I. Miwa, K. Maeda and J. Okuda

Department of Clinical Biochemistry, Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468 (Japan), and Clinical Laboratory, Daini Red Cross Hospital of Nagoya, Showa-ku, Nagoya 466 (Japan), 9 August 1977

Summary. The anomeric compositions of D-glucose in the liver, kidney, heart, blood and plasma of rat were determined by our method for the assay of D-glucose anomers and the percentages of the β-anomers were found to be 61.8, 61.0, 62.4, 62.7 and 62.9, respectively.

D-Glucose is known to exist as an equilibrium mixture of its 2 anomers in aqueous solution; 36% α-D-glucose and 64% β-D-glucose. Almost the same anomeric composition is also found in blood. The percentages of the α- and β-anomers of intracellular D-glucose, however, could not yet be determined, mainly due to technical difficulties, inspite of its necessity for the study of the physiological function of D-glucose anomers.

This paper describes the method for determining the anomeric compositions of D-glucose in tissues and blood samples, and the results obtained on the liver, kidney, heart, brain, adipose tissue, blood and plasma of rat.

Materials and methods. Male Wistar strain rats weighing 200–250 g were given free access to standard rat chow and tap water. Rats fasted for 24 h before use were decapitated to bleed out of tissues as much as possible. Blood was drawn from the heart using a 21-gauge needle, to which 0.2 mL of 10% sodium citrate was added as an anticoagulant.
was collected in a test-tube moistened with heparin solution, cooled in ice and separated to plasma and erythrocytes fractions by centrifugation at 4°C. The liver, kidney, heart, brain and adipose tissue were quickly removed off, and the tissues and blood samples were immediately frozen in dry ice-acetone. It took only 80 sec to finish the removal of tissues after decapitation. Frozen tissues (0.7-1.5 g) and blood samples (around 1 ml) were homogenized in 20 volumes of an ice-cold mixture of chloroform and methanol (10:1, v/v) supplemented with 10 μl of 0.2 M raffinose (see below for the reason of addition) with a Polytron homogenizer (Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 10,000 × g and 4°C for 5 min. The upper layer (aqueous layer) thus obtained served as a sample solution for the assay of D-glucose anomers, because D-glucose in the lower layer (chloroform layer) was only less than 2% of the total D-glucose when determined with D-[14C(U)]-glucose (New England Nuclear, Boston, USA) as a tracer, i.e. D-glucose in tissues and blood samples was almost entirely extracted in the aqueous layer. The assay of D-glucose anomers in sample solutions was carried out by our method using β-D-glucose oxidase (β-D-glucose:oxygen oxidoreductase, EC 1.1.3.4), mutarotase (aldose 1-epimerase, EC 5.1.3.3), and an oxygen electrode within 30 min after the preparation of sample solutions. In the present assay, a sample solution was added to the buffer solution in a vial prior to the addition of β-D-glucose oxidase. The principle of this assay was that β-D-glucose in the sample was oxidized with the consumption of oxygen (referred as A), α-D-glucose remaining in the solution was converted to β-D-glucose by addition of theβ-D-glucose oxidase solution (referred as B) and consequently the percentages of the α and β anomers of D-glucose can be determined as 100B/A + B and 100A/A + B, respectively. The volume of the sample solution was 10 μl for liver and 50 μl for kidney, heart, brain, adipose tissue, blood, and plasma. The total volume of the aqueous layer which is needed for the calculation of D-glucose amount in tissues and blood was determined by the dilution analysis using raffinose as a water-soluble tracer. Raffinose was assayed by the method of Roe et al.

**Results and discussion.** The difficulty in assaying D-glucose anomers in tissues and cells was thought to be mainly due to the rapid mutarotation of D-glucose in homogenates, to the dilution of cell fluid (containing D-glucose) on homogenization and to the presence of D-glucose-forming and D-glucose-consuming enzymes. In order to overcome these problems, we devised a unique treatment of tissues which consisted of rapid freezing of tissues followed by homogenization in a mixture of chloroform and methanol. The usage of 20 volumes (per g wet wt) of a mixture consisting of 10 volumes of chloroform and 1 volume of methanol was chosen as the best method. It was confirmed that the following interfering-proteins and -enzymes were denatured by this treatment; mutarotase (accelerate the mutarotation of D-glucose anomers), catalase (decompose hydrogen peroxide to oxygen and water), hemoglobin (bind with oxygen), hexokinase and glucose-6-phosphate. The mutarotational rate of D-glucose was found to be considerably slower in a sample solution which was obtained from the homogenate of kidney (1.2 g) supplemented with 20 μl of 50 mg/ml α-D-glucose, i.e. the percentages of β-D-glucose in the sample solution just after its preparation and at 1 h after that were 51.2 and 52.1, respectively. Another advantage of this treatment was that D-glucose in tissues was concentrated in small quantities of the aqueous layer, i.e. the volume of the aqueous layer was 0.65-0.75 ml for liver, 0.86-0.95 ml for heart, 0.98-1.07 ml for kidney, 0.82-0.93 ml for blood and 1.30-1.43 ml for plasma when 1 g of each tissue or 1 ml of blood and plasma was used. The anomeric compositions and total amounts of D-glucose in tissues and blood samples are summarized in the table listed in the text. The samples were prepared complete near to mutarotational equilibrium, but had a significant tendency to be abundant in the α anomer. Since both whole blood and its plasma showed almost the same anomeric compositions, D-glucose anomers in erythrocytes would also occur nearly at those compositions. Statistical analysis was performed by means of the Student’s t-test. Since some volume of blood is contained in tissues inspite of the bleeding caused by decapitation, blood D-glucose forms a part of D-glucose found in tissues. Blood D-glucose, however, was supposed to be less than 5% of total D-glucose extracted from tissues. Therefore, the values of the anomeric compositions of D-glucose in tissues listed in the table would be roughly equivalent to that in intracellular fluid of tissues.

