Quantitative Analysis of Metabolic Mixtures by Two-Dimensional ^{13}C Constant-Time TOCSY NMR Spectroscopy

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Introduction

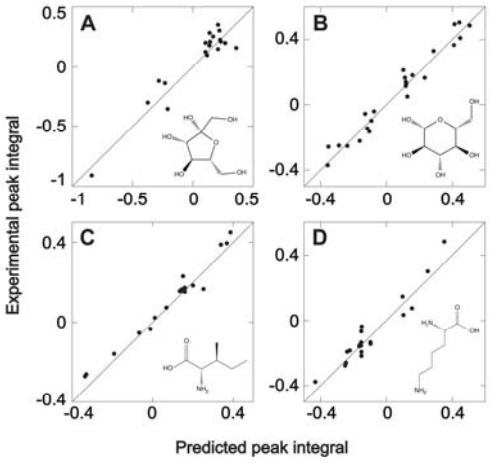
An increasing number of organisms can be fully ^{13}C-labeled, which has the advantage that their metabolomes can be studied by high-resolution 2D NMR ^{13}C-^{13}C constant-time (CT) TOCSY experiments. Individual metabolites can be identified via database searching or, in the case of novel compounds, through the reconstruction of their backbone-carbon topology. Determination of quantitative metabolite concentrations is another key task. Because significant peak overlaps in 1D NMR spectra prevents straightforward quantification through 1D peak integrals, we demonstrate here the direct use of 2D ^{13}C-^{13}C CT-TOCSY spectra for metabolite quantification. This is accomplished through the quantum-mechanical treatment of the TOCSY magnetization transfer at short and long mixing times or by the use of analytical approximations, which are solely based on the knowledge of the carbon-backbone topologies. The methods are demonstrated for carbohydrate and amino-acid mixtures [1].

Experimental

Uniformly ^{13}C labeled carbohydrate and amino acid mixtures were prepared. Quantitative 1D ^{13}C NMR reference spectra and 2D ^{13}C-^{13}C CT-TOCSY spectra at short (τ_m=4.7 ms) and long mixing times (τ_m=47 ms) were recorded. The TOCSY cross-peak volumes were computed by direct integration of the Liouville-von Neumann equation describing the spin dynamics in terms of quantum-mechanics during the 2D experiments. By comparing the computed CT-TOCSY peak volumes with the corresponding experimental volumes the relative concentrations of the different compounds were determined [1].

Result and Discussion

The figure illustrates comparison of experimental and simulated cross-peak integrals of fructose β-furanose, glucose β-pyranose, isoleucine and lysine at long TOCSY mixing-time [1,2]. The peak integrals in the figure align well along the diagonal with a correlation coefficient $R > 0.95$. At shorter mixing times, the accuracy is slightly reduced because of the smaller number of cross-peaks leading to larger statistical errors and distorted peak shapes caused by the presence of zero-quantum effects. For the short time TOCSY experiments, the numerical results were also approximated by analytical relationships providing a rapid means for quantification [1].



Quantitative comparison of experimental and simulated cross-peak integrals of 2D ^{13}C-^{13}C constant-time (CT) TOCSY acquired at long mixing-time (τ_m = 47 ms). The molecules are (A) fructose β-furanose, (B) glucose β-pyranose, (C) isoleucine and (D) lysine.

Conclusion

Introduced techniques show that quantification by 2D ^{13}C-^{13}C CT-TOCSY is feasible, overcoming the requirement of well-resolved resonances in the 1D spectrum, which should prove particularly useful for applications to highly complex mixtures with strongly overlapping peaks as is typical for real-world metabolic samples.

Acknowledgements

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References

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[2] Bingol, A.K., *et al.,* Anal. Chem., (ASAP).