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General acid/base catalysis of sugar anomerization

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ABSTRACT

Based on theoretical and mechanistical considerations, an equation is presented that describes the observed rate of a pH sensitive reaction. In contrast to the commonly used catalytic catenary, the new approach enables the calculation of non-biased thermodynamic activation parameters. Applying this model, the general acid/base catalysis of the ring opening of β–D–fructopyranose was analyzed polarimetrically. Thereby, it could be shown that acids (bases) catalyze the ring opening of anionic (cationic) sugar species. Since anomerization rate constants correlate with the rate of sugar degradation, catalysts of anomerization will increase the sugar's reactivity as well. The most effective catalysts of the ring opening of β–D–fructopyranose in the food relevant pH milieu are weak acids and their conjugated bases. Consequently, the enhanced reactivity of reducing sugars in the presence of amino acids is not solely due to classical Maillard reaction but primarily due to carboxylic acid catalysis of degradation reactions.

1. Introduction

The process of anomerization of aldoses and ketoses is of scientific interest since Dubrunfaut observed the mutarotation of D-glucose in 1846. About 50 years later Lowry (1903) found that the rate of mutarotation can be accelerated by protons and hydroxyl ions. The systematical analysis of the respective dependence by Hudson (1907) showed, that mutarotation rates linearly increase with increasing proton as well as hydroxyl ion concentration. Thus, it could be shown that the change of mutarotation rate constants with a change of proton (hydroxyl) concentration is constant (cf. Eqs. (1) and (2)).

$$\frac{dk}{d[H^+]} = k_{H^+} \tag{1}$$

$$\frac{dk}{d[OH^-]} = k_{OH^-} \tag{2}$$

The integration of these derivatives leads to the well-known catalytic catenary (Eq. (3)):

$$k = k_0 + k_{H^+}\cdot[H^+] + k_{OH^-}\cdot[OH^-] \tag{3}$$

The terms of this equation can be interpreted as reaction equations.

a. *cyclus* → *acyclus*
b. *cyclus* + H^+ → *acyclus* + H^+
c. *cyclus* + OH^- → *acyclus* + OH^-

Interpreting these equations mechanistically, von Euler, Ölander, and Rudberg (1925) proposed that ring opening is initiated by the protonation of the ring oxygen and the deprotonation of the anomeric hydroxyl function, respectively. Thus, they stated that the observed rates of mutarotation depend on the basicity of the ring oxygen and the acidity of the anomeric hydroxyl function. As a result, von Euler et al. (1925) extended the catalytic catenary by the respective acidity constants of the ring oxygen K_{a1} and the anomeric hydroxyl function K_{a2}.

$$k = k_0 + k_1\cdot\frac{[H^+]}{K_{a1}} + k_2\cdot\frac{K_{a2}}{[H^+]} \tag{4}$$

As can easily be seen, Eqs. (3) and (4) are equal for $\frac{k_1}{K_{a1}} = k_{H^+}$ and $\frac{k_2\cdot K_{a2}}{K_W} = k_{OH^-}$, whereby K_W is the ionic product of water. Lowry (1927) criticized von Euler's theory, since he did not describe the whole process of anomerization. According to Lowry, anomerization is not complete, before the cationic (anionic) sugar becomes neutralized. Even though this theory is mechanistically funded, Lowry did not believe that von Euler's equation is better suited for the description of mutarotation than the basic catalytic catenary. Since the equations do not differ mathematically, this is obviously true. However, the catalytic catenary has important limitations. Calculating k_{H^+} and k_{OH^-} for example for the mutarotation of D–glucose leads to the conclusion that the hydroxyl ion

Abbreviations: af, α–D–fructofuranose; *ap*, α–D–fructopyranose; *βf*, β–D–fructofuranose; *βp*, β–D–fructopyranose; β–ala, β–alanine; A^+, acid; A^-, conjugated base; a, open chained keto anomer of D–fructose; B, base; gaba, γ–aminobutyric acid; gly, glycine; L–ala, L–alanine; L–pro, L–proline; qNMR, quantitative nuclear magnetic resonance; SST, selective saturation transfer; Z, sugar

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catalyzed reaction is 40,000 times faster than the respective proton catalyzed one (Hudson, 1907). Nevertheless, such differences have no mechanistic basis. Furthermore, the catalytic catenary allows an unlimited acceleration of mutarotation by just increasing (decreasing) the pH more and more. This however is not realistic.

Based on these limitations we present an updated theory of sugar anomerization that can be regarded as an extension of von Euler's theory.

2. Materials and methods

2.1. Materials

The following compounds were obtained commercially: β–alanine, deuterium oxide, L–proline, taurine (Sigma-Aldrich, Germany); L–alanine, citric acid, D–fructose (Carl Roth, Germany); diluted hydrochloric acid (0.1 M), diluted sodium hydroxide solution (0.1 M) (Bernd Kraft, Germany); D–[2–^{13}C]fructose (Omicron Biochemicals, USA); glycine, malic acid, sodium formate (Fluka, Switzerland); γ–aminobutyric acid (Alfa Aesar, Germany); sodium acetate (Merck, Germany).

2.2. Determination of pK_a values

pK_a values of the analyzed catalysts were determined potentiometrically, using an automated titration unit (TitroLine® 5000, SI Analytics). Therefore, 0.1 M solutions of the respective catalysts were prepared in 0.1 M hydrochloric acid. The solutions were subsequently titrated dynamically with 0.1 M sodium hydroxide solution. pK_a values were extracted from the resulting titration curves based on a graphical analysis of their respective first order derivatives.

