ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Institute of Agricultural Sciences

ETH Zurich
Universitätsstrasse 2, LFW C48.1
8092 Zurich
Switzerland

Dr. Roland A. Werner

Tel. +41 44 632 67 54

roland.werner@usys.ethz.ch
www.gl.ethz.ch

Report on PhD thesis, Ms. Katarzyna M. Romek 15.11.2016

During the practical work for her PhD thesis with the title "Studies of isotopic fractionation of ^{13}C during biosynthesis and enzymatic reactions" Ms. K.M. Romek dealt intensively with the accurate measurement of position-specific carbon isotope distributions in several natural products by irm-NMR (isotope ratio monitoring by ^{13}C Nuclear Magnetic Resonance spectroscopy). For the intramolecular ^{13}C irm-NMR measurement of several amino acids the development of a generally applicable protocol was achieved and refined including suitable chemical prerequisites like quantitative derivatization of the amino acids and finding suitable solvents. Also optimal measurement conditions (e.g. temperature) and NMR acquisition parameters had to be found. With the method presented in the thesis position-specific ^{13}C measurement of many amino acids (ala, val, ser, ile, met, glu, tyr) is now possible. The new method does not work for gln, thr and lys. There is only a limited number of (partial) position-specific isotope analysis on amino acids available in literature, although one of the first papers on site-specific ^{13}C analysis came out already in the early 1960s (pioneering work of Abelson and Hoering on $\delta^{13}C$ of amino acids and their carboxyl groups) and despite the fact that amino acids are not only monomers of proteins but can function also as precursors of many other organic compounds like polyamines and alkaloids, to name only a few. Combining the isotopologue composition of the amino acids with information on their respective biosyntheses may give access to the isotopologue pattern of their precursor molecules like acetyl-CoA, phospho*enol*-pyruvate or organic acid molecules like oxaloacetate (retro-biosynthetic approach). The knowledge of isotopologue profiles of these very basic and central compounds might be further-on used for elucidating the biosynthesis of more complex descendant molecules.
In a further chapter of the thesis by Katarzyna M. Romek the isotope fractionation in methionine (met) metabolism is examined. Besides being a proteinogenic amino acid, the main role of the sulfur-containing amino acid methionine is the synthesis of S-adenosyl methionine (SAM), a cofactor which acts as methyl (C_1) donor in many prominent pathways. The transfer of the methyl group is mediated by the enzyme group SAM-dependent methyl transferases, converting SAM to S-adenosyl-homocysteine. The fact that molecule positions generated by C_1-transfer normally are ^{13}C depleted is known since the late 1970s, but the exploitation of this knowledge has started only recently, since a relatively easy degradation procedure coupled to an IRMS method has been developed. Several publications are showing a common feature with a distinct ^{13}C depletion in O- and N-methyl groups of bio-organic molecules (but with exceptions). Isotope effects on corresponding methyl transfer reactions were held responsible for that (exemplarily shown on catechol-O-methyltransferase COMT). The position-specific ^{13}C pattern of methionine measured by K.M. Romek shows that already the methyl group in methionine is strongly ^{13}C depleted, i.e. the isotope fractionation takes

place already during biosynthesis of methionine, leaving the possibility of an additional isotope fractionation on secondary C_1-group transfer reactions. The cobalamine-independent enzyme L-methionine synthase that transfers the methyl group from 5-methyltetrahydrofolate (5-Me-THF) to L-homocysteine was spotted as the most probable reaction for isotope fractionation. Based on an existing 3-dimensional crystal structure of the methionine synthase enzyme (available from Protein Data Bank) different models of possible reaction mechanism were modelled and the energetically optimized mechanism was further-on used to identify the rate limiting step of the enzyme-catalysed reaction. Based on this the potential energy profile during the reaction progress (reaction coordinate) could be plotted and it was possible to calculate numbers for kinetic isotope effects on the methyl carbon and the nitrogen atom of 5-Me-THF for that specific reaction showing that the breaking of that bond should be part of the rate-limiting step. The expected kinetic isotope effect on the methionine synthase reaction would then be responsible for the well-known ^{13}C depletion of C_1 groups, further secondary isotope effects on secondary methyl transfer reactions might lead to additional ^{13}C depletion.
In another chapter of her thesis Ms. Romek investigated the position-specific carbon isotope pattern in two prominent alkaloids, nicotine and tropine. The pyrrolidine moiety of both alkaloids is derived from the common precursor L-ornithine. Although the biosynthetic pathways for both alkaloids have been described extensively, not all enzymatic steps are completely understood. The carbon positions assigned to the pyrrolidine part of both molecules show similar position-specific carbon isotope ratios because of similar reactions in their biosynthesis. The position-specific ^{13}C information of the "differering" carbon atoms can be related to specific enzyme reactions downstream the setting-up of the common intermediate. The position-specific ^{13}C pattern might help to elucidate reaction mechanisms of some of the not so "well-known" enzymes in the respective biosyntheses. This part of the thesis has been already published in Journal of Biological Chemistry.
The unprecedented approach of suggesting a biosynthetic formation pathway for a natural compound, 'Tramadol', on the basis of position-specific isotope distribution ("retro-bio-synthetic") is described in yet another chapter of the thesis (already published in PNAS). The position-specific carbon isotope pattern of tramadol is used to infer a possible biosynthesis reaction scheme which could explain the measured position-specific carbon isotope pattern in contrast to a chemical-synthetic origin. The scientific debate on the natural-biological or chemical-synthetic origin of tramadol is still on-going.

In my opinion, the high amount of work presented in this thesis and the complete understanding of the subject provided by the discussion and interpretation of the results merit Ms. Romek's progression to the oral defence of her thesis.

Roland A. Werner

Zurich, Switzerland Nov. 15, 2016