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REVIEW PAPER

Natalia Leksa [1(AEF)], Anna Pękala [2(AEF)], Piotr Pardak [2,3(AEF)], Rafał Dziedzic [4(AEF)], Dorota Bartusik-Aebisher [5(BCDEF)], Rafał Filip [2,3(AEF)], Sabina Galiniak [5(AEF)], David Aebisher [6(ADEF)]

The potential of intracellular ^{13}C MR spectroscopy to study the absolute configuration of endogenous and polarized alanine

[1] Department of Human Anatomy, Medical College of Rzeszów University, Rzeszów, Poland
[2] Department of Gastroenterology with IBD Unit, Clinical Hospital No. 2, Rzeszów, Poland
[3] Department of Gastroenerology, Medical College of Rzeszów University, Rzeszów, Poland
[4] Department of Neurosurgey, Hospital MSWiA, Rzeszow, Poland
[5] Department of Biochemistry and General Chemistry, Medical College of Rzeszów University, Rzeszów, Poland
[6] Department of Photomedicine and Physical Chemistry, Medical College of Rzeszów University, Rzeszów, Poland

ABSTRACT

Introduction. Quantitative and accurate monitoring of tumor makes hyperpolarized carbon (^{13}C) Magnetic Resonance Imaging and Spectroscopy (MRI/S) a powerful tool for in vivo metabolic and structural study. Moreover, the studies of the properties and functions in tumor tissue of the compounds of carbon (C) that are organic, are fundamental to tumor biochemistry.

Aim. To review ^{13}C MR spectroscopy to study the absolute configuration of endogenous and polarized alanine

Material and methods. An analysis of literature regarding ^{13}C MR spectroscopy of polarized alanine.

Results. Current evidence suggests that the determination of absolute configurations of amino acids play significant role in physiological mechanisms during tumor growth and treatment.

Conclusions. Nearly 50% nuclear polarization for ^{13}C can be achieved in various organic molecules when Dynamic Nuclear Polarization DNP is performed in a strong magnetic field and at cryogenic temperatures.

Keywords. ^{13}C NMR, dynamic nuclear polarization, hyperpolarized carbon

Introduction

From all types of spectroscopy, Magnetic Resonance Spectroscopy (MRS) can provide a useful analytical tool to determine the absolute configuration (L,D) of some metabolites in biomedical assays.[1-5] All enzyme catalyzed reactions in living matter are stereospecific and provide product in enantiomeric forms. By definition, the enantiomeric forms are mirror images of each other and their high-resolution spectra recorded in achiral media are identical. However, two enantiomers will

Corresponding author: Dorota Bartusik-Aebisher, e-mail: dbartusik-aebisher@ur.edu.pl

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lose the same chemical potential and consequently will have differ MRS spectrum, while interacting with a further chiral entity.[6-9] Moreover, observation of enantiomeric composition of samples, based on MRS in the presence of the chiral shift reagent can induce precise determination of the ratios of both enantiomers and induce its separation. The chiral entity cannot only separate two forms but it also has influence on the sensitivity of recorded spectra by establishing labile diastereomeric interactions with enantiomers.[10-15] The split of the resonances of the two forms can be exploited to assess quantitatively the enantiomeric composition of a living matter. It is obvious that stereochemical considerations of the enantiomeric forms are of high importance for understanding biochemical and physiological mechanisms in cancer tissue. Determination of absolute structure of metabolites, such as amino acids, might be a sufficient way to early detection of metabolic disorders that has implications for diagnosis and treatment. In particular, in order to target a biological receptor, the specificity and chirality of the recognition site must be correct. When such peptides are composed of L-amino acids, they are rapidly degraded *in vivo* by naturally occurring proteases but when composed of D-amino acids, the peptide is resistant to degradation, giving the possibility for therapeutic use.[16-20]

Molecular asymmetry

Molecular asymmetry is fundamental for intracellular processes of chiral components in both healthy and cancer tissue. A molecule is chiral when it cannot be superimposable on its mirror image with the chiral object and its mirror image cannot be referred to as enantiomers. Almost all intracellular reactions are catalyzed by enzyme and usually provide two enanciomeric products with the absolute configuration of right-handed (D-) configuration and left-handed (L-) configuration. Recent studies have shown that both, D- and L- amino acids (AA) are present in animal and human body in high concentrations as building blocks of proteins, intermediates in metabolism and fulfill specific physiological functions. However, the structure and symmetry of organic molecules also play crucial role in many biochemical processes. One of the reasons for the increasing interest in the separation and quantification of both AA configurations lies in clinical diagnostics. The pros and cons of existing methods for enantioselective analysis of AA and their applications to biological samples are recent trends in the field but there are several research questions.[21-28]