It was reported that β-D-glucose was more rapidly transported than α-D-glucose into various cells and tissues studied hitherto, e.g. human erythrocytes, Ehrlich ascites tumor cells, rat pancreatic islets, rat retina, and rat brain slices, so the percentages of β-D-glucose in tissues and erythrocytes were expected to be larger than that in equilibrium D-glucose. The present findings, contrary to anticipation, could be due to the intracellular preferential phosphorylation of β-D-glucose by hexokinase which was suggested by our preliminary experiment.

Anomeric compositions and total amounts of D-glucose in rat tissues and blood samples

<table>
<thead>
<tr>
<th>Tissue and blood sample</th>
<th>No. of experiment</th>
<th>α-D-Glucose (%)</th>
<th>β-D-Glucose (%)</th>
<th>Total D-glucose (μg/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>7</td>
<td>38.2±0.53</td>
<td>61.8±0.53</td>
<td>1490±571</td>
</tr>
<tr>
<td>Kidney</td>
<td>7</td>
<td>39.0±0.83</td>
<td>61.0±0.83</td>
<td>337±56</td>
</tr>
<tr>
<td>Heart</td>
<td>7</td>
<td>37.5±0.35</td>
<td>62.5±0.35</td>
<td>401±70</td>
</tr>
<tr>
<td>Brain</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>Trace</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>Trace</td>
</tr>
<tr>
<td>Whole blood</td>
<td>5</td>
<td>37.3±0.74</td>
<td>62.7±0.74</td>
<td>64±3</td>
</tr>
<tr>
<td>Plasma</td>
<td>5</td>
<td>37.1±0.57</td>
<td>62.9±0.57</td>
<td>67±4</td>
</tr>
</tbody>
</table>

The percentages of the α- and β-anomers of equilibrium D-glucose were 35.6±0.36 and 64.4±0.36 (mean ± SD; 7 determinations), respectively. *Mean ± SD; *p < 0.001 when compared to the values of equilibrium D-glucose; α-D-glucose, i.e. the percentages of β-D-glucose in the sample solution just after its preparation and at 1 h after that were 51.2 and 52.1, respectively. Another advantage of this treatment was that D-glucose in tissues was concentrated in small quantities of the aqueous layer, i.e. the volume of the aqueous layer was 0.65-0.75 ml for liver, 0.86-0.95 ml for heart, 0.98-1.07 ml for kidney, 0.82-0.93 ml for blood and 1.30-1.43 ml for plasma when 1 g of each tissue or 1 ml of blood and plasma was used. The anomeric compositions and total amounts of D-glucose in tissues and blood samples are summarized in the table listed in the text. The samples were prepared complete near to mutarotational equilibrium, but had a significant tendency to be abundant in the α anomer. Since both whole blood and its plasma showed almost the same anomeric compositions, D-glucose anomers in erythrocytes would also occur nearly at those compositions. Statistical analysis was performed by means of the Student’s t-test. Since some volume of blood is contained in tissues inspite of the bleeding caused by decapitation, blood D-glucose forms a part of D-glucose found in tissues. Blood D-glucose, however, was supposed to be less than 5% of total D-glucose extracted from tissues. Therefore, the values of the anomeric compositions of D-glucose in tissues listed in the table would be roughly equivalent to that in intracellular fluid of tissues.

It was reported that β-D-glucose was more rapidly transported than α-D-glucose into various cells and tissues studied hitherto, e.g. human erythrocytes, Ehrlich ascites tumor cells, rat pancreatic islets, rat retina, and rat brain slices, so the percentages of β-D-glucose in tissues and erythrocytes were expected to be larger than that in equilibrium D-glucose. The present findings, contrary to anticipation, could be due to the intracellular preferential phosphorylation of β-D-glucose by hexokinase which was suggested by our preliminary experiment.

1 Clinical Laboratory, Daini Red Cross Hospital of Nagoya, Showa-ku, Nagoya 466, Japan.