MIT Open Access Articles

Measuring Deuterium Enrichment of Glucose Hydrogen Atoms by Gas Chromatography/Mass Spectrometry

Massachusetts Institute of Technology

Measuring deuterium enrichment of glucose hydrogen atoms by gas chromatography mass spectrometry

Maciek R. Antoniewicz[1], **Joanne K. Kelleher**, and **Gregory Stephanopoulos**[*]
Department of Chemical Engineering, Bioinformatics and Metabolic Engineering Laboratory, Massachusetts Institute of Technology, Cambridge MA 02139, USA

Maciek R. Antoniewicz: mranton@udel.edu

Abstract

We developed a simple and accurate method for determining deuterium enrichment of glucose hydrogen atoms by electron impact gas chromatography mass spectrometry (GC-MS). First, we prepared 18 derivatives of glucose and screened over 200 glucose fragments to evaluate the accuracy and precision of mass isotopomer data for each fragment. We identified three glucose derivatives that gave six analytically useful ions: (1) glucose aldonitrile pentapropionate (*m/z* 173 derived from C4–C5 bond cleavage; *m/z* 259 from C3–C4 cleavage; *m/z* 284 from C4–C5 cleavage; and *m/z* 370 from C5–C6 cleavage); (2) glucose 1,2,5,6-di-isopropylidene propionate (*m/z* 301, no cleavage of glucose carbon atoms); and (3) glucose methyloxime pentapropionate (*m/z* 145 from C2–C3 cleavage). Deuterium enrichment at each carbon position of glucose was determined by least squares regression of mass isotopomer distributions. The validity of the approach was tested using labeled glucose standards and carefully prepared mixtures of standards. Our method determines deuterium enrichment of glucose hydrogen atoms with an accuracy of 0.3 mol%, or better, without the use of any calibration curves or correction factors. The analysis requires only 20 μL of plasma, which makes the method applicable for studying gluconeogenesis using deuterated water in cell culture and animal experiments.

INTRODUCTION

Measuring positional enrichment of glucose hydrogen atoms in combination with stable-isotope tracer experiments is a powerful technique for quantifying intracellular metabolic fluxes in the study of liver cell physiology[1–3]. In hepatic gluconeogenesis labeled hydrogen atoms are incorporated into glucose from medium containing deuterated water[4]. The amount of deuterium labeling incorporated at each carbon position depends on the deuterium enrichment of the medium, the relative contributions of gluconeogenesis and glycogenolysis to glucose production, and the extent of equilibration of several reactions in the gluconeogenesis pathway, in particular phosphoglucose isomerase and triose phosphate isomerase[5]. It has been suggested that the relative contribution of gluconeogenesis to *in vivo* glucose production can be determined from the ratio of deuterium enrichment on carbon atoms C5 vs. C2 of glucose, while deuterium labeling on C6 vs. C2 corresponds to the contribution of phosphoenolpyruvate to glucose production[6]. The enrichment of glucose hydrogen atoms can be measured by NMR or GC-MS. The NMR technique is well established, however requires expensive equipment and a fairly large amount of sample[7]. In contrast, GC-MS is a less costly and more sensitive technique for measuring stable-isotope

labeling. Guo et al.[8] developed a GC-MS method for determining positional deuterium enrichment of glucose from mass isotopomer data of eight fragments of glucose derivatives. The aldonitrile pentaacetate derivative of glucose yielded fragments at m/z 328, 242, 217, 212, 187, and 145; and the pentaacetate derivative yielded fragments at m/z 331 and 169. The authors derived expressions to calculate the enrichment of glucose from the mass isotopomer distributions. However, the method required the use of calibration curves and correction factors to account for artifacts in the spectra. As an example, significant deuterium-hydrogen exchange was observed for m/z 187 fragment that required an empirical correction. The authors also reported isotope discrimination for m/z 169 and 328 fragments in samples containing ^{2}H-labeled glucose diluted with unlabeled glucose. Furthermore, the mass spectrum of m/z 145 fragment contained a constant contaminating ion amounting for up to 30% of the total ion counts. The authors proposed to correct for this by dividing the observed enrichment at m/z 145 by 0.7 to obtain the corrected value. However, even with these corrections the estimated deuterium enrichments of glucose deviated up to 25% from the expected values. Desage et al.[9] developed a procedure to determine the ^{13}C-labeling pattern of glucose based on six methyloxime trimethylsilyl fragments of glucose at m/z 103, 160, 205, 217, 262, 319, and the assumption that glucose was labeled symmetrically. Beylot et al.[10] provided an improved method for determining ^{13}C-labeling based on 21 selected fragments from four glucose derivatives: aldonitrile pentaacetate fragments at m/z 314, 242, 225, 217, 212, 200, 187; methyloxime trimethylsilyl fragments at m/z 319, 217, 205, 160, 117, 103; bis-buthylboronate acetate fragments at m/z 297, 210, 181, 168; and permethyl glucose fragments at m/z 149, 101, 88, 75. However, these methods relied on fragments that were previously identified to be inaccurate due to artifacts in mass spectral data[8]. More recently, Price[11] compared several derivatives for analysis of ^{13}C-labeling of glucose, including dialkyldithioacetal acetate derivatives that gave rise to fragment resulting from C1–C2 bond cleavage, which was advantageous for measuring ^{13}C-enrichment at C1 of glucose.

