13C NMR isotopomer distribution analysis: a method for measuring the synthesis of biological polymers

Caterina Puccetti[1], Tommaso Aureli[2], Cesare Manetti[1], Filippo CONTI[3]

[1]Università 'La Sapienza', Dipartimento di Chimica, Roma, Italy; [2]Department of Endocrinology and Metabolism, Sigma-Tau S.p.A. Research Labs, Via Pontina Km 30.400, Pomezia Italy; [3]Università 'La Sapienza', Diparimento di Chimica, Roma, Italy;

Introduction

^{13}C NMR spectroscopy associated with the use of ^{13}C-enriched substrates is a powerful tool to investigate intracellular metabolism because of the wealth of information contained in the distribution of isotopes in key metabolites. NMR spectroscopy measures both the amount of ^{13}C label at a single carbon positions and at neighbouring carbons by ^{13}C-^{13}C couplings, thus, information on isotopomer distribution within metabolites pool can be obtained. We present a new method of using measurements of isotopomer distribution to estimate fluxes through metabolic reaction networks. It can be applied to condensation biosynthesis reactions of the type A→nB (1) where n is the number of precursor A molecules needed to synthesize one molecule of product B. NMR isotopomer distribution analysis (NMR-IDA) involves introduction of a ^{13}C-labeled precursor and measurements of the ^{13}C positional enrichments at only one carbon atom position of polymeric product B via ^{13}C NMR spectroscopy. Information on the isotopomer distribution of B are obtained and data are analysed according to a combinatorial probability model by comparing experimental results to theoretical distribution predicted by multinomial expansion.

Methods

In this poster we present a new method of isotopomer distribution analysis (NMR-IDA). It can be applied to condensation biosynthesis reactions (polymerisation) of the type (1). Examples include the synthesis of fatty acids as the palmitate from acetate (n=8). The mathematical analysis used here for ^{13}C NMR data treatment derives from that previously proposed for mass spectrometry ones [1]. We expanded the approach used in mass spectrometry by introducing the unique concept of positional isotopomer, which can be easily estimated by ^{13}C NMR spectroscopy. The physiological model used in our analysis is representative of different conditions where the reaction described by Eq. 1 occurs in isolated living cells or whole organisms. Essentially, it reflects the synthesis of the product B from a mixture of ^{12}C- and ^{13}C-enriched precursor A molecules, which combine in an intracellular location (MixA and Synthesis B), with all the newly synthesised B molecules flowing to a compartment (Measured B) where the product B can be sampled. Tracer A and Natural A are compartments of the externally added source of ^{13}C-enriched A molecules and the endogenous sources of precursor A, respectively. In Tracer A compartment the isotopomer distribution of administered precursor A molecules is known or can be measured, while in Natural A isotopomer distribution is based on natural 1.1% abundance of ^{13}C isotope. Tracer A and Natural A supply a rapidly turning over MixA compartment which is the precursor for product B synthesis. Synthesis B is the compartment of newly synthesised ^{13}C-enriched B molecules deriving from MixA compartment. Natural B is the compartment of B molecules synthesised by metabolic pathways not involving 13C-enriched precursor A molecules, and hence, the isotopomer distribution of Natural B molecules is based on naturally occurring ^{13}C (1.1%). Measured B is a slowly turning over compartment which is supplied from both Synthesis B and Natural B compartments. In Measured B the product B molecules are sampled for ^{13}C label distribution measurements. The precursor dilution D is defined as the fractional steady-state flux of Tracer A to the rapidly turning over compartment MixA, while 1-D is the fractional steady-state flux of Natural A to MixA. The D value provides quantitative information about the relative importance of tracer versus endogenous pathway as sources of precursor A used for the synthesis of product B. The product dilution F is defined as the fractional steady-state flux from Synthesis B to Measured B compartment, while 1-F is the fractional steady-state flux of Natural B to Measured B. An important feature of NMR-IDA method is that the amount of each isotopomer of both A and B molecules is calculated as a fraction of all the isotopomers, so that the sum of these mole fractions within each compartments then equals 1 (PE). Moreover, the modelling process develops an equation for the probability of each isotopomer of A and B molecules, and because these equations are probabilities and all the probabilities are considered, then the equations sum to 1. On these bases, it is possible to compare the experimentally obtained isotopomer mole fractions with the probability-based fractions produced by the model.

