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Analysis of the antidiabetic drug acarbose by capillary electrophoresis

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Abstract

This study describes the derivatization of the pseudooligosaccharide acarbose and its main metabolite, component 2, with 7-aminonaphthalene-1,3-disulfonic acid (ANDS) in human urine. Their efficient separation was possible by means of capillary zone electrophoresis, using a capillary tube of fused-silica containing 100 m*M* triethylammonium phosphate buffer, pH 1.5. On column laser-induced fluorescence allowed the detection of the pseudooligosaccharides in human urine in the nanomolar range. With this method, acarbose and component 2 were quantified in human urine after application of 300 mg of acarbose. © 1997 Elsevier Science B.V.

Keywords: Acarbose; 7-Aminonaphthalene-1,3-disulfonic acid

1. Introduction

Acarbose (Glucobay) represents a new therapeutic concept for the treatment of Diabetes mellitus [1]. As a glucosidase inhibitor, the compound exerts its activity within the gastrointestinal tract of humans [2]. The drug delays glucose absorption and, thus, reduces the postprandial blood glucose peaks [3].

Acarbose and its inhibitory effective metabolite, component 2, are pseudooligosaccharides [4]. Both compounds contain an unsaturated cyclitol residue, which is bound to an amino sugar. This moiety is linked to one or two glucose residues via α-1,4 glycosidic bonds (Fig. 1) [5,6].

Bayer (Wuppertal, Germany) determined the unchanged acarbose and component 2 by their inhibitory action against sucrase isolated from porcine intestinal mucosa. *p*-Nitrophenyl-α-D-maltoheptoside

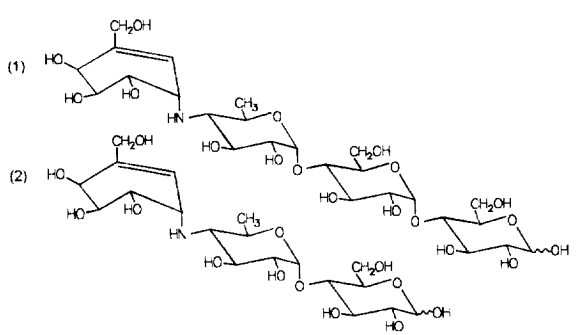

Fig. 1. Structure of acarbose (1) and component 2 (2).

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was used as a substrate for this enzyme [7]. The formation of *p*-nitrophenol was measured spectrophotometrically. The detection limit was 150 ng/ml in urine (rel. imprecision 5.3%) [4]. In 96 h, about 0.5–2% of the applied dose was excreted unchanged in urine [8]. After the application of 300 mg of acarbose, the concentration of both glucosidase inhibitory compounds in urine was determined to be 0.15–1 μg/ml, the human plasma concentration to be about 10 ng/ml. A separate assay of acarbose and component 2 was not possible.

Capillary electrophoresis is used as an alternative to many current techniqes for the analytical separation of sugars. Acarbose and component 2 can be quantified in acidic phosphate buffer using UV detection. At pH 3 the secondary amino group is protonated. Because the electrophoretic migration time increases with the number of glucose residues, acarbose has a higher migration time compared to component 2. However, the limits of detections for the concentrations of acarbose and component 2 (about 10 μg/ml) are insufficient for their quantification in human urine and in serum after the application of acarbose.

Therefore precolumn derivatization with fluorogenic reagents was used for the assay. The derivatization is based on reductive amination, where the reducing end of a saccharide reacts with the primary amino group of a fluorophoric compound.

In order to improve the sensitivity, UV laser-induced fluorescence detection with a He–Cd laser emitting in the UV region at 325 nm was used. After the derivatization with fluorescent agents like naphthalene derivatives [9] acarbose and component 2 are substituted with fluorophoric and ionic functions. Among various reagents 7-amino-naphthalene-1,3-disulfonic acid (ANDS) revealed the highest fluorescence yields. High speed and efficient separations for ANDS derivatized oligosaccharides were obtained with a triethylammonium phophate buffer at low pH in free solution using uncoated fused-silica capillaries [10].

