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Published in:
Analyst

Link to article, DOI:
[10.1039/d0an00555j](https://doi.org/10.1039/d0an00555j)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):

Elliot, S. G., Jessen, B. M., Taarning, E., Madsen, R., & Meier, S. (2020). Sensitive NMR Method for Detecting Carbohydrate Influx into Competing Chemocatalytic Pathways. *Analyst*, 145, 4427-4431. <https://doi.org/10.1039/d0an00555j>

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Communication

Sensitive NMR Method for Detecting Carbohydrate Influx into Competing Chemocatalytic Pathways

Received 00th January 20xx,
Accepted 00th January 20xx

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DOI: 10.1039/x0xx00000x

Reaction pathways are often tracked with stable isotopes in order to determine the provenance of products in the pathway and to deduce mechanistic information. NMR spectroscopy can provide direct insight into the specific labelling position of the stable isotope. We suggest a simple assay that allows rapid quantitative measurements of isotope distributions in biomass-derived products using commercially available carbohydrate substrates and routine instrumentation. In the assay, biomass-derived products in post reaction material are quantitatively reduced with NaBH₄ to install hydrogens at each carbon site in the product. In this manner, the detection of ¹³C and ¹²C sites becomes possible in multiplets of the sensitive 2D ¹H-¹H TOCSY experiment. The approach detects the usage of competing upstream reactions from isotope patterns in chemically identical reaction products. Changing influx into Sn-Beta-catalysed carbohydrate conversion reactions in the absence and in the presence of K⁺ was quantitatively assessed, showing how the presence of K⁺ alters the initial reactions towards methyl lactate.

Heterogeneous catalysts bear considerable promise for the sustainable conversion of renewable substrates to fuels and materials.¹⁻⁴ An important question in the development and optimization of suitable catalysts is the selectivity of the desired processes, including the influence of reaction conditions or additives on selectivity.⁵⁻⁸ Direct studies of catalyst-substrate complexes have remained difficult. The short-lived nature of the complexes and the difficulty in rapidly obtaining sufficient chemical detail has often proven too challenging, especially in heterogeneously catalysed conversion. Catalytic mechanisms can be often inferred, however, by using isotope-enriched substrates to measure kinetic effects or isotope distributions in the products.⁹⁻¹⁵

Among the heterogeneous catalysts employed for carbohydrate conversion, the zeotype Sn-Beta has attracted considerable attention. Sn-Beta is able to convert carbohydrates to prospective polymer building blocks and precursors.¹⁶⁻²⁰ The widely abundant glucose, fructose and sucrose substrates can be converted to high yields of methyl lactate, especially in the presence of alkali ions at amounts comparable to the number of active sites.^{16, 21, 22} Considerable strides have therefore been made towards understanding Sn-Beta active site structures and functions, both in the absence and in the presence of alkali ion modification.²³⁻³⁰

Insight into the pathway usage under different conditions is a prerequisite to understand substrate-catalyst interaction, and to rationally optimize substrate influx into desired pathways. Such insight can become accessible by tracking isotope distributions either by mass spectrometry³¹ or by NMR spectroscopy.^{10, 31} NMR spectroscopy permits direct insights into the specific labelling positions of the stable isotope. Thus, isotope distributions in methyl lactate deriving from [1-¹³C] xylose have previously shown that the substrate remains coordinated to the active site and does not react through a symmetric dihydroxyacetone form.³² In consequence, methyl lactate gets formed with ¹³C enrichment predominantly at the C3 position. A challenge for the routine application of isotope tracking methods is that NMR spectroscopic measurements of ¹³C isotope distributions through quantitative 1D ¹³C NMR (qNMR) is slow and insensitive, especially for small molecules with long-T₁ carbon atoms such as methyl lactate with its quaternary ester carbon. As an added grave disadvantage, the abundance of NMR silent ¹²C sites is not accessible from quantitative 1D ¹³C NMR.

