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Hyperpolarization: Sensitivity Boost in Magnetic Resonance Spectroscopy and Imaging

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Abstract Hyperpolarization methods are the most emerging techniques in the field of magnetic resonance (MR) researches since they make a contribution to overcoming sensitivity limitation of MR spectroscopy and imaging, leading to new fields of researches, real-time *in vivo* metabolic/molecular imaging and MR analysis of chemical/biological reactions in non-equilibrium conditions. Make use of enormous signal enrichments, it becomes feasible to investigate various chemical and biochemical systems with low γ nuclei in real-time. This review deals with the theoretical principals of common hyperpolarization methods and their experimental features. In addition, more detailed theories, mechanisms, and applications of dissolution dynamic nuclear polarization (D-DNP) are discussed.

Keywords Hyperpolarization, dissolution DNP, MRS, MRI, real-time measurement

Introduction

Magnetic resonance technique (MRS, MRI) is one of the most important analytical and diagnostic tools in the fields of chemistry, biology, and clinic. The techniques play significant roles in the investigation of structural features of small or macro-molecules, and molecular dynamic information.[1-3] Characteristics of the molecules are reflected in various magnetic resonance (MR) parameters including chemical shifts, relaxation rates, scalar or dipolar coupling, linewidth, and others, which are useful to probe the information. In spite of extensive applicability of the MR method in these fields, its use is occasionally restricted due to an intrinsically low sensitivity limitation. The sensitivity limitation is originated from a small Zeeman energy splitting of nuclear spin states, resulting in a slight population difference between of them (low polarization level). Since the first development of a commercial NMR spectrometer in 1952, there have been many efforts to improve the sensitivity of MR methods. First of all, the approaches have been mainly relied on building stronger superconducting magnets over the past few decades. Nevertheless, the polarization level of ^{1}H spins at room temperature is just 8×10^{-5} even in the highest superconducting magnet (1000 MHz ^{1}H frequency). Moreover, a wide variety of experimental and technical approaches such as Fourier transform, polarization transfer, transverse relaxation optimized spectroscopy (TROSY), cryogenically cooled probe technology, and others, have been extensively developed. These efforts have made the MR methods increasingly powerful analytical tools. Nevertheless, none of them directly address the intrinsically low polarization level of nuclear spins. As a consequence, significant current attempts are focused on a new

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category of sensitivity enhancing methods, namely hyperpolarization techniques. This review deals with theoretical aspects and practical applications of common hyperpolarization techniques. Furthermore, more detailed polarization mechanisms, experimental arrangements, and various applications of dissolution dynamic nuclear polarization (D-DNP) method, the most emerging hyperpolarization technique, are discussed.

Common Hyperpolarization Techniques

A hyperpolarized nuclear spin state denotes the spin polarization level, which is far beyond the level in Boltzmann equilibrium conditions. The hyperpolarized nuclear spin states can be accomplished by several different ways: (*i*) chemical reaction, (*ii*) optical pumping, and (*iii*) microwave irradiation. In this section, hyperpolarization methods in particular parahydrogen induced polarization, optical pumping, chemically induced dynamic nuclear polarization, and dynamic nuclear polarization, are discussed.

(1) Parahydrogen induced polarization (PHIP)

Parahydrogen induced polarization (PHIP) was first demonstrated by Bowers and Weitekamp in 1986. They found that the incorporation of parahydrogen enhances the NMR signals of unsaturated acrylonitrile with Wilkinson's catalyst.[4] Hydrogen molecules exist in two isomeric spin states, a triplet state (total nuclear spin I=1; orthohydrogen) and a singlet state (total nuclear spin I=0; parahydrogen); the ratio between the former and the latter is around 3:1 at a thermal equilibrium. Since the total nuclear spin of parahydrogen is zero, it cannot provide any NMR signals. However, the introduction of the parahydrogen into the target molecules produces enhanced population differences of the nuclear spin states of the analyte. According to experimental criteria, the PHIP is categorized into three methods, PASADENA (Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment),[5] ALTADENA (Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment),[6] and SABRE (Signal Amplification By Reversible Exchange).[7] In the PASADENA method, the hydrogenation reaction occurs inside of a high magnetic field, resulting in populated $\alpha\beta$ and $\beta\alpha$ states in the reaction product. Two hyperpolarized anti-phase doublet signals are detected since four NMR transitions are available ($\alpha\beta \rightarrow \beta\beta$ & $\alpha\alpha$, and $\beta\alpha \rightarrow \beta\beta$ & $\alpha\alpha$). In the ALTADENA method, the hydrogenation proceeds outside of the NMR magnet followed by an adiabatic transport of the product into the NMR magnet. In this method, either the $\alpha\beta$ or the $\beta\alpha$ state becomes populated. As a result, only two NMR transitions are available ($\alpha\beta \rightarrow \beta\beta$ & $\alpha\alpha$, or $\beta\alpha \rightarrow \beta\beta$ & $\alpha\alpha$). In the SABRE method, the parahydrogen is first incorporated with metal centers reversibly binding to the substrate, without a direct contact with the substrate. At a subsequent time, the hyperpolarization derived from the parahydrogen with the metal center is transferred to the substrate. Since the SABRE method does not require cooperation with the hydrogenation reaction between the parahydrogen and substrate, its use is not limited to specific substrates containing unsaturated functional groups (double, or triple bonds). The parahydrogen induced polarization has been widely applied to investigate organometallic chemistry, and magnetic resonance imaging.