^{13}C MRS

In particular, breast cancer cells show varied concentrations of AA using mostly chromatographic techniques; however their chirality was not studied yet. Therefore, the detection of D- and L- AA is of particular interest for our study since the determination of absolute configurations play significant role in physiological mechanisms during tumor growth and treatment. It is generally held that breast cancer cells express strong glycolytic activity and release large amounts of lactate, which can be produced or consumed with regard to the oxygenation of cells. D- and L-alanine was already found in gland and epithelial cells of mammals, but is typically quantified as the sum of their D- and L-enantiomers.[29-33] Moreover, alanine is known to be the predominant amino acid for the growth of breast cancer cells. Because the intracellular functions of alanine such as cell regeneration by L- form and damage by D- form are associated with absolute configurations, their ratio can be useful in diagnostic and therapeutic processes. We focused on the detection of absolute configuration of intracellular alanine *ex vivo* using carbon-13 magnetic resonance spectroscopy (^{13}C MRS). The chemical shift range for 13C (~250 ppm) is much larger than that for proton (~15 ppm) and the T_1 relaxation time of ^{13}C in small molecules is much longer than that of ^{1}H. However, in nature, about 1.11% of naturally occurring C is ^{13}C isotope, which is magnetically active and can be applied to probe molecular structures that correspond to physiological changes in tumor tissues. More than 98.89% of naturally occurring C is ^{12}C with no MR signal.[35-41] Several techniques have been used to overcome the ^{13}C MRS limitation by means of enhancing the polarization of nuclear spins. One of the techniques is proton decoupling, which eliminates the coupling of ^{1}H with ^{13}C by irradiating the entire ^{1}H resonance absorption range and consequently collapsing ^{13}C resonances to singlets. Although the MR signal of ^{13}C measured intracellularly corresponds to metabolic changes, signal intensity (SI) is too low to be relevant for quantitative and longitudinal study. However, the visualization of ^{13}C nuclei concentration may result in images and spectra with high signal to noise ratio (SNR) due to the hyperpolarization process. Nearly 100% nuclear polarization for ^{1}H and 50% for ^{13}C can be achieved in various organic molecules when Dynamic Nuclear Polarization (DNP) is performed in a strong magnetic field and at cryogenic temperatures. Replacing the ^{12}C (98.9% natural abundance) isotope with the ^{13}C isotope at a specific carbon or carbons in a metabolic substrate does not affect the substrate's biochemistry. With ^{13}C MRS, the body tissues are virtually invisible, and only regions where the hyperpolarized ^{13}C-labeled substance is present will appear in the generated images.[36-39] In nature, C is abundant in all forms of life and all dead organic materials. Although the MR signal of ^{13}C *in vivo* corresponds to metabolic changes, signal intensity (SI) of naturally occurring ^{13}C is too low to be relevant for quantitative and longitudinal study. However, the visualization of ^{13}C nuclei concentration may result in images and spectra with high signal to noise ratio (SNR) due to the hyperpolar-

ization process. Hyperpolarization does not change any chemical or physical properties of the substances, however it allows acquisition of ^{13}C images and spectra in a relevant time frame. Hence, hyperpolarized ^{13}C MRI/S can directly inform about the biochemical tissue composition and chemical structure by generating frequency and spatial distribution of hyperpolarized atoms. In general, most suitable ^{13}C compounds for MR are small molecules (molecular weight ~120 $gmol^{-1}$) with a possibility to obtain information about molecular behavior *in vivo* due to rapid uptake in tissue. We review the applications of ^{13}C hyperpolarized techniques such as dynamic-nuclear-polarization (DNP) to monitor tumor targeting giving an overview of rapid ^{13}C MRI/S sequences used which is followed by the hardware enhancement such as coil design.[29-33] Hyperpolarization does not change any chemical or physical properties of the substances; however it allows acquisition of ^{13}C images and spectra within a relevant time frame. Hence, hyperpolarized ^{13}C MRI/S can directly inform about the biochemical tissue composition and chemical structure by generating frequency and spatial distribution of hyperpolarized atoms. In general, most suitable ^{13}C compounds for MR are small molecules (molecular weight ~120 $gmol^{-1}$) with a possibility to obtain information about molecular behavior *in vivo* due to rapid uptake in tissue. Prior to ^{13}C MRS *ex vivo* experiments, can be use presently available ^{13}C D-, L- alanine and ^{13}C L- Alanine to confirm ^{13}C resonance assignment in water solution phantoms. In these studies, we propose the utilization of at least three chiral lanthanide complexes such as (1) lanthanum(III)-tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate, $C_{30}H_{30}F_{21}LaO_6$, (2) europium tris[3-(heptafluoropropylhydroxymethylene)-(−)-camphorate, $C_{42}H_{42}EuF_{21}O_6$ and (3) ytterbium(III)-tris[3-(heptafluoropropylhydroxymethylene)-l-camphorate, $C_{42}H_{42}F_{21}O_6Yb$, as a probe for differentiation of the enantiomeric chirality on the racemic D- and L- spectra and enantiomeric (D or L) spectra of amino acids solution.[33-36] Proposed chemical shift agents are highly water-soluble and suitable for study in the living matter. The chiral entity (1-3) cannot only separate two absolute forms of D- and L- alanine but it also has influence on the sensitivity of the recorded spectra by establishing labile diastereomeric interactions with enantiomers. The split of resonances of the two forms can be exploited to assess quantitatively the enantiomeric composition of the living matter.[37-41]

Conclusion

Due to the beneficial impact of intracellular ^{13}C MR spectroscopy to study the absolute configuration of endogenous and polarized alanine in medicine there is a growing interest in analytical identification and quantification in vitro and in vivo.

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