Food Chemistry 265 (2018) 222–226

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

NMR analyses of complex D-glucose anomerization

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ARTICLE INFO

Keywords:
D-Glucose
Furanose
Pyranose
NMR spectroscopy
Saturation transfer
Anomerization
Ring opening
Thermodynamics

ABSTRACT

Analyzing the ^{1}H NMR spectrum of D-glucose, the resonance frequencies of the anomeric protons of five D-glucose anomers could be determined in dependence on temperature. Besides, the relative concentrations of all cyclic D-glucose anomers could be quantified. Based on that, thermodynamic parameters were calculated. In addition, ring opening rate constants of all cyclic D-glucose anomers were measured for the first time using ^{1}H selective blind saturation transfer NMR spectroscopy. The results presented here give rise to the assumption that furanoid anomers highly influence the reactivity of total D-glucose. Finally, the complex anomeric equilibration curves for a freshly prepared solution of crystalline α-D-glucopyranose are presented. Based on that, it is hypothesized that the reactivity of a solution of a reducing sugar in general and D-glucose in particular depends on time until the thermodynamic equilibrium state is reached.

1. Introduction

D-Glucose is the most common sugar and has therefore high impact on the quality of food, especially regarding its degradation during Maillard reaction and caramelization. Besides, D-glucose plays a central role in biochemistry and physiology. Due to its high importance in life sciences, studies on the reaction behaviour of D-glucose are of major interdisciplinary interest. Since 1846, when Dubrunfaut (1846) observed the mutarotation of freshly prepared solutions of D-glucose, its molecular characteristics are under investigation. In this regard, the knowledge of the molecular structure of D-glucose is the most basic information to describe its chemical behaviour. Besides crystallographic analyses of the molecular structure (e.g. Sponsler & Dore, 1931), NMR spectroscopy was intensively used to elucidate the geometry of D-glucose anomers (references shown below). Thereby, its pyranoid anomers are one of the most common text book examples to describe the dependence of vicinal homonuclear *J*-couplings on the dihedral angle. Nevertheless, D-glucose forms another four anomeric structures in aqueous solution which have never been described based on ^{1}H NMR spectroscopy before. This is remarkable since plenty of ^{13}C NMR spectroscopic data is available. Perlin and Casu (1969) as well as Perlin, Casu, and Koch (1970) published the ^{13}C chemical shifts of the D-glucopyranoses. Williams and Allerhand (1977) were the first who detected ^{13}C NMR signals of the β-D-glucofuranose (βf), before Maple and Allerhand (1987) detected the anomeric ^{13}C NMR signals of the remaining three anomers and quantified the relative concentrations of all six anomers in dependence on temperature. Zhu, Zajicek, and Serianni (2001) repeated the quantification of all anomers at 30 °C using ^{13}C NMR spectroscopy. Regarding ^{1}H NMR spectroscopy, first results concerning D-glucopyranoses were published by Lenz and Heeschen (1961). Later Angyal and Pickles (1972) investigated the ^{1}H NMR spectra of all D-aldopentoses and -hexoses to locate the anomeric proton resonances of their respective furanose and pyranose forms. Nevertheless, they could not detect any anomeric furanose signal in the case of D-glucose. More recent publications mostly focus on the assignment of spectral NMR information of D-glucopyranoses (Roslund, Tähtinen, Niemitz, & Sjöholm, 2008). Based on this lack in spectral information of minor anomers of D-glucose, we considered to re-investigate the ^{1}H NMR spectrum of D-glucose in aqueous solution.

2. Materials and methods

2.1. Materials

The following compounds were obtained commercially: 1,2-ethanediol (80%) in DMSO-*d6*, deuterium chloride in D_2O, deuterium oxide (Sigma-Aldrich, Germany); D-glucose (Carl Roth, Germany).

