A new direction for polarized carbon-13 MRI

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The implementation of hyperpolarized contrast agents in MRI provides the opportunity to combine the flexibility and safety of MR-based imaging with the large signal-to-noise achievable through external nuclear polarization. Nuclei aligned in a magnetic field are termed "hyperpolarized" when they are forced into an artificially-created nonequilibrium distribution among Zeeman levels with respect to thermal equilibrium. Once removed from the magnetic field, this distribution becomes transient, exhibiting a lifetime dependent on the nuclear spin-lattice relaxation time, T_1. Hyperpolarization can be performed by using a variety of techniques such as optical pumping [spin exchange (1) and metastablity (2)], parahydrogen-induced polarization (PHIP) (3, 4), and dynamic nuclear polarization (DNP) (5) in the solid state followed by rapid dissolution (6). Although the optically-pumped noble gases ^{3}He and ^{129}Xe have exhibited potential for clinical utility in imaging lungs and sinuses (7, 8), the investigation of DNP-hyperpolarized ^{13}C liquid-phase agents for medical and diagnostic use has only recently begun. Most of the published work in this field has focused on acquiring real-time metabolic information. However, the recent work in this issue of PNAS (9) takes a completely new direction for the application of polarized ^{13}C.

DNP results when spin polarization is transferred from electrons to nuclei. Nuclear spins then become aligned to the extent that the electron spins are aligned, which at a given magnetic field and temperature is normally described by the Boltzmann distribution under thermal equilibrium. If, however, the electron-spin polarization deviates from its thermal equilibrium value, polarization transfer between electrons and nuclei can occur spontaneously through electron–nuclear cross-relaxation and/or spin-state mixing among electrons and nuclei. Accordingly, when a system with unpaired electrons, e.g., a mixture containing free radicals, is in thermal equilibrium, continuous microwave irradiation at a frequency close to the corresponding electron paramagnetic resonance frequency is required to accomplish the polarization transfer. The corresponding mechanisms for the microwave-driven polarizing processes are categorized into the Overhauser effect, the solid effect, the cross effect, and thermal mixing. Any of these mechanisms can be exploited to hyperpolarize substrates containing heteronuclei. DNP therefore requires mixing the substrate with a substance containing an unpaired electron (radical) at low temperatures (close to absolute zero) in a magnetic field. The sample is then irradiated with microwaves close to the electron resonance frequency. The process allows the transfer of the polarization (currently up to ≈10–30%) from the radical species to the labeled substrate (10–12).

Wilson et al. use "secondary labeling" to hyperpolarize a variety of amino acid derivatives.

DNP is the only known method of hyperpolarization that is not subject to severe limitations in terms of its applicability, primarily because the physical basis of DNP is not significantly influenced by the chemical properties of the substrate. Therefore, in principle, it is reasonable to expect that nearly any molecule with a high abundance of a magnetically-active nucleus is hyperpolarizable. Of course, the total achievable polarization is still subject to interplay among spin diffusion, polarization transfer, and relaxation. Successful medical imaging of hyperpolarized contrast agents, however, requires that the compound be soluble in aqueous solution and have a sufficiently long T_1 relaxation time to permit transport, administration, redistribution, and/or metabolism in vivo, and the acquisition of images and/or spectra, perhaps for several times. As a result, the practical implementation of hyperpolarized MRI or magnetic resonance spectroscopy (MRS) studies using many biologically important molecules has been hindered. Historically, these limitations have been the primary obstacles in the field of nuclear polarization, particularly with respect to the DNP method for nuclear enhancement.

A New Direction

To address these limitations, Wilson et al. (9) discuss a completely new approach to DNP. They have demonstrated that it is possible to use a simple molecule that is readily polarizable, in this case acetic anhydride, to label another molecule through a rapid chemical reaction. The concept of hyperpolarizing a fast-reacting substrate and forming biochemically interesting derivatives has clear merits. Most notably, multiple derivatives can be hyperpolarized simultaneously, which is often not possible to do directly in a DNP system. Furthermore, the difficulties with water solubility, crystallization, and polarization decay can be addressed by delaying the synthesis of the molecule of interest until the last possible moment. The system chosen here is strikingly simple, widely applicable and, as such, is quite elegant.

Wilson et al. (9) use "secondary labeling" to hyperpolarize a variety of amino acid derivatives. First, a sample of ^{13}C acetic anhydride is hyperpolarized and then it is quickly mixed with 5 amino acids. A rapid reaction occurs between the hyperpolarized acetic anhydride and the amino acids, resulting in the simultaneous production of 5 hyperpolarized amino acid derivatives. This approach introduces the potential for the application of DNP in high-throughput research. Likewise, small peptides have been hyperpolarized by using this method, even though the lifetime of the hyperpolarized state decreases with increasing size or chain length of the target tracer molecule.

