



DNP NMR of carbohydrate converting enzymes

Kjeldsen, Christian; Ardenkjær-Larsen, Jan Henrik; Duus, Jens Øllgaard

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Kjeldsen, C., Ardenkjær-Larsen, J. H., & Duus, J. Ø. (2017). *DNP NMR of carbohydrate converting enzymes*. Abstract from 19th European Carbohydrate Symposium, Barcelona, Spain.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



DNP NMR of carbohydrate converting enzymes

Christian Kjeldsen¹, Jan Henrik Ardenkjær-Larsen², Jens Duus¹

¹Technical University of Denmark, Department of Chemistry, Lyngby, Denmark.

²Technical University of Denmark, Center for Hyperpolarization in Magnetic Resonance, Lyngby, Denmark
e-mail: chkje@kemi.dtu.dk

Dissolution dynamic nuclear polarization (DNP) NMR can be used to increase the sensitivity of ¹³C NMR signal by up to four orders of magnitude [1]. This allows for real time monitoring of reactions and observation of intermediates [2]. The biggest drawback of the method is the loss of polarization with T₁ relaxation, but even with this limitation, it is possible to obtain detailed reaction parameters in less than one minute. The enzyme investigated was β-galactosidase from *E. coli* (E.C. 3.2.1.23). It is well described and the mechanism is generally accepted to be a double displacement with a covalently bound intermediate, however, this evidence is based on mutant of X-ray crystallography and simulations [3]. As the natural substrate lactose does not have any quaternary carbon with long T₁, the unnatural substrate *o*-nitrophenyl β-D-galactopyranoside was used (figure 1) as the quaternary positions have T₁ relaxations of ca. 15 s instead of <2 s.

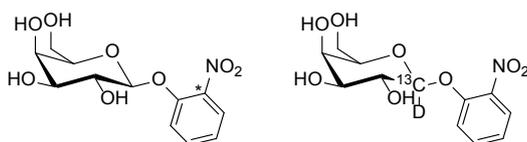


Figure 1. Left: *o*-nitrophenyl β-D-galactopyranoside. The marked (*) position is the one monitored in figure 2. Right: Doubly isotopically labelled substrate on the 1-position which increases the T₁ from <2 s to ca. 10 s.

The DNP NMR monitoring of the hydrolysis of this substrate can be seen in figure 2, and another use of this substrate is for optimizing the conditions for a labelled substrate (figure 1), which would further increase the signal and allow monitoring of the carbohydrate instead of the aglycon. This is, however, not commercially available and had to be synthesized from doubly labelled galactose.

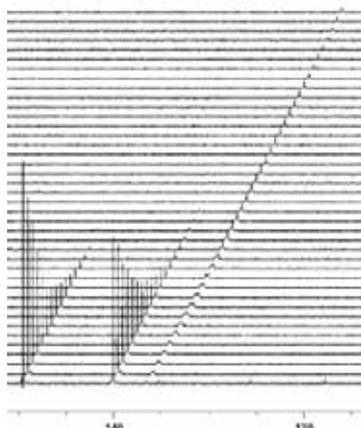


Figure 2. DNP NMR of the enzymatic hydrolysis of *o*-nitrophenyl β-D-galactopyranoside. There is 2 seconds between each 10° flip angle scan and each new spectrum is shifted 0.5 ppm.

[1] Ardenkjær-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. Increase in Signal-to-Noise Ratio of > 10,000 Times in Liquid-State NMR. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10158–10163.

[2] Jensen, P. R.; Meier, S.; Ardenkjær-Larsen, J. H.; Duus, J. Ø.; Karlsson, M.; Lerche, M. H. Detection of Low-Populated Reaction Intermediates with Hyperpolarized NMR. *Chem. Commun. (Camb)*. **2009**, 5168–5170.

[3] Juers, D. H.; Matthews, B. W.; Huber, R. E. LacZ β-Galactosidase: Structure and Function of an Enzyme of Historical and Molecular Biological Importance. *Protein Sci.* **2012**, *21*, 1792–1807.