This method has been applied to determine acarbose and component 2 in human urine from two volunteers after oral administration of 300 mg of acarbose. However, the sensitivity of this method was not yet sufficient for the analysis in human plasma.

2. Experimental

2.1. Chemicals and reagents

Acarbose (1) and component 2 (2) were kindly provided by Bayer (Wuppertal, Germany). 7-Aminonaphthalene-1,3-disulfonic acid was obtained from Aldrich (Steinheim, Germany) and dimethylsulfoxide (DMSO) was from Merck–Schuchardt (Hohenbrunn, Germany). Sodium cyanoborohydride, glucose, maltose, maltotriose, maltotetraose and maltohexaose were purchased from Sigma (St. Louis, MO, USA). Analytical grade phosphoric acid, triethylamine and acetic acid were obtained from Baker (Deventer, Netherlands).

2.2. Apparatus

CE-separations were performed on a P/ACE 2100 system (Beckman, Fullerton, CA, USA). All determinations were carried out in 50 μm I.D. uncoated fused-silica capillaries (Grom, Herrenberg, Germany) with an overall length of 37 cm and an effective separation length of 30 cm. Between each analysis the capillary was rinsed with 100 m*M* phosphoric acid for 1 min followed by a rinse with the running buffer for 1 min.

Samples were introduced into the capillary by pressure injection with 0.5 p.s.i. for 5 s with a second injection of water for 5 s for sharper peaks. The measurements were performed at 25°C with an applied voltage of 20 kV. The power supply polarity was reversed with the anodic site at the detector. The detection was carried out with a Beckman LIF-detector equipped with a He–Cd laser (10 mW, Omnichrome, Chino, CA, USA) operated at an excitation wavelength of 325 nm. Emission was measured at 450 nm. Data were collected and integrated using Beckman System Gold software 7.11.

2.3. Buffer and sample preparation

The running electrolyte was prepared by dissolving an appropriate amount of phosphoric acid in deionized water to yield a final concentration of 100 m*M* and adjusting the pH to 1.5 by the addition of triethylamine.

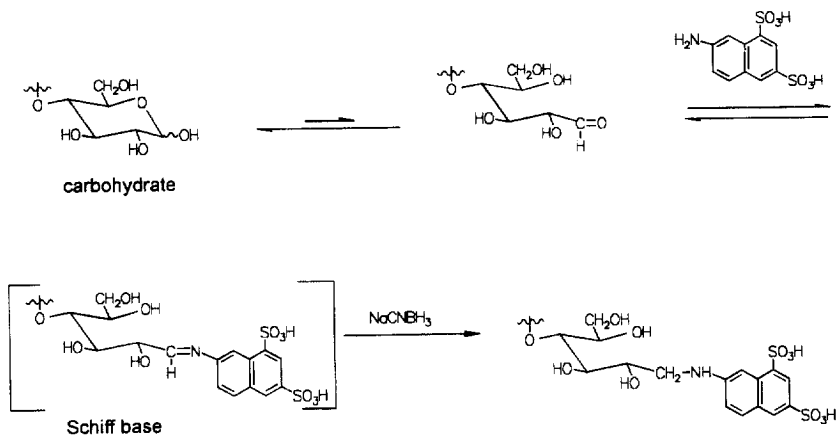

Fig. 2. Reaction scheme for the derivatization of carbohydrates with ANDS by reductive amination.

Before derivatization, 0.5-ml samples of urine and 0.5-ml samples of spiked urine were evaporated to dryness in Eppendorf tubes.

2.4. Precolumn derivatization of carbohydrates with 7-aminonaphthalene-1,3-disulfonic acid (ANDS)

The reductive amination with ANDS was adapted from the method described by Jackson [11] according to the reaction scheme outlined in Fig. 2.

Lyophilized samples in Eppendorf tubes were mixed with 100 μl of 0.08 *M* ANDS solution in acetic acid–water (3:17, v/v) and 50 μl of 0.9 *M* NaCNBH3 solution in dimethylsulfoxide. The reagent solutions were freshly prepared before derivatization. The reaction tubes were vortexed and then incubated overnight at 40°C. After lyophilization, seven different carbohydrates in the range of 1.5–20 nmol were derivatized as described above. The labelled oligosaccharides were analysed by capillary electrophoresis without sample clean-up from an excess of derivatizing agents and other components of the reaction mixture.