Here, we present a simple and accurate method for measuring deuterium enrichment of glucose by GC-MS. In this study, we critically evaluated the accuracy and precision of over 200 fragments from 18 glucose derivatives. In addition to the four widely used derivatization methods we synthesized and analyzed 14 additional derivatives of glucose that have not been reported previously. We tested the mass isotopomer data for accuracy and precision and validated the assumed fragmentation patterns using ^{13}C and ^{2}H-labeled standards. The majority of analyzed fragments were found to yield inaccurate data, including several of the most widely used fragments. From the 200+ analyzed fragments we selected the six most accurate fragments that provided sufficient information to determine positional labeling. Combined analysis of the six fragments allowed determination of deuterium enrichment of glucose hydrogen atoms over the entire range of enrichments with an accuracy of 0.3 mol%, or better.

MATERIALS AND METHODS

Materials

[1-^{2}H]glucose (97%), [2-^{2}H]glucose (97%), and [6,6-^{2}H$_2$]glucose (98%) were purchased from IsoTec (Miamisburg, OH). [3-^{2}H]glucose (98%), [4-^{2}H]glucose (94%), [5-^{2}H]glucose (99%) were purchased from Omicron Biochemicals (South Bend, IN). [U-^{13}C$_6$]glucose (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA). Stock solutions of glucose standards were prepared at 10 mM in distilled water. For each derivatization, 50 μL of glucose solution was evaporated to dryness under airflow at room temperature.

Preparation of plasma samples

20 μL of blood was collected from a BL6 mouse via the tail. After centrifugation, the plasma was mixed with 200 μL of cold acetone and centrifuged for 10 min. The supernatant was divided into three micro-centrifuge tubes: 50 μL for aldonitrile pentapropionate derivatization; 50 μL for methyloxime pentapropionate derivatization; and 100 μL for di-*O*-isopropylidene propionate derivatization. The samples were evaporated to dryness under airflow at room temperature and stored at -20°C.

GC-MS analysis

GC-MS analysis was performed using HP 5890 Series II GC equipped with a DB-XLB (30 m × 0.25 mm i.d. × 0.25 μm; Agilent J&W Scientific) capillary column, interfaced with a HP 5971 MSD operating under ionization by electron impact at 70 eV. The mass spectrometer was tuned using the 'Max Sensitivity Autotune' setting. The injection volume was 1 μL and samples were injected in splitless mode. Helium flow was maintained at 0.88 mL/min via electronic pressure control. The injection port temperature was 250°C. The temperature of the column was started at 80°C for 1 min, increased to 280°C at 20°C/min, and held for 4 min. The interface temperature was maintained at 300°C. Mass spectra were recorded at *m/z* 100–500 at a rate of 2.0 scans/sec. Mass isotopomer distributions (MIDs) were obtained by integration[12]. Reported MIDs of fragments are averages from at least four injections per sample.

Preparation of glucose derivatives

The procedure is based on Biemann et al.[13] and Laine and Sweeley[14]. Evaporated glucose samples were dissolved in 50 μL pyridine, followed by addition of 100 μL of acetic, propionic, or butanoic anhydride to obtain the respective esters, or 70 μL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) + 1% trimethylchlorosilane (TMCS) to obtain the trimethylsilyl (TMS) ether. After 30 min incubation at 60°C, samples were evaporated to dryness and dissolved in 100 μL of ethyl acetate.