Here, we suggest a simple assay that provides rapid quantitative insight into isotope distributions of biomass-derived products. Commercially available carbohydrate substrates and routine detection instruments can be used. The approach installs protons at the C1 position of methyl lactate through reduction with NaBH₄³³⁻³⁵ prior to the indirect detection of ¹³C and ¹²C sites in multiplets of the sensitive 2D

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Scheme 1. (A) Schematic overview of [1-¹³C]xylose conversion to various methyl lactate isotopomers by Sn-Beta catalysis in methanol at 160 °C. (B) Depiction of methyl lactate forms with different isotopic composition (different isotopologues; left) and the corresponding ¹H-¹H TOCSY cross-peak structures on the right.

¹H-¹H TOCSY experiment as schematically depicted in Scheme 1.^{10, 12, 36, 37} This Scheme depicts that a cross signal between two different hydrogens can show four different fine structures, depending on the presence of ¹³C directly bonded to either, both or none of the hydrogens. The experimentally observed signal is a weighted average of these four forms.

The described methodology was used to inspect the upstream reaction selectivity of the Sn-Beta-xylose complex in the presence and in the absence of alkali ions, where possible initial reactions towards methyl lactate may include 1,2-intramolecular hydride shift (1,2-HS), 1,2-intramolecular carbon shift (1,2-CS) or retro-aldol cleavage (Scheme 1). Use of [1-¹³C] xylose as the substrate results in an asymmetric retro-aldol cleavage that reveals, if methyl lactate in an unpurified reaction mixture derives from the aldose or from the ketose form. While other products such as longer alpha-hydroxy acids can be formed as byproducts, the methyl lactate product has been obtained at highest yield and arguably has attracted most commercial interest in this reaction. The [1-¹³C] xylose substrate (36 mg) was converted for five minutes at 160 °C by Sn-Beta (hydrothermally synthesized with Si/Sn=200 18mg) in methanol using a microwave reactor, as previously described.³² In order to account for the population of methyl lactate without ¹³C, isotope-labelling was detected using a ¹H-¹H TOCSY spectrum. A representative cross-peak between the protons in the methyl and alcohol group of methyl lactate in post reaction material is shown in Fig. 1.

The cross-peak distinguishes between the absence of ¹³C

Fig. 1. ¹H-¹H TOCSY cross-peak between H2 and H3 in methyl lactate deriving from Sn-Beta catalysed conversion of [1-¹³C] xylose. Reaction conditions: D-[1-¹³C]-xylose (36 mg), Sn-Beta (HT) (18 mg), methanol (500 μL), and dimethyl sulfoxide (5 mg), which were heated to 160 °C for 5 min.

label, presence of ¹³C labelling at the C2 position only, at the C3 position only, or both at the C2 and the C3 position. Relative signal areas show that the majority of methyl lactate was ¹³C-labelled at the C3 position. A smaller prevalence was found of the isotopic form (isotopologue) without ¹³C at either position or with ¹³C labelling at C2 (see fractions in Figure 1). Double labelling at the C2 and C3 positions was negligible, as no intermolecular reaction occurred that channelled ¹³C atoms from two substrate molecules into one methyl lactate product molecule.

The high abundance of ¹³C labelling at the C3 position was consistent with previous observations indicating a concerted mechanism converting the C1 site in the carbohydrate substrate to the C3 site in methyl lactate, with minor scrambling to the C2 position due to 1,2-CS.³² Relative amounts of isotopic forms as determined from indirect observation through ¹H-¹H TOCSY cross-peaks were subsequently compared with quantifications from less sensitive and more time-consuming quantitative ¹³C NMR experiments. Quantitative ¹³C NMR experiments were obtained with an inter-scan relaxation delay of one minute using inverse-gated decoupling in the ¹³C NMR spectrum of 130 min experiment time. This quantitative ¹³C NMR assay has previously been validated gravimetrically.³⁸ The correlation between indirect and direct measurements of isotopologue abundance is shown in Fig. 2. Determinations through ¹H-¹H TOCSY cross-peaks and through quantitative ¹³C data yielded a Pearson correlation coefficient above 0.99. TOCSY determinations may further be improved by accounting for possible different relaxation behaviour in different isotopomers and isotopologues (Fig. S2, S3).¹¹ The ¹H-¹H TOCSY approach permits the reliable isotopic analysis of less than 90 nanomole analyte in the NMR sample on an 800 MHz instrument equipped with a TCI cryoprobe and of sub-micromole amounts on a standard 400 MHz instrument within 10 minutes (Fig. S1, ESI).