3. Results and discussion

3.1. Derivatization of carbohydrates with ANDS

The compounds were derivatized in order to increase the sensitivity of detection. The derivatization scheme is based on the reductive amination of the reducing carbonyl group of the oligosaccharide with the primary amino group of a fluorophore like ANDS.

The primary reaction product is a Schiff base which is subsequently reduced to a secondary amine by sodium cyanoborohydride (Fig. 2). The main advantage of ANDS as a labelling agent is that it facilitates highly sensitive fluorescence detection of trace amounts.

3.2. Separation

The capillary zone electrophoretic separation of a mixture of 7 ANDS-derivatized monosaccharides and oligosaccharides, composed of glucose, maltose, maltotetraose, maltohexaose, acarbose and component 2 is depicted in Fig. 3.

All experiments were carried out using the running electrolyte (TEA phosphate, pH 1.5) as described above. According to the literature, positively charged buffer additives are adsorbed resulting in a positive charge at the capillary inner wall [10]. In this case electroosmotic flow is directed toward the anodic end of the capillary and therefore in the same direction of the electrophoretic migration of the negatively charged ANDS-derivatized saccharides. The peak of excess derivatizing reagent peak (R) was the first one to appear in the electropherogram (Fig. 3).The

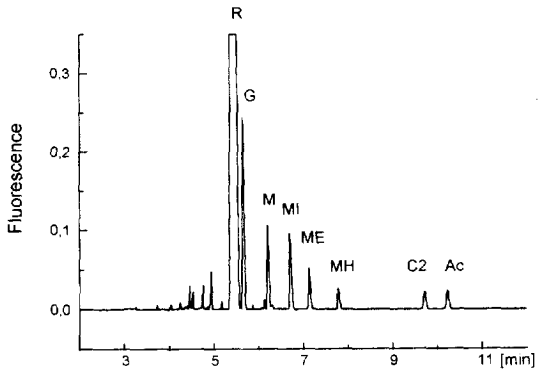

Fig. 3. Electropherogram of 7 ANDS-derivatized carbohydrates [ANDS (R), glucose (G), maltose (M), maltotriose (MI), maltotetraose (ME), maltohexaose (MH), component 2 (C2), acarbose (Ac)]. For electrophoretic conditions see Section 2. Peaks with migration times shorter than that of R are due to impurities of the derivatizing agent.

electrophoretic migration times of derivatized glucose and its 2–6 oligomers increased according with the number of glucose residues. In the case of acarbose and component 2 lower electrophoretic mobilities are probably due to the additional positive charge. Separation of all components was achieved in less than 11 min.

3.3. Application for human urine samples

Blank urine spiked with five different concentrations of each analyte, acarbose and component 2, from 0.05 to 1.3 μg/ml was derivatized as described above and analysed three times. A linear correlation was found between the concentration and the corrected peak areas. Parameters of the calibration are summarized in Table 1. The limits of detections for

Table 1
Typical parameters of the calibration for acarbose and component 2 in human urine

	Slope (mean±S.D.)	Intercept (mean±S.D.)	Correlation coefficient
Acarbose	4.40±0.04	0.029±0.026	0.999
Component 2	5.48±0.09	0.096±0.060	0.999

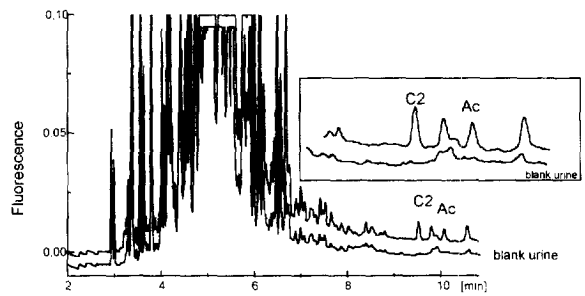

Fig. 4. Electropherogram of ANDS-derivatized acarbose (0.278 μg/ml) and component 2 (C2, 0.319 μg/ml) in a 6-h human urine of volunteer 2. For electrophoretic conditions see Section 2.

the concentrations in human urine were about 30 ng/ml for acarbose and 24 ng/ml for component 2 (signal-to-noise ratio of 3). At the corresponding migration times of acarbose and component 2, no interfering peaks from urine were observed.

