Catalytic Cycle of Carbohydrate Dehydration by Lewis Acids: Structures and Rates from Synergism of Conventional and DNP NMR

Pernille Rose Jensen and Sebastian Meier

Lewis acids play key roles in many chemical reactions. Structural and functional (kinetic) detail in Lewis acid catalysed fructose conversion are derived herein by the combined use of conventional and dissolution dynamic nuclear polarization (D-DNP) NMR. Structural information obtained with D-DNP NMR was used to identify conditions that stabilize an elusive initial intermediate and to determine its chemical structure. Carbohydrate dehydration through this intermediate had been predicted computationally. Complementary kinetic NMR assays yielded rate constants spanning three orders of magnitude for the three biggest energy barriers in the catalytic cycle.

Lewis acidic metals catalyse many central chemical reactions, including hydride shift, aldol, retro-aldol, Michael and retro-Michael reactions in the conversion of carbohydrates. However, conversion pathways especially in carbohydrate dehydration are still debated controversially. Increased insights into the reaction steps could simplify the improvement of Lewis acid catalysis, but most routine methods either lack the time resolution, sensitivity or chemical detail to support such insight. Accordingly, tracing conversion pathways, especially their fast and irreversible reactions, has remained a daunting task.

In situ NMR spectroscopy has often been the method of choice for retrieving structural information and concentrations in ongoing organic reactions. A particular stronghold of NMR spectroscopy is the ability to resolve equilibria of isomeric forms, for instance in carbohydrate substrates. Many of the relevant reactions converting carbohydrates occur by coordination of the low populated acyclic carbohydrate form to the catalyst. Moderate sensitivity and time resolution therefore aggravate molecular studies of the initial steps of catalytic carbohydrate conversion by conventional NMR spectroscopy. Considering these problems, the use of enhanced NMR methods should improve the discovery of the formation and conversion of intermediates in the Lewis acid catalysed reaction cycle. Specifically, dissolution dynamic nuclear polarization for enhancing NMR signals (D-DNP-NMR) has shown promise in detecting previously elusive states in bio- and chemocatalytic carbohydrate conversion. This approach generates substrates with hyperpolarized nuclear spins and triggers reactions by rapid injection of the hyperpolarized substrate to a chemical or biological catalyst, while providing a signal increase by DNP on the order of 10 000. Synergistic applications of D-DNP and conventional NMR techniques to characterize catalytic cycles on complementary time scales have remained very rare, however.

The current work aims to extend the mechanistic and kinetic understanding of carbohydrate dehydration pathways. D-DNP NMR was employed to detect transient early intermediates in the Lewis acid catalysed conversion of fructose. The fine structure of the transient signal was used to obtain insight into the structural motif that is initially formed in the reaction. This information was used to optimize the detection of the species in a temporarily stabilized sample using conventional NMR spectroscopy (Scheme 1). In this manner, a structural assignment of the transient intermediate could be achieved.

Scheme 1. Schematic overview of rationales used herein to detect an elusive intermediate, either through sample stabilization at low temperature or through the use of hyperpolarized substrate, and to trace NMR detectable intermediates in the catalytic cycle.

**a** Department of Health Technology, Technical University of Denmark Elektrovej 349, 2800 Kgs. Lyngby, Denmark
**b** Department of Chemistry, Technical University of Denmark, Kemitorvet, Building 207, 2800 Kgs Lyngby (Denmark), E-mail: semei@kemi.dtu.dk

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The finding experimentally validates that the lowest energy reaction pathway encompasses 3-deoxyglucos-2-ene (3-DGE, Scheme 2) as an on-pathway intermediate. Dehydration through this pathway intermediate had recently been predicted using machine learning based reaction sampling of carbohydrate dehydration. Subsequently, time resolved D-DNP and conventional NMR spectra were combined in a first experimental determination of three catalytic rate constants in the catalytic cycle converting fructose to a prospective polymer building block.

Experiments employing hyperpolarized [2-13C] fructose and a rapid injection setup were used in order to attempt detecting transient species that were formed immediately after mixing the substrate and the catalyst. The experiments indicated the formation of an enolic species with a characteristic 13C chemical shift of 151.5 ppm for the hydroxyl-bearing C2 in the double bond. This serendipitous observation encouraged attempts to determine the structure of the intermediate and of obtaining sufficiently good time resolved data to determine the kinetics in the catalytic cycle (Scheme 1). This enol signal was split into a doublet by a scalar coupling of 30.3±0.3 Hz (Figure 1A). Coupling constants of 30 Hz to an enolic carbon, in turn, are characteristic for two-bond couplings to the proton in an adjacent aldehyde group. Having established the likely presence of an aldehyde proton coupling through a 13C2 in order to maximize the detected signal (Figure 2). The 13C NMR (A) and though the same H NMR (B).

Figure 1. Detection of an enolic group adjacent to an aldehyde group through the 13C splitting of the 1H enol signal in D-DNP 13C NMR (A) and though the same splitting of the 1H aldehyde signal in conventional 1H NMR (B).

Figure 2. 1H-13C HMBC spectrum and chemical shift assignment for the structure determination of compound 2. Minor species from the cyclic pathway to HMF (Scheme 2) are indicated.

