Identification and Metabolic Implication of 1-Deoxyglucose (1,5-Anhydroglucitol) in Human Plasma

Shigetake Yoshioka, Shizuko Saitoh, Tomoo Fujisawa, Akihiko Fujimori, Osamu Takatani, and Masuo Funabashi

1-Deoxyglucose (1,5-anhydroglucitol), a metabolite related to diabetes mellitus, was identified in human plasma by gas–liquid chromatography and by gas–liquid chromatography/mass spectrometry. Plasma polyols were accurately determined with a gas–liquid chromatograph equipped with an all-glass capillary column. The plasma content of 1-deoxyglucose in healthy persons varies with age. Although the precise physiological role of 1-deoxyglucose remains obscure, the method described here for determining the minor polyol components of plasma, as well as the findings of 1-deoxyglucose in the plasma of healthy subjects, may be useful for investigating the metabolic roles of 1-deoxyglucose.

Additional Keyphrases: chromatography, gas–liquid - gas chromatography/mass spectrometry - diabetes - age-related effects - polyols

1-Deoxyglucose (1,5-anhydroglucitol) has been detected in plants, a species of bacteria (1), and human cerebrospinal fluid (2). Recent reports indicate that its concentration in human plasma varies in some metabolic states, such as diabetes mellitus, uremia, and others (3, 4).

Here we discuss the identification of 1-deoxyglucose in human plasma and describe an improved method for determining it by gas–liquid chromatography (GLC)3 and with a gas–liquid chromatograph/mass spectrometer (GLC-MS) equipped with an all-glass capillary column. We also report our findings regarding 1-deoxyglucose in the plasma of apparently healthy subjects of various ages.

Materials and Methods

Samples: Eight samples of cord blood were obtained and 342 blood samples from healthy subjects of various ages. All samples were stored at −80 °C until used.

Apparatus: We used a GLC (Model 6MPF) equipped with an automatic calculator (Chromatopac CR1A) and capillary column holder (Model CHL-4M), all from Shimadzu Seisakusho Co., 160 Tokyo, Japan. The GLC-MS was a Shimadzu Automatic, Model 6020.

Reagents: Absolute ethanol (grade S), pyridine (grade S), and other chemical reagents were all purchased from Wako-Junyaku Co., 130 Tokyo, Japan. Pyridine was distilled twice and stored at 4 °C until used. Reagents for trimethylsilyl (TMS) derivatization were obtained from Gaskuro-Kogyo Co., 160 Tokyo, Japan. The gases used for analysis (H2, He, N2, and NH3) were all of 99.9999% purity and were from Nihon-Sanso Co., 105 Tokyo, Japan.

Sample preparation: To deproteinize the samples, mix 100 μL of plasma with 200 μL of 99.9% ethanol, and let stand for 60 min. Centrifuge the mixture at 16 000 × g for 20 min, transfer the supernates to mini-vials, and evaporate them under reduced pressure. Store the residues at 4 °C over silica gel in a desiccator.

Before analysis, derivatize samples with TMS or trifluoroacetic acid (TFA) as follows. Dissolve 0.4 mL of hexamethyldisilazane and 0.2 mL of trimethylchlorosilane in 2 mL of anhydrous pyridine; add 100 μL of the resulting solution to each dried sample and stir for 30 s with a magnetic stirrer. Allow the sample to stand for 3 h at room temperature to form TMS derivatives. Derivatize samples with TFA according to the procedure of Tamura and Imanari (5). In brief, this involves mixing 0.1 mL of ethyl acetate and 0.1 mL of TFA and letting the mixture stand for 30 min at room temperature; add 2 μL of the resulting solution to the dried sample and mix vigorously for a few seconds with a magnetic stirrer to form the TFA derivatives.

For determination of the compound in blood cells: Wash the heparinized whole blood three times with physiological saline solution by centrifugation (3000 rpm, 30 min). Dilute the collected cells about 10-fold with distilled water and shake vigorously until the blood cells are completely disrupted. Treat the resulting product the same as for plasma before analysis. Treat the standard 1-deoxyglucose solution in the same manner.