Preparation of aldonitrile derivatives of glucose

The procedure is based on Szafranek et al.[15]. Evaporated glucose samples were dissolved in 50 μL of hydroxylamine hydrochloride solution (20 mg/mL in pyridine), heated at 90°C for 60 min, followed by addition of 100 μL of acetic, propionic, or butanoic anhydride to obtain the respective aldonitrile glucose esters, or 70 μL of MSTFA + 1% TMCS to obtain the TMS derivative. After 30 min incubation at 60°C, samples were evaporated to dryness and dissolved in 100 μL of ethyl acetate.

Preparation of methyloxime derivatives of glucose

The procedure is based on Laine and Sweeley[14], and is identical to the aldonitrile method, except that *O*-methylhydroxylamine hydrochloride solution (20 mg/mL in pyridine) was used instead of hydroxylamine hydrochloride.

Preparation of di-*O*-isopropylidene derivatives of glucose

The procedure is based on Hachey et al.[16]. Glucose samples were transferred to a 10 mL screw-cap culture tube and evaporated to dryness. 500 μL of 0.38 M sulfuric acid in acetone was added, and samples were incubated at room temperature for 60 min. 400 μL of 0.44 M sodium carbonate was added to neutralize the reaction, followed by addition of 1 mL of saturated sodium chloride. The di-*O*-isopropylidene derivatives were extracted by partitioning with 1 mL of ethyl acetate. The upper, organic layer was evaporated to dryness. 100 μL of acetic, propionic, or butanoic anhydride was added to obtain the respective esters,

or 70 μL of MSTFA + 1% TMCS to obtain the TMS derivative. After 30 min incubation at 60°C, samples were evaporated to dryness and dissolved in 100 μL.

Preparation of permethyl and perethyl derivatives of glucose

The procedure is based on Ciucanu and Kerek[17] and recently updated by Ciucanu and Caostello[18]. A cloudy suspension of NaOH powder in DMSO was prepared (0.25 g/mL), vortexed vigorously, and 100 μL of the suspension was added to the glucose samples, followed by incubation at room temperature for 3 min. 75 μL of iodomethane, or iodoethane was then added to the samples, which were incubated at room temperature for 6 min to obtain the respective permethyl and perethyl derivatives. 1 mL of chloroform and 2 mL of distilled water were added and the samples were vortexed vigorously. The top, aqueous layer was removed and the organic layer was washed three times with 2 mL of distilled water, or until the aqueous layer was no longer basic. The organic layer was then evaporated to dryness and dissolved in 100 μL of ethyl acetate.

Calculation of positional deuterium enrichments

The amount of deuterium at each carbon position of glucose was determined by least-squares regression using Matlab (Mathworks Inc.). The details of the calculation method are given in Supporting Information. In short, theoretical MIDs were calculated for the selected glucose fragments and all isotopomers of glucose. With seven stable (i.e. carbon bound) hydrogen atoms, there are 128 ($=2^7$) possible isotopomers of glucose hydrogen atoms. Isotopomer fractions were estimated by minimizing the difference between measured and predicted mass isotopomers. The deuterium enrichments of glucose hydrogen atoms were then determined by summation of appropriate isotopomer fractions. We denote the deuterium enrichment at C1 of glucose as D_1, the enrichment at C2 as D_2, etc. Since there are two hydrogen atoms at C6 that cannot be distinguished by GC-MS we determined the average enrichment at C_6 as $D_{66}/2$.

The calculation method for determining positional enrichments was implemented into a Matlab program that accepts as input the measured MIDs for the six selected glucose fragments and returns as output the estimated deuterium enrichments of glucose hydrogen atoms and a statistical analysis of the goodness-of-fit. The program is freely available for academic use and can be obtained from the authors upon request.