Figure S2, S3) spectra were acquired to obtain structural assignments. The 1H-13C HMBC spectra benefitted from the knowledge of the scalar coupling between the aldehyde 1H and the 13C2 in order to maximize the detected signal (Figure 2). The 1H-13C HMBC spectra further profited from the use of non-uniform sampling of the indirect dimension in order to rapidly collect data on the temporarily stabilized intermediate. This approach validated that the aldehyde group detected in the spectrum shown in Figure 1B indeed is adjacent to the enol group detected in the spectrum shown in Figure 1A, as evidenced by the cross signal between the 1H and the 13C2 signal in the intermediate. Full chemical shift assignment and structure determination of the intermediate were subsequently achieved by a combination of assignment spectra (Figure 2, inset). The determination of the covalent structure for this previously postulated intermediate 3-DGE (2) complements the detection of the main reaction intermediate trans-3,4-DGE (3), which is converted to THA (4), the recently discovered product of the cycle and a prospective polymer building block (Scheme 2; chemical shift assignments for 2-4 are given in Figure S5). The observation of 3-DGE rationalizes the previous detection of its keto tautomer 3-deoxyglucosone (3-DG) in reaction mixtures of SnO2-catalysed fructose
conversion,\textsuperscript{4,23} as the on-pathway intermediate 3-DGE can tautomerise to 3-DG upon dissociation from the catalyst (Figure S3 and S4). Other Lewis acids than Sn\textsuperscript{IV} had proven less active in catalysing the acyclic pathway of carbohydrate conversion.\textsuperscript{23}

D-DNP and conventional NMR are useful for kinetic assays on different time scales. Hyperpolarized signal fades on the seconds to minutes time scale and D-DNP NMR is best suited for the detection of fast reactions amongst multi-step transformations. Kinetic experiments were thus conducted under identical reaction conditions in order to quantify reaction rate constants by the complementary use of conventional NMR, thermal NMR, and computational modelling (Scheme 3). The real-time experiments are displayed in Figure 3. While conventional NMR captured the late intermediate and product signals, D-DNP NMR with optimized fructose polarization and sample delivery to the reaction mixture was successful in quantifying signal from the initial intermediate in a time-resolved manner with one second time resolution (Figure 4). Corresponding signals for the molecular species of Scheme 3 were integrated. Integrals for assays conducted on both time scales were separately fitted to models for the conversion of fructose to THA upon sequential elimination of the hydroxyl groups at the 3- and 4-positions. Kinetic data and their fits to the model of Scheme 3 are displayed in Figure 4. Data were well described by a simple model of the reaction cycle encompassing sequential, irreversible elementary steps with 2 as an on-pathway intermediate (detailed accounts of the fitted models are given in the Supporting Information). Conventional NMR assays were suitable for determining an apparent kinetic rate constant for the conversion of 1 to 3 and for the reaction step converting intermediate 3 to 4. D-DNP NMR resolved the two kinetics steps converting 1 to 3 by successive elimination of the 3- and 4-hydroxyl groups. Notably, excellent kinetic data could be obtained from single D-DNP NMR transients, although signals of 2 and 3 accumulate to less than 0.05% of the substrate signal in the D-DNP NMR assay owing to the slow initial reaction.

As a proof of concept, conventional and D-DNP NMR assays could be shown to return nearly identical values for the rate constant of substrate dehydration ($k_{1\text{app}} = 0.00017 \pm 0.00002$ s$^{-1}$ and $k_1 = 0.00018 \pm 0.00001$ s$^{-1}$, respectively). Rate constants in the catalytic cycle were thus $k_1 = 0.00018$ s$^{-1}$, $k_2 = 0.20(\pm0.02)$ and $k_3 = 0.0016(\pm0.0002)$ s$^{-1}$ (Figure 4, Scheme 4). Thus, rate constants differing by three orders of magnitude were derived. Replication indicated that the fitted values could be precisely determined. The initial conversion of fructose with $k_{1\text{app}}$ has previously been described to encounter an activation energy of 107.2 kJ/mol.\textsuperscript{23} Using the rate constants $k_2$ and $k_3$ and assuming comparable pre-exponential factors in transition state theory, the subsequent energy barriers can be estimated to 87.5 kJ/mol and 100.6 kJ/mol, respectively. Kinetic models of D-DNP NMR data also account for the general fading of hyperpolarization through pulsed excitation and $T_1$ relaxation (see Supporting Information for the kinetic expressions).\textsuperscript{29, 30} Fitted $T_1$ values for...
the quaternary C2 carbon in compounds 1 to 3 were realistic (~40 s) at the assay temperature of 70 °C (Figure 4B).

In conclusion, an initial dehydrated intermediate was detected with D-DNP NMR, and spectral information was used to screen for conditions that allowed the structure of the previously postulated intermediate in the catalytic cycle to be determined as 3-deoxyglucos-2-ene. To this end, scalar coupling constant information retrieved from the D-DNP experiment was determined as 3-deoxyglucos-2-ene. To this end, scalar coupling constant information retrieved from the D-DNP experiment was applied to a temporarily stabilized sample (10 °C). The structure determination validates recent computational predictions of a carbohydrate dehydration pathway through the enol tautomer of 3-deoxyglucosone. Combined use of D-DNP and conventional NMR under relevant reaction conditions (70 °C) yielded insight into the rate constants (differing by three orders of magnitude) and thus into the three main energetic barriers (differing by 20 kJ/mol) of the catalytic cycle. Consistency with conventional NMR under relevant reaction conditions (70 °C) and spectral information was used to screen for conditions that allowed the structure of the previously postulated intermediate in the catalytic cycle to be determined as 3-deoxyglucos-2-ene. To this end, scalar coupling constant information retrieved from the D-DNP experiment was applied to a temporarily stabilized sample (10 °C). The structure determination validates recent computational predictions of a carbohydrate dehydration pathway through the enol tautomer of 3-deoxyglucosone. Combined use of D-DNP and conventional NMR under relevant reaction conditions (70 °C) yielded insight into the rate constants (differing by three orders of magnitude) and thus into the three main energetic barriers (differing by 20 kJ/mol) of the catalytic cycle.

Conflicts of interest
There are no conflicts to declare.

References