GLC procedure: In analyzing TMS derivatives, we used adonitol as the internal standard. The sample volume injected was 1 μL, and nitrogen was the carrier gas, at the rate of 50 mL/min (0.27 mL/min in the column). The temperature of both detector and sample injection port was 240 °C. The column temperature was set at 160 °C initially, then linearly programmed at the rate of 0.5 °C/min, up to 195 °C. The split ratio, sensitivity, and range were 1:180, 10 MΩ, and 0.002 or 0.004 V, respectively. For analyzing TFA compounds, the flow rate of the carrier gas was 40 mL/min, the temperature of both detector and sample injection port was 160 °C and that of the column was 120 °C. A flame ionization detector was used. The column, 0.25 mm i.d. × 20 m, was coated with OV-101 and used as a coated-wall, open-tube column.

GLC-MS procedure: In analyzing TMS compounds, we used a 2 mm i.d. × 1 m chromatographic column packed with 15% JXR (methyl silicone; Gaskuro-Kogyo) and kept at 160 °C. The support was Gas-Chrom Q (Applied Sci. Laba. Inc., State College, PA 16801), and the flow rate of the carrier gas was 15 mL/min. In analyzing TFA compounds, we used a 0.35

Received Dec. 14, 1981; accepted Mar. 11, 1982.

Department of Pediatrics, National Defense Medical College, 3-2, Namiki, Tokorozawa, 359 Saitama, Japan.

1 Department of Third Internal Medicine, National Defense Medical College, and 2 Department of Chemistry, College of Arts and Sciences, Chiba University, 1-3, Yayo, Chiba, 260 Chiba, Japan.

3 Nonstandard abbreviations: GLC, gas-liquid chromatography; GLC-MS, GLC-mass spectrometer (or "spectrometry"); TMS, trimethylsilyl; TFA, trifluoroacetic acid; EI, electron impact ionization; CI, chemical ionization.
mm i.d. × 25 m chromatographic column packed with SE-20 (Appl. Sci.), column temperature was 120 °C and the split ratio was 1:1.

The conditions used with the mass spectrometer in EI analysis were as follows. The temperatures of injector, separator, and ion source were 300, 300, and 250 °C, respectively. Electric energy and electric current was 70 eV and 100 μA, respectively. Acceleration high voltage was 3.5 kV, and the scan speed was 3 s.

In CI analysis, with use of NH₃ as the reaction gas, the conditions were as follows. The temperatures of the injector, separator, and ion source were 280, 300, and 210 °C, respectively. Electric energy was 200 eV and electric current was 250 μA. Acceleration high voltage was also 3.5 kV. Scan speed was 3 s. The mass range was m/e 10–600 in both EI and CI analysis.

**Results**

**Identification of plasma 1-deoxyglucose**: GLC analysis of TMS derivatives of plasma polyols showed peaks originating from α- and β-glucoses (Figure 1). In addition, there was an unidentified peak (arrow) whose retention time differed from

---

**Fig. 1.** Gas–liquid chromatogram of TMS derivatives of polyols in the plasma of a 24-year-old woman
AD: adonitol (internal std.); GLC, glucose; arrow, unknown peak. x-axis units: min of retention time (R)

**Fig. 2.** Comparison of peaks of TMS derivatives of unknown origin (sample) with peaks of 1-deoxyglucose standard with respect to mass numbers as measured by CI and EI
Note that the mass spectra of the unknown substance and synthetically obtained 1-deoxyglucose totally coincide

**Fig. 3.** Mass spectrum of TMS derivative of α-ᴅ-glucose.
The mass range observed was m/e 41–525. The molecular ion and m/e 452 were not detected. The spectrum was quite different from that of TMS derivatives of 1-deoxyglucose
those for hexoses, pentoses, and their deoxy derivatives or of the corresponding alcohols. This peak was also observed in plasmas of both maternal and cord-blood samples, but not in analyses of blood-cell components.

In the GLC-MS analysis of TMS derivatives, the unidentified substance produced a peak at m/e 453 (EI), which coincided with the mass number of the molecular ion of the TMS derivative of deoxyhexose (Figure 2).

In CI with NH₃ as a reaction gas, the corresponding peak was at m/e 453, which suggests that this was (M+H)⁺ ion of the TMS derivative of deoxyhexose.