Abbreviations: αf, α-D-glucofuranose; βf, β-D-glucofuranose; αp, α-D-glucopyranose; βp, β-D-glucopyranose; a, acyclic aldehyde; *ACuSTiC*, approximated carbohydrate milieu stability time constant; NMR, nuclear magnetic resonance; STD, saturation transfer difference

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https://doi.org/10.1016/j.foodchem.2018.05.100
Received 10 January 2018; Received in revised form 5 May 2018; Accepted 22 May 2018
Available online 23 May 2018
0308-8146/

2.2. Sample preparation

D-Glucose was dissolved in D_2O to produce a solution with a concentration of 200 mM, whereby pD was adjusted to 2.5 by adding diluted deuterium chloride (in D_2O). Thereby, pH^* was measured with a thin glass electrode, calibrated with aqueous (H_2O) calibration buffers. pD was calculated according to Krężel and Bal (2004). The solution was transferred into a 5 mm NMR tube and equilibrated at ambient temperature for about three days to make sure that the thermodynamic anomeric equilibrium was reached.

2.3. Temperature calibration

To calibrate the variable temperature unit (VTU) (Bruker BVT 3000 Digital) of the NMR spectrometer, a standard high temperature NMR thermometer (80% 1,2-ethanediol in DMSO-*d6*, Sigma-Aldrich) was used according to Findeisen and Berger (2014). Thereby, ^{1}H NMR spectra were acquired at temperatures between 300 and 360 K using an increment of 5 K.

2.4. NMR spectroscopic experiments

All ^{1}H NMR experiments were acquired using a Bruker AVANCE 600 NMR spectrometer operating at 599.89 MHz equipped with a 5 mm BBI probe. The acquisition time was fixed to approximately 5.45 s and the relaxation delay to 30 s. Spectra were acquired with a FID resolution of about 0.18 Hz per point, collecting 64 k data points in the time domain. Zero filling to 128 k data points was applied prior to Fourier transformation. Selective blind saturation of the anomeric aldehyde proton was achieved using a 90° Gaussian soft-pulse cascade with an excitation bandwidth of 212 Hz. In order to determine ring opening rate constants, sixteen different saturation times ranging from 0 s to 73 s were employed.

2.5. Data analysis

^{1}H NMR spectra were deconvolved applying mixed Lorentzian-Gaussian lineshapes using TopSpin 3.5 pL2 as processing software. All further calculations were carried out using MS Excel 2016. For non-linear regression analysis (calculation of thermodynamic parameters as well as ring opening rate constants) the MS Excel Add-In XLSTAT 2017 was used. Calculations of eigenvalues and eigenvectors were carried out using the matrix-extension "matrix.xla" of the MS Excel Add-In XNUMBERS Ver 6.0.5.6 M. The particular solution for the integrated system of homogeneous differential equations (time dependent equilibration after the dissolution of crystalline α-D-glucopyranose) was found numerically using the MS Excel Solver Add-In.