(2) Optical pumping

In optical pumping, circularly polarized laser light is used to excite electrons of alkali metals (typically rubidium, or cesium) from a lower energy state to a higher energy state. A high level of the electron spin polarization of the alkali metal can then be transferred to nuclear spins of the noble gases (^{129}Xe, or ^{3}He) through collisions. This theory is known as the spin exchange optical pumping process (SIOP).[8] Optical pumping is mostly applied to the field of medical research in areas such as magnetic resonance imaging (MRI). The spin exchange polarized ^{3}He or ^{129}Xe is utilized for images of a human lung and other organs. In addition to the spin exchange optical pumping process, the polarization level of nuclear spins in a solution state can be increased by interacting with a polarized noble gas through the

spin polarization-induced NOE (SPINOE) effect.[9] Time-resolved MRIs showed spatially selective enhancements of the ^{1}H nuclear spins in solution due to the regional interactions with the hyperpolarized ^{129}Xe. The SPINOE in combination with optical pumping has been applied to the study of protein surfaces, lipid membranes, and porous materials

(3) Chemically induced dynamic nuclear polarization
Chemically induced dynamic nuclear polarization (CIDNP) was discovered by Ward and Lawler in 1967. The enhanced absorption and emission of NMR intensities were detected from chemical reactions with organolithium compounds. The radical pair mechanism, which was proposed by Closs, Kaptein, and Oosterhoff, explained this behavior of the CIDNP.[10,11] A photo-excited dye (typically a flavin mononucleotide or its derivative) in its triplet state induced by laser irradiation takes a single electron from a specific amino acid residue (normally tryptophan, histidine, or tyrosine) in polypeptides or proteins. The residue is oxidized after the interaction with the dye, resulting in the formation of a transient triplet radical pair. Two competing reaction paths that are identified with the recombination (singlet-triplet mixing) product and the escape product (radical separation) affect the outcome of the transient radical pair. Thus, the nuclear spin states of the residue can be controlled by the exchange rate between the two products, which gives rise to enhanced NMR intensities. Since the CIDNP provides site specific enhancements, the technique is mainly applied to the study of protein structures, especially for surface exposed residues, protein folding, and their interactions with oligonucleotide and carbohydrates.

Dynamic Nuclear Polarization (DNP)

(1) Overhauser DNP (O-DNP)
The Overhauser DNP (O-DNP) is the only DNP method that polarize nuclear spins directly in liquid state (usually at room temperature). The polarization transfer originates from cross relaxation processes (flip-flop (W_0) and flip-flip (W_2)), following a saturation of an EPR line of the electron (W_S) as shown in Figure 1a (Overhauser effect; energy diagram of an electrons-nuclei coupled system).[12]

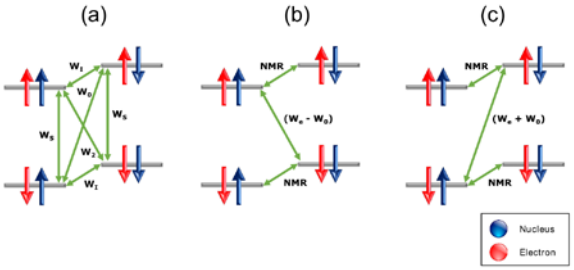

Figure 1. Energy level diagrams for the Overhauser effect (a) and solid effect (b; positive enhancement, c; negative enhancement). W_S and W_I are EPR and NMR transitions, respectively. W_0 and W_2 are zero and double quantum transitions.