RESULTS AND DISCUSSION

Preparation of glucose derivatives and analysis of glucose fragments

We prepared 18 derivatives of glucose based on four commonly used derivatization methods (Figure S-1). In the first step, the carbonyl group at C1 of glucose was derivatized to produce aldonitrile and methyloxime derivatives via reaction with hydroxylamine and methylhydroxylamine, respectively. Alternatively we prepared the di-*O*-isopropylidene derivative via reaction with acetone. In the second step, the hydroxyl groups of glucose were derivatized with acetic, propionic, or butanoic anhydride to obtain the respective esters, or with iodomethane, iodoethane, or *N*-methyl-*N*-trimethylsilyltrifluoroacetamide to obtain the permethyl, perethyl, and trimethylsilyl ethers of glucose, respectively. All 18 glucose derivatives were then analyzed by electron impact GC-MS (Figure S-2). We obtained mass spectra for natural glucose and specifically labeled glucose standards: [1-^{2}H], [2-^{2}H], [3-^{2}H], [4-^{2}H], [5-^{2}H], [6,6-^{2}H$_2$], and [U-^{13}C$_6$]glucose. For each glucose derivative we identified the most abundant fragments (Table S-3), and quantified MIDs for each fragment. To assign structural positions of glucose carbon and hydrogen atoms to each fragment, mass spectra of specifically labeled glucose standards were analyzed. As an example, the mass spectrum of aldonitrile pentapropionate glucose was characterized by fragments arising from

bond cleavage at C5–C6 (m/z 240 and 370), C4–C5 (m/z 173 and 284), C3–C4 (m/z 259), and C2–C3 (m/z 345) (Figure 1). These assignments were apparent from the shift in mass spectra of singly labeled glucose standards. Based on these assignments we postulated chemical formulae for the fragments, calculated theoretical MIDs and compared these to measured MIDs. Fragments for which the measured and predicted MIDs deviated more than 0.5 mol% were considered inaccurate and were discarded. We evaluated over two hundred glucose fragments this way.

Selection of glucose fragments for determining positional enrichments of glucose

From these fragments we selected the six most accurate fragments that provided sufficient information to quantify deuterium labeling on all six carbon positions of glucose. The selected fragments were derived from three pripionic anhydride derivatives of glucose: aldonitrile pentapropionate fragments at m/z 173 (C5-6, H5-6), m/z 259 (C4-6, H4-6), m/z 284 (C1-4, H2-4), and m/z 370 (C1-5, H2-5); di-*O*-isopropylidene propionate fragment at m/z 301 (C1-6, H1-6); and methyloxime pentapropionate fragment at m/z 145 (C1-2, H1-2). Figure 1 shows the electron impact mass spectra of the three derivatives and Figure 2 illustrates schematically the positional information obtained from the fragments. The derivatization reactions were complete for all three derivatization methods. The GC chromatograms contained a single large peak for each derivative. Table 1 compares the measured and theoretical MIDs for the six fragments. The agreement was excellent for all fragments; the maximum deviation was 0.2 mol% and measurement precision was less than 0.1 mol%.

Determining deuterium labeling of glucose hydrogen atoms

To assess the accuracy and precision of our methodology for determining deuterium enrichment of glucose hydrogen atoms we first analyzed MIDs of six deuterated glucose standards (Table S-4). Table 2 shows the estimated enrichments using the least-squares regression technique described in the Methods section. The determined enrichments corresponded well with the expected enrichments based on manufacturers' specifications. In addition, the estimated enrichments for natural glucose were not significantly different from zero on all carbon positions. The highest estimated enrichment was 0.1 mol% at C1.

For the study of gluconeogenesis it is especially important to determine deuterium labeling at C2 and C5 of glucose accurately. To test the accuracy of our method, we prepared a mixture of [2-^{2}H]glucose and [5-^{2}H]glucose (1:1 mol/mol) and diluted it with non-enriched glucose to obtain final deuterium enrichment at C2 and C5 of 1%, 5%, 10%, and 50%. The measured MIDs for the four mixtures of glucose standards are given in Table S-5. The estimated enrichments at C2 and C5 corresponded well with the expected values (Table 3). The deviation from expected enrichments was less than 0.3 mol% for the diluted mixtures. Furthermore, the estimated enrichments at C1, C3, C4 and C6 were not significantly different from zero. The observed 1.5 mol% enrichment at C1 in the 50:50 mixture could be explained by the presence of deuterium labeling at C1 in [2-^{2}H]glucose and [5-^{2}H]glucose standards (Table 2). Taken together, these results suggest that proton-deuterium exchange was not significant for the selected glucose fragments.

To demonstrate that the method can be applied with biological samples, we analyzed glucose from plasma without any tracers. 20 μL of plasma was sufficient to complete the analysis. Half of the sample was used for di-*O*-isopropylidene propionate derivatization of glucose to compensate for losses due to the extraction steps, and the other half of the sample was used for aldonitrile pentapropionate and methyloxime pentapropionate derivatizations of glucose. The injection volume for GC-MS analysis was 5 μL for each derivative and samples were injected in splitless mode. The measured MIDs of glucose from plasma were

identical to MIDs obtained from pure glucose standard, thus demonstrating that there were no artifacts resulting from analysis of a real biological sample (data not shown).