Quantitative ¹³C spectra and indirect observation in ¹H-¹H TOCSY cross-peaks provide complementary, but incomplete

information. The 1D ^{13}C spectra detect quaternary carbon positions but do not contain information on NMR-silent ^{12}C sites. The ^1H - ^1H TOCSY cross-peaks report on ^{12}C sites attached to CH groups, but do not monitor quaternary carbons. An approach to combine the sensitivity and information content of ^1H - ^1H TOCSY with the ability to detect quaternary sites was therefore sought. To this end, hydrogen was installed at the C1 position of methyl lactate. To this end, a direct reduction of the post-reaction material was conducted with NaBH_4 in the presence of catalytic amounts of sodium methoxide at room temperature. In basic solution, this approach predominantly formed borate esters of the 1,2 diol species formed by reduction of methyl lactate (Fig. 3A and Fig. S3, ESI). NMR spectroscopy of the post-reaction material validates the near-complete conversion of the methyl lactate ester group (Fig. 3B). This approach leaves olefinic positions in byproducts intact (Fig. S4 and S5, ESI),³⁵ while reducing ester groups by hydrogenation. The conversion transforms methyl lactate to a product that

Fig. 2. Correlation between isotope distributions determined with quantitative ^{13}C and with ^1H - ^1H TOCSY multiplet structures, yielding a correlation coefficient of $Q > 0.99$. A line with a slope of 1 is shown as a guide to the eye.

Fig. 3. (A) Reaction used to install protons at the C1 position of methyl lactate for detecting isotope distributions from ^1H - ^1H TOCSY cross-peaks also for the quaternary C1 site in methyl lactate (the reaction procedure is detailed in the ESI). (B) Near-quantitative conversion and identical isotope pattern in substrate and product as shown for the cross-peaks between H2 and H3 in methyl lactate.

Fig. 4. ^1H - ^1H TOCSY cross-peak for the methyl lactate signal deriving from Sn-Beta catalysed conversion of $[1-^{13}\text{C}]$ xylose, subject to reduction of the C1 positions as displayed in Fig. 3A. Resultant TOCSY signals are shown for conversions of methyl lactate in the presence of 0.5 mM K_2CO_3 .

permits the quantification of ^{13}C at all sites through ^1H -based detection (^1H - ^1H TOCSY).

TOCSY cross-peaks resulting from methyl lactate after its reduction are shown in Fig 4, allowing to determine ^{12}C and ^{13}C populations in methyl lactate. The cross-peak between H3 and H1 protons shows that the C1 position is less enriched in ^{13}C compared to the C2 and C3 positions, as little splitting by a one-bond coupling is observed in the H1 dimension of the cross peak. This observation is consistent with previous interpretations arguing against a free symmetric substrate as an on-pathway intermediate towards methyl lactate.

The novel assay was used herein to gain insight into the isotope redistribution upon changing reaction selectivity in the absence and in the presence of 0.5 mM K_2CO_3 in the reaction mixture. Alkali ions have previously been found to affect Sn-Beta action on carbohydrates, favouring 1,2-CS over 1,2-HS near 80-100 $^\circ\text{C}$,³⁹ and retro-aldol cleavage over dehydration reactions at 160 $^\circ\text{C}$.²¹ Populations of methyl lactate isotopologues were thus quantified with the ^1H -NMR based assay developed herein. Methyl lactate without ^{13}C is formed from $[1-^{13}\text{C}]$ xylose upon direct retro-aldol cleavage (between C2 and C3) of the aldose, generating a C3-fragment without ^{13}C . Methyl lactate with ^{13}C at the C1 or C3 position is formed from the ketose form after a 1,2-HS in the substrate. Methyl lactate with ^{13}C labelling of the C2 position results from an initial 1,2-CS leading to $[2-^{13}\text{C}]$ pentose, followed by aldose-to-ketose isomerization and retro-aldol cleavage of the $[2-^{13}\text{C}]$ ketopentose to a C3 fragment with ^{13}C still at the C2 position (Fig. 5).