Results

The modelling process develops an equation for the probability of each isotopomer of sampled product B, thus, considering both the contributing pathways to Measured B compartment we can write: MBjCi=F SBjCi+(1-F) NBjC (2). It is worth noting that SBjCi and NBjCi refer to the same kind of positional isotopomer (i.e. SB2C12 and NB2C12). NBjCi=$(0.011)^a(0.989)^b$(3) where the probability of occurrence of ^{13}C (0.011) and of ^{13}C (0.989) are elevated to the number of carbon isotopes (a for ^{13}C and b for ^{13}C) present in the considered isotopomer. The term SBjCi is the probability of B isotopomers present in Synthesis B which derive from MixA compartment. In order to quantify the probability of each isotopomer SBjCi, another flux parameter should be considered. In fact, into the MixA compartment flow together two mixtures of precursor A molecules which have different isotopomer distributions Natural A compartment and Tracer A compartment were the probability of each isotopomer TAjCi is known. On these bases, in order to calculate the probability of each precursor A isotopomer in MixA compartment, we should keep into account the mixing parameter D: MixAjCi=D TAjCi+(1-D) NAjCi (4) Using the appropriate probabilities MixAjCi, it is possible to calculate the probability of any isotopomer of newly synthesised B molecules (SBjCi) as follow: SBjCi=MixAjCi ' MixAjCi " (5) As an example, to obtain the probability of the isotopomer SB3C123, the product of the probability MixA2C12 MixA1C1 should be considered.

Discussion

In polymerisation reactions of n monomeric units the probability of formation of new spin couplings between adjacent ^{13}C atoms that occurs as a consequence of condensation event remain constant during the overall biosynthetic process. Thus, to obtain information on all possible isotopomers of the polymeric product B, it is required to measure the different positional enrichments at only one of two carbon atoms directly involved in a single condensation reaction. In ^{13}C NMR spectra we can calculate the positional enrichments at a single carbon position and equal them to the sum of suitable MBjCi: PE Cimy= SMBjCi=F $\sum$ SBjCi+(1-F) $\sum$NBjCi (6) Equation 6 can also be written as: F=(PE Cimy-$\sum$NBjCi) / ($\sum$SBjCi - $\sum$NBjCi) (7) Each PE equation will contain distinct coefficients and power terms producing independent equations. Because there are only two unknowns (D and F) and yet multiple isotopomers of B, an over determined set of equations results. independent equations. Because there are only two unknowns (D and F) and yet multiple isotopomers of B, an over determined set of equations results. The terms D and F are separable and it is possible to obtain a plot of F vs D. In fact substituting the PE values for a specific carbon position in different equations in the form of Eq. 7, we can obtain curves of F using D values ranging from 0 to 1. The intersection point of the curves represents the solution set of D and F values. In the real conditions, dispersion of experimental data will produce a relative curve shift and it will be only possible to find in the D vs F plot a zone of possible solutions. In this case, to estimate the couple of D and F values, a best-fit procedure should be used. This procedure requires an initial guess parameter values that may be obtained by inspecting the plot of D vs F. The D and F values obtained by the best visual estimate are used as input for a non-linear least-squares fit procedure to obtain actual parameter estimates. Non-linear least-squares fit procedure was developed with the assistance of Sigma-Plot (version 2.0 Jandel Scientific), a PC-based computer program with the capacity for symbolic algebraic manipulation and graphical solutions plotting. The Marquardt-Levenberg algorithm was used in the fitting procedure.

References

[1] Kelleher, J.K. & Masterson, T.M. (1992) Model equations for condensation biosynthesis using stable isotopes and radioisotopes. Am. J. Physiol., 262, E118-125.