3. Results and discussion

3.1. ^{1}H NMR analysis of D-glucose

Analyzing the ^{1}H NMR spectrum of an equilibrated aqueous solution of D-glucose, it becomes immediately apparent, that there is an anomeric low intensity signal downfield from the anomeric pyranoid resonances. This signal shows a doublet splitting with a coupling constant of 3.9 Hz and a weak roof effect directing to higher field. This in principle is in accordance with the general observation that furanoses resonate downfield relative to pyranoses. Furthermore, the furanoid 1,2-*cis* anomer (*here*: α-D-glucofuranose, αf) is usually shifted downfield relative to the furanoid 1,2-*trans* anomer (*here*: β-D-glucofuranose, βf). Finally, furnaoid 1,2-*cis* anomers usually show a doublet splitting with a coupling constant of 3–5 Hz (Angyal & Pickles, 1972; Hayward & Angyal, 1977; Kiely & Benzing-Nguyen, 1975). Based on this information, the observed anomeric signal can preliminarily be assigned to αf. Nevertheless, there is no additional signal that could be assigned to the anomeric proton of βf, even though its relative concentration should be comparable to that of αf according to Maple and Allerhand (1987). It is well known that the relative concentration of open chained as well as furanoid sugar anomers substantially increases with increasing temperature (Angyal & Pickles, 1972; Maple & Allerhand, 1987). For that reason, a series of ^{1}H NMR spectra of D-glucose was subsequently measured at different temperatures ranging from ambient temperature up to 87 °C. At 37 °C a shoulder at the high field end of the anomeric ^{1}H NMR signal of α-D-glucopyranose (αp) becomes apparent which has a comparable intensity to the anomeric signal of αf. A further increase in temperature leads to a low field shift of all ^{1}H NMR resonances, whereby the anomeric αp resonance is shifted to a higher extent than the above-mentioned shoulder. Thus, the resolution of the shoulder and the anomeric αp signal increases with increasing temperature. At 87 °C the distance between the maximum of the shoulder and the anomeric αp signal at the height of the shoulder's maximum is more than 10 Hz. Therefore, it can be concluded that the observed signal is a singlet. Nevertheless, it is substantially broader than the anomeric αf signal at 47 °C. Furthermore, its lineshape is not that of a pure Lorentzian. It rather seems to be a non-resolved doublet. This is in accordance with the general observation of anomeric 1,2-*trans* furanoid coupling constants which are in the order of 0–2 Hz (Kiely, & Benzing-Nguyen, 1975). Thus, the observed anomeric signal can preliminarily be assigned to βf, showing a coupling constant of less than 1 Hz.

To exclude the possibility that the discussed signals arise from sample impurities, ^{1}H saturation transfer difference (STD) NMR experiments were performed. Lewis, Choytun, Schramm, and Bennet (2006) showed that the blind saturation of the ^{13}C NMR resonance of the acyclic anomer of D-glucose can be used to measure ring opening rate constants according to the method described by Forsén and Hoffman (1963). In analogy to Lewis et al. (2006), we selectively saturated the expected range of ^{1}H resonances of aldehyde protons using a 90° Gaussian soft-pulse cascade. The saturation time was fixed to a total of 45 s. The excitation bandwidth of the soft-pulse was 212 Hz. Since this is a small excitation window for a blind saturation experiment, we systematically increased the offset of the frequency by 50 Hz by means of a pseudo 2D NMR experiment (frequency sweep saturation transfer). When the offset frequency is on-resonance with the ^{1}H NMR frequency of the aldehyde proton, a decrease in the signal intensities of the cyclic anomers becomes apparent, indicated by an intensity dip in the pseudo 2D matrix. Thereby, the decrease in signal intensities depends on ring opening rate constants and the specific spin-lattice relaxation times T_1 of the anomeric protons of the cyclic D-glucose anomers. The faster the ring opening rates and the higher the T_1 times, the higher is the decrease in signal intensities. Since D-glucose is a fast tumbling small molecule, an increase in temperature will most probably induce an increase of T_1 (Bloembergen, Purcell, & Pound, 1948). Likewise, ring opening rate constants increase with increasing temperature. To get a pronounced effect, we therefore performed the first experiment at 87 °C. As expected, a decrease in all anomeric signal intensities could be observed for the same soft-pulse offset frequency. This frequency was subsequently used to execute an STD NMR experiment with a saturation time of 60 s. Subtracting this spectrum form a regular ^{1}H NMR spectrum leads to a difference spectrum, showing dispersive signals for resonances which do not underlie saturation transfer as well as in-phase signals for resonances that are affected by saturation transfer. As shown in Fig. 1, the saturation transfer from the aldehyde proton to the anomeric protons of the furanoid and pyranoid anomers proves that all anomers belong to the same anomeric system.

In order to facilitate the identification of the anomeric resonances of D-glucose, Table 1 shows the chemical shift differences of the anomeric protons of the acyclic aldehyde (a), αf, βf and αp relative to that of βp in dependence on temperature.

Since exchange-related line broadening is low under the experimental conditions described here, the chemical shift differences provided in Table 1 should not depend on the spectrometer field strength