The cross relaxation processes is due to the dipolar and scalar interactions between electron and nuclear spins, which are governed by rotational and translational molecular motions in the liquid state. These processes raise the population differences of the nuclear spins, giving rise to hyperpolarization of the nuclear spins. Overall polarization enhancement (ε, Equation 1) is shown through three parameters (ξ: coupling factor, f: leakage factor, s: saturation factor) and gyromagnetic ratio between the electron and nucleus.

$$\varepsilon = \frac{\langle I_Z \rangle}{\langle I_{0,eq} \rangle} = 1 - \xi \cdot f \cdot s \cdot \frac{|\gamma_e|}{\gamma_N} \qquad (1)$$

The elements and represent the expectation value of the nuclear spin polarization and the value in thermal equilibrium, respectively. The coupling factor illustrates an impact of the transition probability which is described by a ratio between the cross-relaxation rates and auto-relaxation rates. Since the coupling factor is influenced by an electron Larmor frequency ω_e, the requirement of the effect ($\omega_e * \tau_e < 1$) is well fulfilled at a low magnetic field (τ_e: electron rotational correlation time). The leakage factor represents an influence of the paramagnetic electron spin on the nuclear spin relaxations. The saturation factor explains the degree of saturation of the electron transitions by the influence of the microwave irradiation. Since the overall polarization

enhancement becomes to be less efficient at a high magnetic field strength, the polarization process and data acquisition in O-DNP method are usually conducted at low magnetic fields near ~ 0.3 T.[13] In addition, several groups proposed continuous flowing / shuttling technique where hyperpolarization first takes place in a low magnetic field (0.23 T ~ 0.34 T), and the NMR data is acquired in a high magnetic field (4.7 T ~ 14.09 T) by transferring the polarized sample.[14] Even there have been many efforts to extend the applicability of the O-DNP technique, it is generally restricted to the study of small molecules because of limited signal enhancements and sample heating problems. From the point of view, hyperpolarization of water is extensively studied for a MRI contrast agent.[15] Hyperpolarized water provided a contrast of signals from the background bulk water due to a substantial signal gain. Spin relaxation of the hyperpolarized water was utilized to study local water dynamics on the surface of bio-macromolecules with site directed spin-labeling of water with nitroxide radicals.

(2) Solid-state DNP (SS-DNP)

Solid state MAS spectroscopy in combination with the DNP technique (SS-DNP) was initially utilized for signal enhancements of polymers, carbonaceous materials, and diamonds. However, Griffin group has been expanded applicability of the SS-DNP to the study of biological macromolecules by a gyrotron oscillator producing 100 W of 140 GHz irradiation.[16] Overall 50 ~ 200 signal enhancements driven by thermal mixing mechanism was observed by employing cross polarization pulse sequences. Thermal mixing (TM) mechanism was developed with Provotov theory (thermodynamic spin ensemble models) in which a polarization transfer expressed with three interacting thermodynamic reservoirs: nuclear Zeeman (NZ), electron Zeeman (EZ), and electron spin-spin interaction (ESSI).[17] Microwave irradiation firstly cools down a spin temperature of the EZ reservoir, and followed by cooling of the ESSI reservoir which is thermally contacted with the EZ and NZ reservoir sequentially. Form the consecutive processes, polarization level of the nuclear spins can successively be enhanced. This mechanism is very efficient when high radical concentrations are involved. In this case, homogeneously broadened EPR linewidth is much larger than nuclear Larmor frequency. Since high concentrations of electrons are involved in the DNP process, the thermal mixing is a good description when the concept is too complex to define specific energy states.

On the basis of Griffin's pioneering work in DNP-MAS spectroscopy, the SS-DNP technique has been extensively applied to study several different bio-macromolecules like amyloid fibers, peptides, membrane proteins, and bacteriophases. In addition to the one dimensional (1D) SS-DNP experiments, several two dimensional (2D) homo/heteronuclear correlation spectroscopy (^{1}H-^{13}C, ^{13}C-^{13}C, and ^{15}N-^{13}C) were proposed. Since the 2D correlation spectroscopy provides a spin connectivity, the method can potentially be used for investigations of resonance assignments and intermolecular structures of proteins. Furthermore, the SS-DNP technique has also been utilized for a characterization of various solid materials. Characteristics of adsorbed substrates, distribution of surface bonding, and their interactions were studied by a surface enhanced NMR spectroscopy in cooperation with SS-DNP cross polarization spectroscopy.