In the mass spectra of TMS derivatives of α-D-glucose in EI mode (Figure 3) the molecular ion and m/e 452 were not observed. We therefore concluded that the unknown peak did not originate from TMS derivatives of glucose in the course of analytical operations, nor from any substance contained in erythrocytes, leukocytes, or platelets. Because the retention time for this peak was not changed when the samples were reduced with NaHB₄ and derivatized with TFA, we concluded that the substance was probably difficult to reduce to an alcohol. Also, a single peak of the GLC measurement suggested that the substance has no anomer, i.e., no anomeric carbon atom. We deduced from these findings that the substance is most likely 1-deoxyglucose, for which the structural formula, in the form of the TMS derivative, is shown in Figure 2. This identification was confirmed by the total coincidence between the substance and synthetically prepared 1-deoxyglucose (6) in terms of retention time in GLC and mass spectrum obtained with GLC-MS (Figures 2 and 4).

**Table 2. 1-Deoxyglucose Concentration in Plasma from Cord Blood and from Healthy Subjects**

<table>
<thead>
<tr>
<th>Age of Subject</th>
<th>n</th>
<th>1-Deoxyglucose concn, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>cord blood</td>
<td>8</td>
<td>9.3</td>
</tr>
<tr>
<td>1 wk</td>
<td>48</td>
<td>3</td>
</tr>
<tr>
<td>2 wk</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3 wk</td>
<td>7</td>
<td>0.6</td>
</tr>
<tr>
<td>1 mo</td>
<td>33</td>
<td>12.3</td>
</tr>
<tr>
<td>2–5 mo</td>
<td>20</td>
<td>22.4</td>
</tr>
<tr>
<td>6–11 mo</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>1–2 yr</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>3–6 yr</td>
<td>58</td>
<td>32</td>
</tr>
<tr>
<td>7–10 yr</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>11–15 yr</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>Adults</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>

The differences among various age groups were statistically significant at p ≤ 0.01 for: cord blood vs age groups of 1–2 yr, 3–6 yr, 11–15 yr, and adults; 1–3 wk vs all age groups, 1 mo vs 1–2 yr, 3–6 yr, 7–10 yr, 11–15 yr, and adults; 6–11 mo vs 3–6 yr, and 7–10 yr; 1–2 yr vs 3–6 yr, and 7–10 yr; 7–10 yr vs 11–15 yr. Significantly different at p ≤ 0.05 for: 2–5 mo vs 1 mo, 3–6 yr, and 7–10 yr; 7–10 yr vs adults; 11–15 yr vs 6–11 mo, 1–2 yr, and 3–6 yr.

**Discussion**

1-Deoxyglucose was first detected in human cerebrospinal fluid by Pitkänen (2). To our knowledge, ours is the first report of its presence in human plasma.

Previous studies of plasma polyols other than glucose have involved a rather complicated method. In contrast, the method we described here is quite simple: deproteinization of plasma with absolute ethanol, evaporation to dryness under reduced pressure produced with a water aspirator, and derivatization to TMS forms before determination on an all-glass capillary column with high resolution capacity, which avoids the peak contaminations that preclude determination of plasma polyols with GLC.

The 1-deoxyglucose content of plasma varies with age and occasionally it was not detected in plasma from the newborn period. Servo and Pitkänen (7) stated that diabetics receiving insulin have low concentration of this compound in plasma, but they did not give conclusive evidence that plasma 1,5-anhydroglucitol was what was in fact being measured. According to our investigations (8), plasma 1-deoxyglucose, at the onset of diabetes, before any insulin have been received, is already low or undetectable. However, after therapy with insulin is begun, the concentration of this compound in plasma
sometimes increases or becomes detectable (8). 1-Deoxyglucose in plasma collected less than four weeks postnatally may be of maternal origin; its gradual increase later is presumably related to the induction of enzymes that synthesize it. All of these findings suggest that the compound may play some important role in the carbohydrate metabolism of human.

Recently, 1-deoxyglucose has become a subject of interest as a metabolic parameter in diabetes mellitus and other disease states (8). The simple, rapid, and reliable method we describe here for its determination and our findings of its occurrence in the plasma of healthy persons may be useful for further elucidation of its physiological and clinical significance and perhaps that of other minor polyol components of plasma.

We acknowledge Professor F. Iwanami for his valuable advice. This study was supported in part by a grant from Morinaga-Hoshikai Foundation, Japan.

References