CONCLUSIONS

Accurate assessment of stable-isotope enrichments is critical for determining *in vivo* metabolic fluxes and elucidation of metabolic pathways [12, 19–21]. It is therefore of the utmost importance that analytical procedures for measuring isotopomers are critically evaluated and optimized using specifically labeled standards that reflect the range of expected enrichments in biological samples. In GC-MS, measured MIDs may deviate from expected isotopomer abundances for many reasons, such as unresolved metabolite peaks, overlapping mass spectra of adjacent fragments, hydrogen abstraction, deuterium-hydrogen exchange, gas phase ion/molecule chemistry in ionization chamber, isotope discrimination during ionization and detection, sample size effects, and imprecision due to low signal-to-noise ratio and background noise[8, 12, 22]. In the presence of these errors, observed isotopomer data may underestimate or overestimate true enrichments. Since small errors in measured MIDs can propagate to large errors in estimated positional enrichments, MIDs of individual fragments should be as accurate and precise as possible[23]. Our strategy to overcome these problems was to screen a large library of fragments and select only the most reliable fragments for analysis. We evaluated the accuracy and precision of mass isotopomer data using both natural glucose and specifically labeled glucose standards. For the estimation of deuterium enrichment of glucose hydrogen atoms, combined analysis of at least six fragments is required. The six fragments we identified were derived from three new derivatives of glucose that have not been described previously. The method we present here determines deuterium enrichment of glucose hydrogen atoms with an accuracy of 0.3 mol%, or better, over the entire range of expected deuterium enrichments. In addition, our method doesn't require the use of calibration curves and correction factors that were needed in previous methods. The procedure for measuring labeling in biological samples is straightforward: the sample is deproteinized, divided into 3 tubes, evaporated, and derivatized in parallel. We demonstrated that 20 μL of plasma was sufficient to complete the analysis. We anticipate that due to the higher sensitivity of GC-MS compared to NMR this method will be preferred for analysis of glucose labeling in cell culture experiments and in animal studies, where enrichments up to 5–10% can be achieved and the amount of sample is limited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Kelleher JK. Metab Eng. 2004; 6:1–5. [PubMed: 14734250]

2. Antoniewicz MR, Stephanopoulos G, Kelleher JK. Metabolomics. 2006; 2:41–52. [PubMed: 17066125]

3. Fernandez CA, Des Rosiers C. J Biol Chem. 1995; 270:10037–10042. [PubMed: 7730305]

4. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC. J Clin Invest. 1995; 95:172–178. [PubMed: 7814612]

5. Malaisse WJ, Malaisse-Lagae F, Liemans V, Ottinger R, Willem R. Mol Cell Biochem. 1990; 93:153–165. [PubMed: 2345541]

6. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC. J Clin Invest. 1996; 98:378–385. [PubMed: 8755648]

7. Burgess SC, Nuss M, Chandramouli V, Hardin DS, Rice M, Landau BR, Malloy CR, Sherry AD. Anal Biochem. 2003; 318:321–324. [PubMed: 12814639]

8. Guo ZK, Lee WN, Katz J, Bergner AE. Anal Biochem. 1992; 204:273–282. [PubMed: 1443525]

9. Desage M, Guilluy R, Brazier JL, Riou JP, Beylot M, Normand S, Vidal H. Biomed Environ Mass Spectrom. 1989; 18:1010–1015. [PubMed: 2620153]