(3) Dissolution DNP (D-DNP)

The first dissolution DNP (D-DNP) was reported in 2003 by Ardenkjær-Larsen and coworkers where a high level of nuclear spin polarization of ^{13}C-labeled urea (37 % polarization) was achieved by the DNP process and fast dissolution set-up.[18] Instrumental features of the D-DNP are that: (*i*) polarization increments at low magnetic fields (~ 3.4 T) and cryogenic temperatures (1.1 ~ 1.5 K), (*ii*) data acquisition at higher magnetic fields (> 9.4 T) and room temperatures followed by a fast dissolution process. During the polarization time, a small volume (1 ~ 20 μL) of analyte solution (a mixture of analyte, organic free radical, and glassing agent) is irradiated with microwave sources (~ 94 GHz; $\omega_e \pm \omega_N$). Subsequently, the polarized sample is dissolved with

a pre-heated solvent (~ 200 °C under 10 bar pressurization), and rapidly injected into the NMR spectrometer by applying high pressures of He and N_2 gases. Finally, the NMR spectra are acquired at a room temperature. Figure 2 represents a schematic diagram of the dissolution DNP-NMR arrangement with fast injection system.

Main mechanisms of the sensitivity improvements in D-DNP are the solid effect and thermal mixing. In the solid effect, forbidden transitions (W_0 and W_2 transitions; see Figure 1b, c) enhance the nuclear polarization level as high as a gyromagnetic ratio between the electron and nucleus under the appropriate assumptions ($W_e \gg W_2$ *and* $W_0 \gg W_N$). The hyperfine coupling gives rise to a mixing of spin states of the electron-nucleus coupled system under the influence of microwave irradiation. As the hyperfine term becomes time-independent, the enhancement is directly driven by saturating these forbidden flip-flop (W_0) and flip-flip (W_2) transitions at the microwave frequencies of $\omega_e \pm \omega_N$. The solid effect is potentially successful when the electron paramagnetic resonance (EPR) linewidth of the radical is much smaller than the resonance frequency of nuclear spin ($\omega_{e,1/2} \ll \omega_N$). In addition, frequencies of the forbidden transitions ($\omega_e \pm \omega_N$) should be completely resolved in order to obtain the maximum enhancement. Simultaneous saturations with opposite enhancement signs may cancel each other when they are partially overlapped. Maximum enhancement factor (Equation 2) can be higher than 10,000 times due to a large temperature factor.

$$\varepsilon' = \varepsilon \cdot \frac{B_{DNP}}{B_{NMR}} \cdot \frac{T_{NMR}}{T_{DNP}} \qquad (2)$$

With this signal enhancements by the D-DNP method, a wide variety of applications have been currently studied. In the D-DNP technique, MR spectroscopy or imaging of hyperpolarized substrates is significantly different to conventional MR methods. The hyperpolarized signals are only accessible at the beginning of the experiment because the lifetimes of the hyperpolarized states are limited to longitudinal relaxation times for the spins of interest. Consequently, particular nuclear spins (carbonyl carbon and quaternary carbon) representing long T_1 times are generally applicable for the D-DNP applications. The first D-DNP research was initially proposed by Golman and coworkers for the purpose of *in vivo* ^{13}C metabolic magnetic resonance spectroscopy (MRI) and imaging (MRI) in real-time.[19] Over the past few decades, ^{1}H based MR approaches have been played significant roles as diagnostic tools for several diseases including cancers in the clinic. In particular, the technique determines the relative concentrations of particular metabolites (biomarkers) in a given voxel. However, total number of the cancer biomarkers detectable using ^{1}H based MRS is relatively limited due to a low spectral resolution (small chemical shift dispersion, approximately 10 ppm).

