indole (100 μL) was added, and the mixture was heated at 100° for 5 min and then cooled, ethanol (1 mL) was added, and the absorbance at 492 nm was measured.

ACKNOWLEDGMENTS

We thank Mr. M. Masuda and Miss N. Muroi for technical and secretarial assistance.

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