10. Beylot M, Previs SF, David F, Brunengraber H. Anal Biochem. 1993; 212:526–531. [PubMed: 8214596]

11. Price NP. Anal Chem. 2004; 76:6566–6574. [PubMed: 15538778]

12. Antoniewicz MR, Kelleher JK, Stephanopoulos G. Anal Chem. 2007; 79:7554–7559. [PubMed: 17822305]

13. Biemann K, de Jongh DC, Schnoes HK. Am J Chem Soc. 1963; 85:1763–1770.

14. Laine RA, Sweeley CC. Anal Biochem. 1971; 43:533–538. [PubMed: 5141097]

15. Szafranek J, Pfaffenberger CD, Horning EC. Carbohydr Res. 1974; 38:97–105. [PubMed: 4447950]

16. Hachey DL, Parsons WR, McKay S, Haymond MW. Anal Chem. 1999; 71:4734–4739. [PubMed: 10546538]

17. Ciucanu I, Kerek F. Carbohydr Res. 1984; 131:209–217.

18. Ciucanu I, Costello CE. J Am Chem Soc. 2003; 125:16213–16219. [PubMed: 14692762]

19. Crown SB, Indurthi DC, Ahn WS, Choi J, Papoutsakis ET, Antoniewicz MR. Biotechnol J. 10.1002/biot.201000282

20. Choi J, Antoniewicz MR. Metab Eng. 10.1016/j.ymben.2010.1011.1006

21. Young JD, Walther JL, Antoniewicz MR, Yoo H, Stephanopoulos G. Biotechnol Bioeng. 2008; 99:686–699. [PubMed: 17787013]