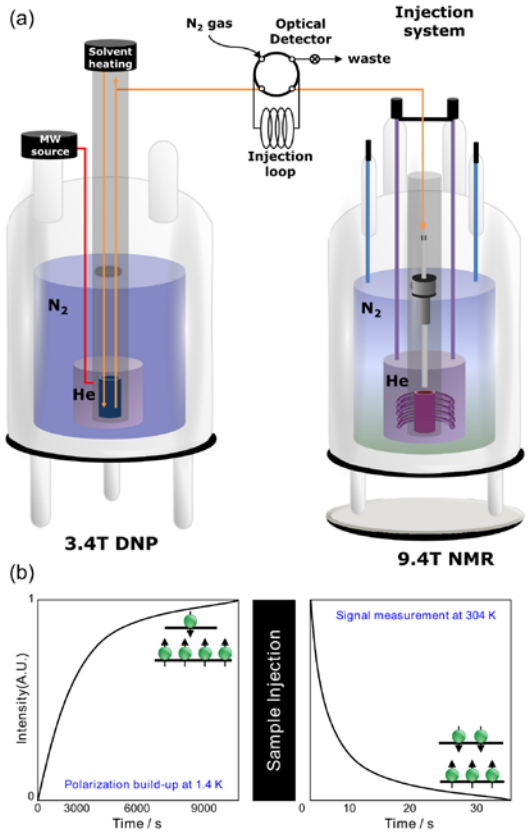

Figure 2. (a) Schematic diagram of the D-DNP NMR arrangement and injection system. (b) Solid state build-up and liquid state decay of the hyperpolarized signal

In addition, the standard technique can only provide average concentrations of the biomarkers (not a dynamic or real-time information). In this sense, ^{13}C based MRS/MRI (200 ppm chemical shift dispersion) in combination with the D-DNP technique (signal enhancement) is of particular interest since specific information about the identity and structure of biologically important compounds can potentially be obtained in real time. Thus, the ^{13}C hyperpolarization technique makes a contribution to overcoming sensitivity and selectivity limitations of the typical ^{1}H based MR methods, leading to investigate several *in vivo* metabolisms in real time.

In this area of research, hyperpolarized pyruvate can routinely be used as a metabolic tracer of cancers since the agent plays leading roles in determining the level of glycolysis, metabolic flux, and tumor responses to therapy.[20-22] The hyperpolarized substrate follows the metabolic to lactate, alanine, and bicarbonate. The high glycolysis rate of cancer cells represents an abnormally high lactate concentration in the cancer tumors. Therefore, information of the lactate distribution or mapping provides clues about the cancer location, size, and enzyme activity. The utility of DNP polarized pyruvate in metabolic imaging has been extensively studied in various cancer animal models (prostate, hepatocellular carcinoma, glioma, lymphoma, and others), and the first clinical trial was concluded recently by Nelson and colleagues.[23] The metabolic profiles and flux rates of hyperpolarized pyruvate *in vivo* substantially increase current understanding of detailed metabolisms in cancer.

In addition to the *in vivo* applications, applicability of the D-DNP technique can be extended to the field of fundamental chemistry or biology area. Investigating a chemical or biological reaction, particularly systems in non-equilibrium conditions, has commonly been a scope of optical spectroscopies by reason of high sensitivity of the optical detection. However, caused by several orders of signal enhancement, D-DNP method makes it possible to study the non-equilibrium reactions while the reactions carried on. Hilty group initially presented a time-resolved ^{13}C DNP-NMR spectroscopy where the real-time enzyme catalyzed reaction was examined under near-physiological conditions.[24] The process was monitored by observing consumption of the reactant and formation of products by using a small flip angle pulse scheme. The linear fitting of the time dependent reactant signal characterizes a catalytic property of the enzyme (k_{cat}).

The most significant feature of the real-time DNP-NMR measurement is the potential to study a reaction mechanism and kinetic information concurrently. To make a close investigation for reaction mechanisms, a temporal correlation pulse scheme (selective inversion or saturation pulses on spins of interest) was proposed to several case studies of Grignard addition, Diels-Alder reaction, polymerization reaction, and others.[25] With the pulse scheme, a spin in the reactant is selectively encoded at the beginning of the reaction, and its transfer to the reaction intermediates and products is observed. Since the hyperpolarized NMR signals flow through the reaction pathways, the treatment of the selective inversion on the reactant provides direct correlations with the intermediates and products, resulting in elucidating mechanistic and kinetic information of the chemical reactions.

Conclusions

Dissolution dynamic nuclear polarization affords the high level of nuclear spin polarization through polarization transfers from free electron spins to nuclear spins. The enhancement factor is maximized by conducting the polarization process at a cryogenic temperature and by determining optimal microwave frequencies. The resulting signal enrichment has made it feasible to acquire time-resolved MR spectroscopic data and imaging in real time, beyond out of the scope of the conventional MR approach. These capabilities of the D-DNP led to a major breakthrough in the sensitivity boost of MRI/MRS, and make the D-DNP a powerful method for the study of a variety of chemical and biological reactions

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