The relative recovery of about 95% for both compounds was estimated using the corrected peak areas obtained from derivatized spiked human urine samples compared to corrected peak areas from injection of pure standard solutions. Urine spiked with four different concentrations of the analytes was investigated with three repetitions for the day to day precision. For the determination of drugs in biological fluids, precision and accuracy should always be within ±15% [12]. In our experiments the relative standard deviations were about 2.7–5.0% for acarbose and 2.0–5.8% for component 2 and therefore always within this range.

Following oral administration of 300 mg of acarbose, urine of two Caucasian volunteers was collected for at least 24 h in intervals of 6 h. In the urine fraction from 18 to 24 h, the concentrations of acarbose and component 2 were below the limit of detection. A typical electropherogram of a 6-h human urine is shown in Fig. 4. The urine concentrations were about 0.32 μg/ml for acarbose and 0.28 μg/ml for component 2. The results for both volunteers are shown in Table 2. About 0.18% of the applied dose were excreted unchanged in urine. A dose percentage of 0.06 was found for component 2 in urine of volunteer 1 compared with 0.12 of volunteer 2.

Table 2
Concentration–time values of acarbose and component 2 in human urine

	Volunteer 1		Volunteer 2	
	Acarbose concentration (μg/ml)±S.D. $n=3$	Component 2 concentration (μg/ml)±S.D. $n=3$	Acarbose concentration (μg/ml)±S.D. $n=3$	Component 2 concentration (μg/ml)±S.D. $n=3$
0–6 h	0.312±0.010	0.120±0.007	0.278±0.013	0.319±0.014
6–12 h	0.193±0.009	0.104±0.004	0.431±0.015	0.581±0.017
12–18 h	0.095±0.008	0.046±0.004	n.d.	0.082±0.008
18–24 h	n.d.	n.d.	n.d.	n.d.

n.d.=not detected

4. Conclusion

This study demonstrated that capillary electrophoresis is a very useful technique to analyse carbohydrates like acarbose and its main metabolite in biological fluids. In order to obtain high sensitivity, acarbose and its metabolite were labelled with 7-aminonaphthalene-1,3-disulfonic acid. The derivatization allowed the use of laser-induced fluorescence detection, and with triethylammonium phosphate buffer as running electrolyte, the analysis of acarbose and component 2 in human urine was achieved.

Acknowledgements

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References

[1] H. Morck, Pharm. Ztg. 139 (1994) 1.
[2] W. Puls, U. Keup, H.P. Krause, G. Thomas, F. Hoffmeister, Naturwissenschaften 64 (1977) 536.
[3] H. Bischoff, Akt. Endokr. Stoffw. 12 (1991) 25.
[4] H.J. Ahr, M. Boberg, H.P. Krause, W. Maul, F.O. Müller, H.J. Ploschke, H. Weber, C. Wünsche, Arzneim.-Forsch. Drug Res. 39 (1989) 1254.
[5] B. Junge, F.R. Heiker, J. Kurz, L. Müller, D.D. Schmidt, C. Wünsche, Carbohydr. Res. 128 (1984) 235.
[6] K. Bock, H. Pedersen, Carbohydr. Res. 132 (1984) 142.
[7] J.A. Balfour, D. McTavish, Drugs 46 (1993) 1025.
[8] F.O. Müller, Acta Pharmacol. Toxicol. (Suppl. V) (1986) 1.
[9] C. Chiesa, R.A. O'Neill, Electrophoresis 15 (1994) 1132.
[10] C. Chiesa, C. Horváth, J. Chromatogr. 645 (1993) 337.
[11] P. Jackson, J. Biochem. 270 (1990) 705.
[12] V.P. Shah, K. Midha, S. Dighe, I.J. Mcgilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. Mcdowell, K.A. Pittman, S. Spector, Eur. J. Drug Metab. Pharmacokin. 4 (1991) 249.