22. Fagerquist CK, Hellerstein MK, Faubert D, Bertrand MJ. J Am Soc Mass Spectrom. 2001; 12:754–761. [PubMed: 11401166]

23. Antoniewicz MR, Kelleher JK, Stephanopoulos G. Metab Eng. 2006; 8:324–337. [PubMed: 16631402]
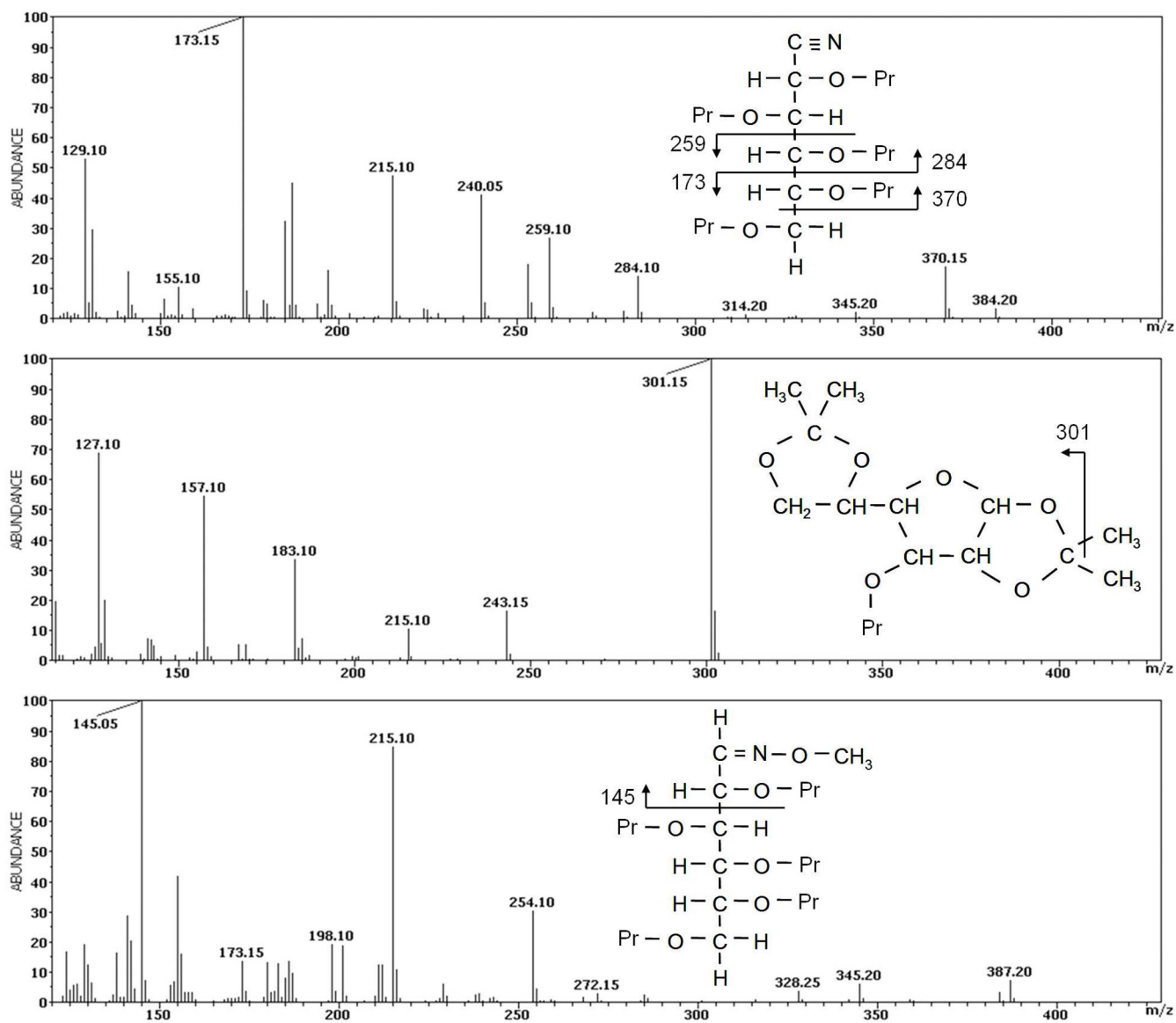
Figure 1.
Electron impact mass spectra of three selected glucose derivatives, aldonitrile
pentapropionate glucose (top), di-*O*-isopropylidene propionate glucose (middle), and
methyloxime pentapropionate glucose (bottom).
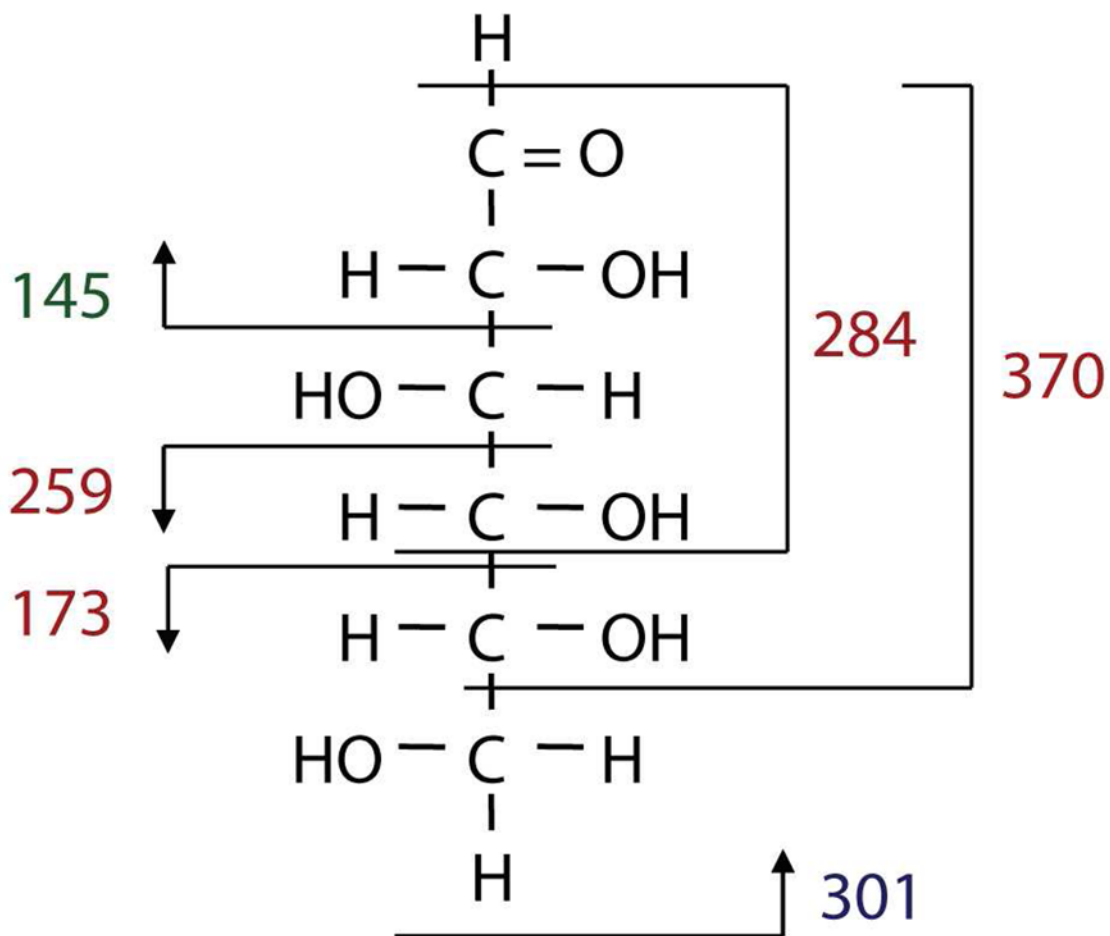
Figure 2.
Schematic overview of positional information obtained from GC-MS analysis of the selected glucose fragments, aldonitrile pentapropionate glucose fragments *m/z* 173, 259, 259, 284; di-*O*-isopropylidene propionate glucose fragment *m/z* 301; methyloxime pentapropionate glucose fragment *m/z* 145.