Comparison of isotope distributions in the presence and in the absence of added K_2CO_3 shows that methyl lactate formed at conditions that are relevant for prospective chemocatalytic processes (160 $^\circ\text{C}$) results from an aldol cleavage of the aldose form of $[1-^{13}\text{C}]$ xylose for 22% of substrate molecules in the absence of added alkali ions: 22% of methyl lactate does not contain ^{13}C . Of the remaining 78% substrate molecules reacting through a ketose intermediate, 7% reacted from the $[2-^{13}\text{C}]$ ketose form upon a 1,2-CS to form $[2-^{13}\text{C}]$ methyl lactate. The remaining 71% substrate reacted through a $[1-^{13}\text{C}]$ ketopentose to $[1-^{13}\text{C}]$ methyl lactate or $[3-^{13}\text{C}]$ methyl lactate.

In the presence of 0.5 mM added K_2CO_3 , the fraction of substrate that was converted through an aldol cleavage of $[1-^{13}\text{C}]$ xylose increased from 22% to 31%. Most significantly, the

Fig. 5. Formation of different isotopologues of methyl lactate from [1-¹³C] xylose by different initial reactions of the substrate at the catalyst, in the absence and in the presence of added K₂CO₃.

fraction reacting through a [2-¹³C] ketopentose upon a 1,2-CS increased from 7% to 28%, while the fraction reacting through a [1-¹³C] ketopentose decreased from 71% to 41%. These distributions showed that the propensity of reaction flux through 1,2-CS increases relative to 1,2-HS at 160 °C, similar to previous observations in Sn-Beta catalysed isomerization reactions.^{7, 32} In addition, retro-aldol cleavage of the substrate in the aldose form is enhanced. This observation is consistent with a lower activity for competing aldose-to-ketose isomerization (1,2-HS) and higher activity for C-C breaking reactions in the presence of added alkali ions. Despite these changes in reaction preference in the presence of 0.5 mM K₂CO₃, initial 1,2-HS at Sn-Beta active sites still accounts for 41% of substrate influx into the methyl lactate pathway at 160 °C (Fig. 5).

Conclusions

In conclusion, changing preferences for the initial conversion of carbohydrate were inferred under conditions that favour conversion to methyl lactate. The conclusions were afforded by (i) site specific isotope labelling, (ii) the use of a C5 substrate leading to asymmetric aldol cleavage and (iii) a novel analytical approach permitting the distinction of all isotopic forms of methyl lactate. This approach combines the reduction of carboxylic ester groups and ¹H-based detection, and can notably be conducted using standard low-to-medium field NMR instrumentation. Isotope distributions could be measured within 10 minutes on a 400 MHz NMR instrument, if non-uniform sampling was employed in the indirect dimension (Fig. S2, ESI). Overall, these results encourage the use of indirect detection schemes and chemical introduction of hydrogen groups for probing isotope enriched positions with routine NMR instrumentation and methodology. The approach concomitantly provides high information content, quantitiveness and sensitivity, thus reducing the material demand for expensive substrates in isotope tracking studies of chemocatalytic pathways.

Financial support from the Innovation Fund Denmark (case number 5150-00023B) is gratefully acknowledged. All 800 MHz NMR spectra were recorded on the spectrometer of the NMR Center DTU supported by the Villum Foundation.

Conflicts of interest

There are no conflicts to declare.

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