The choice of acetic anhydride for secondary labeling is a great one, not only because of its fast reaction kinetics but also because of the attractively long ^{13}C relaxation time of its carbonyl carbons. Furthermore, appropriately ^{13}C-labeled acetic anhydride is not only readily available but also reasonably affordable compared with other potential reaction partners. Another attractive aspect of the implementation of secondary labeling is its relatively universal nature; although the posthyperpolarization chemistry is in some ways similar to that of the PHIP approach, potential precursors are generally commercially available, effectively eliminating the requirement for expensive custom syn-

Author contributions: R.R. wrote the paper.
The author declares no conflict of interest.
See companion article on page 5503.
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www.pnas.org/cgi/doi/10.1073/pnas.0901843106

thesis. Most amino groups will react sufficiently fast to provide hyperpolarized N-acetylated derivatives. The only drawback to this approach is that so far it requires an organic solvent, here *p*-dioxane, a consequence of acetic anhydride's high reactivity with water, which precludes in vivo use. Nevertheless, the concept is sound and minor modification is likely to open up new areas for the application of hyperpolarization. As it stands, the technique can be used for in vitro measurements, e.g., increasing the detection threshold of the relevant molecules to allow parallel quantification of a wide variety of amino-containing molecules at very low concentration.

Future Possibilities

To put the recent progress into perspective, it is worthwhile to note that the field of polarized ^{13}C is still at a very early stage, and this method will likely be combined with other ongoing work before an approach with clear clinical utility is developed. Each of these areas could benefit, and benefit from, the methods described by Wilson et al. (9). Examples of these improvements include: polarization at higher magnetic field, methods for relaxation time elongation, and techniques to address the low aqueous solubility of many interesting compounds.

The DNP system used by Wilson et al. (9) is commercially available and operates at a static field of ≈3.35 T. The basic principles of DNP suggest that increasing the strength of the magnetic field will improve the production rate and the ultimate achievable polarization level. Additionally, increased throughput of highly-polarized samples has been deemed essential for advanced animal research with DNP ^{13}C MRI and MRS. Recent experimental results by Johannesson et al. (13) suggest that a substantial increase in percentage polarization can be achieved by just increasing the magnet field from 3.35 to 4.6 T. Specifically, these studies show that this increase in magnetic field strength resulted in an improved polarization of pyruvic acid in solid state to ≈64%. The results of this study suggest that the polarization level dependence on magnetic field is surprisingly strong, and increasing the field strength is one of the most attractive means of enhancing the polarization of nuclear spins.

The implied benefit for the present work is substantial, because the signal gains achieved in substrate hyperpolarization translate directly to the signal achievable in the derivative molecule. Suppression of the thermal processes that cause relaxation to thermal equilibrium is perhaps the most challenging aspect of this research. However, even here, methods have been proposed and demonstrated that may help alleviate this obstacle substantially, including decoupling of high-gamma nuclei (14, 15), minimization of chemical-shift anisotropy at low field (16, 17), and preparation of symmetric states of multiple nuclei that persist longer than the individual nuclear T_1s (18).

A third factor that plays an important role in practical DNP experiments is the solubility of the target molecule, both because a large final concentration is typically desirable and spin diffusion in the DNP process itself is more rapid when the average distance between ^{13}C-labeled molecules is kept small. As demonstrated by Gallagher et al. (19), attempts to polarize sodium bicarbonate were much more successful when the more soluble cesium bicarbonate was used, and the counterion was exchanged after dissolution. Broadly analogous and applicable to the present work, similar techniques can been used to overcome the solubility limitations of many interesting biological molecules.

Progress in DNP and hyperpolarized NMR research in general will likely require application of some or all of these methods. The approach by Wilson et al. (9) adds a new, well-thought-out avenue for exploration that is specifically applicable to an interesting class of molecules; i,e. any species that can be acetylated reasonably, efficiently, and quickly can now also be hyperpolarized. It is conceivable that the hyperpolarization introduced into the ^{13}C nucleus of the carbonyl carbon of the acetylated products may subsequently be transferred to other appropriate, NMR-active nuclei, for instance, its adjacent ^{15}N neighbor. Moreover, consideration of the general approach may lead to other precursors, functional groups, and classes of targets, which are amenable to an analogous chemical transformation.

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