Table 1

Evaluation of the accuracy and precision of MIDs of the selected glucose fragments

Fragment (formula)		M+0	M+1	M+2	M+3	M+4
m/z 301	Exp	84.1 ± 0.10	13.5 ± 0.10	2.2 ± 0.10	0.2 ± 0.08	0.0 ± 0.01
(C₁₄H₂₁O₇)	Theory	84.2	13.4	2.2	0.2	0.0
m/z 145	Exp	92.3 ± 0.05	6.8 ± 0.03	0.8 ± 0.01	0.1 ± 0.01	0.0 ± 0.00
(C₆H₁₁O₃N)	Theory	92.5	6.7	0.8	0.0	0.0
m/z 173	Exp	90.6 ± 0.02	8.2 ± 0.02	1.1 ± 0.02	0.1 ± 0.01	0.0 ± 0.00
(C₈H₁₃O₄)	Theory	90.6	8.3	1.1	0.1	0.0
m/z 259	Exp	86.3 ± 0.09	11.7 ± 0.07	1.8 ± 0.02	0.2 ± 0.01	0.0 ± 0.00
(C₁₂H₁₉O₆)	Theory	86.3	11.8	1.8	0.2	0.0
m/z 284	Exp	85.2 ± 0.10	12.7 ± 0.05	1.9 ± 0.03	0.2 ± 0.04	0.0 ± 0.02
(C₁₃H₁₈O₆N)	Theory	85.0	12.8	1.9	0.2	0.0
m/z 370	Exp	81.1 ± 0.02	15.7 ± 0.05	2.9 ± 0.01	0.3 ± 0.03	0.0 ± 0.01
(C₁₇H₂₄O₈N)	Theory	80.9	15.9	2.8	0.4	0.0

Shown are the experimental (Exp) and theoretical mass isotopomer abundances for di-*O*-isopropylidene propionate fragment at *m/z* 301, methyloxime pentapropionate fragment at *m/z* 145, and aldonitrile pentapropionate fragments at *m/z* 173, 259, 284, and 370 (molar percent abundances, mol%; mean ± SD, n=6).

Table 2

Deuterium enrichment of glucose hydrogen atoms determined for natural glucose and six deuterated glucose standards (molar percent enrichments, mol %)

	[1-^{2}H] glucose (97 At%)	[2-^{2}H] glucose (97 At%)	[3-^{2}H] glucose (98 At%)	[4-^{2}H] glucose (94 At%)	[5-^{2}H] glucose (98 At%)	[6,6-^{2}H$_2$] glucose (98 At%)	Natural glucose
D$_1$	99.8	2.2	1.0	0.5	0.8	2.6	0.1
D$_2$	2.9	98.6	3.2	4.6	0.4	1.5	0.0
D$_3$	0.2	0.0	95.3	0.4	0.1	1.5	0.0
D$_4$	0.0	0.5	0.3	90.7	0.0	0.2	0.0
D$_5$	0.1	0.0	0.3	0.0	99.7	3.7	0.0
D$_{66}$/2	0.1	0.0	0.3	0.4	0.1	96.6	0.0

Numbers in parentheses denote manufacturers' specification of isotopic purity.

Table 3

Deuterium enrichment of glucose hydrogen atoms determined for mixtures of [2-^{2}H]glucose, [5-^{2}H]glucose and natural glucose (molar percent enrichments, mol%; estimated value ± SD, n=6)

	[2-^{2}H]glucose + [5-^{2}H]glucose + natural glucose					
	0:0:100	1:1:98	5:5:90	10:10:80	50:50:0
D$_1$	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	1.5 ± 0.1
D$_2$	0.0 ± 0.1	0.9 ± 0.1	4.9 ± 0.1	9.5 ± 0.2	48.5 ± 0.2
D$_3$	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
D$_4$	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
D$_5$	0.0 ± 0.0	1.1 ± 0.1	5.1 ± 0.1	10.1 ± 0.2	51.1 ± 0.2
D$_{66'}$/2	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1