2.3. NMR spectroscopic experiments

For NMR spectroscopic experiments a 0.5 M solution of D–[2–^{13}C] fructose in deuterium oxide was used. The solution pD was adjusted to 4.0. 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 for two days to make sure that the thermodynamic anomeric equilibrium was reached. Ring opening rate constants of the cyclic anomers of D–[2–^{13}C]fructose were determined by pseudo-2D ^{13}C selective saturation transfer (SST) qNMR experiments using a 90° Gaussian soft pulse cascade for selective saturation of the carbonyl species of D–[2–^{13}C]fructose. 16 Different saturation times ranging from 0 s to 73 s were employed. All NMR experiments were performed on a Bruker AVANCE 600 equipped with a 5 mm BBI probe. NMR spectra were deconvolved using Lorentzian line shapes applying Bruker's TopSpin 3.5 pl2 as processing software. Equation (5) was non–linearly fitted to the integrals of pseudo-2D ^{13}C SST qNMR spectra (Forsén and Hoffman, 1963) using the MS Excel Add-In XLSTAT 2017 to extract ring opening rate constants.

$$M_z^i(\tau) = M_z^i(0)\cdot\left(\frac{1 + T_1^i\cdot k_{obs}^{open}\cdot exp\left(\frac{-\tau\cdot(1 + T_1^i\cdot k_{obs}^{open})}{T_1^i}\right)}{1 + T_1^i\cdot k_{obs}^{open}}\right) \quad (5)$$

Equilibrium constants were calculated quantifying ^{13}C NMR signals of anomeric carbons. Ring closing rate constants were calculated using ring opening rates and equilibrium constants. 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 β–D–fructopyranose) was found numerically using the MS Excel Solver Add-In.

2.4. Polarimetric experiments

For polarimetric experiments 0.5 M solutions of the catalysts were prepared at different pH values (pH 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5). An aliquot of each solution was used to dissolve crystalline β–D–fructopyranose under vigorous stirring to produce a 0.5 M solution of D–fructose containing equimolar concentrations of the respective catalyst. A part of the solution was transferred into a 2 dm polarimeter cuvette immediately after complete dissolution of D–fructose. Subsequently, the optical rotation of the solution was measured in dependence on time until the equilibrium rotation was reached using a manual polarimeter (P1000–LED, KRÜSS OPTRONIC). The residual solution was used to check for pH stability. Equation (21) was non–linearly fitted to the time dependent optical rotation to calculate ring opening rate constants of β–D–fructopyranose. Equation (19) was subsequently non–linearly fitted to the pH dependent ring opening rates to extract the ring opening rates of ionic species of β–D–fructopyranose. Thereby, the acidity constant of the anomeric hydroxyl function was assumed to be 11.96 according to Feng, Bagia, and Mpourmpakis (2013). The proton affinity of the ring oxygen of β–D–fructopyranose is comparable to that of *tert*-butanol (Feng et al., 2013). Since acidity constants are not published for ring oxygens of reducing sugars and cannot be calculated using commercial software (like e.g. Percepta, ACD/Labs), pK_a of the ring oxygen of β–D–fructopyranose was assumed to be approximately that of protonated *tert*-butanol ($pK_a = -2.6$) (Deno & Turner, 1966). Non–linear regression analysis was performed using the MS Excel Add-In XLSTAT 2017.

3. Results and discussion

3.1. Theoretical considerations

It is generally accepted that anomerization of reducing sugars proceeds via the protonation (deprotonation) of the ring oxygen (anomeric hydroxyl function). Thus, the ring oxygen can be regarded as a weak base, whereas the anomeric hydroxyl function is a weak acid. Therefore, acidity constants can be defined that describe the relation of the concentration of ionic to non–ionic species. Anionic (Z^-) and cationic (Z^+) sugar species are both stabilized via two resonance structures – one cyclic and one acyclic (cf. Scheme 1).

In other words, cyclic ionic sugar species will open spontaneously. A consecutive step of protonation or deprotonation, respectively, terminates the anomerization process. Consequently, the observed rate of ring opening (closing) k of a reducing sugar anomer can be described as the product of the concentration of ionic species and their respective ring opening rates $k_{Z^\pm}$, k_{Z^+} and k_{Z^-} (cf. Eq. (6)).

$$k = k_{Z^\pm}\cdot[Z^\pm] + k_{Z^+}\cdot[Z^+] + k_{Z^-}\cdot[Z^-] \quad (6)$$

Thereby, the concentrations of ionic (zwitterionic) species can be calculated based on their acidity constants K_{a1} (acidity constant of protonated ring oxygen) and K_{a2} (acidity constant of anomeric hydroxyl function) using the law of mass action (cf. Eqs. (7)–(9)). Besides, the ring opening rates $k_{Z^\pm}$, k_{Z^+} and k_{Z^-} can be determined experimentally.

$$[Z^+] = \frac{[H^+]^2}{[H^+]^2 + [H^+]\cdot K_{a1} + [H^+]\cdot K_{a2} + K_{a1}\cdot K_{a2}} \quad (7)$$

$$[Z^-] = \frac{K_{a1}\cdot K_{a2}}{[H^+]^2 + [H^+]\cdot K_{a1} + [H^+]\cdot K_{a2} + K_{a1}\cdot K_{a2}} \quad (8)$$

$$[Z^\pm] = \frac{[H^+]\cdot K_{a2}}{[H^+]^2 + [H^+]\cdot K_{a1} + [H^+]\cdot K_{a2} + K_{a1}\cdot K_{a2}} \quad (9)$$

Comparing Eq. (6) with the catalytic catenary, it can be seen that ring opening rates are accelerated in dependence on pH by just