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Nuclear Spin Hyperpolarization of the Solvent Using Signal Amplification by Reversible Exchange (SABRE)

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Abstract

Here we report the polarization of the solvent OH protons by SABRE using standard iridium-based catalysts under slightly acidic conditions. Solvent polarization was observed in the presence of a variety of structurally similar N-donor substrates while no solvent enhancement was observed in the absence of substrate or para-hydrogen (p-H₂). Solvent polarization was sensitive to the polarizing field and catalyst:substrate ratio in a manner similar to that of substrate protons. SABRE experiments with pyridine-d₅ suggest a mechanism where hyperpolarization is transferred from the free substrate to the solvent by chemical exchange while measured hyperpolarization decay times suggest a complimentary mechanism which occurs by direct coordination of the solvent to the catalytic complex. We found the solvent hyperpolarization to decay nearly 3 times more slowly than its characteristic spin-lattice relaxation time suggesting that the hyperpolarized state of the solvent may be sufficiently long lived (~20 s) to hyperpolarize biomolecules having exchangeable protons. This route may offer future opportunities for SABRE to impact metabolic imaging.

Graphical Abstract

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Keywords

hyperpolarization; SABRE; solvent enhancement; iridium catalyst; MR imaging; chemical exchange

1. Introduction

In the last few decades magnetic resonance (MR) techniques have greatly expanded our understanding of metabolism and metabolic flux [1–4]. A disadvantage of MR, however, has been its lack of sensitivity compared with other imaging modalities like optical, positron emission tomography (PET), and single-photon emission computed tomography (SPECT). MR sensitivity can be enhanced by 4 to 6 orders of magnitude by means of hyperpolarization, whereby a fraction of the population in the excited state is redistributed to the ground state. One of the most widely used methods of hyperpolarization, dynamic nuclear polarization (DNP), uses microwave irradiation to transfer electron polarization from an added free radical to nuclear polarization at ultra low temperature after which the sample is quickly warmed and transferred to a high field magnet for spectroscopic imaging [5,6]. The DNP method is able to polarize a wide variety of molecules which can be used to follow metabolism in cells [7,8] animals [9] and even to diagnose cancer in human subjects [10]. The biggest drawback of DNP is the lengthy time for sample polarization, typically ranging from 1–4 hours, and the expense and size of the instrumentation. Other methods for hyperpolarization include spin-exchange optical pumping [11] and para-hydrogen induced polarization (PHIP) [12,13]. Using PHIP para-hydrogen ($p\text{-H}_2$), the spin singlet state of H_2 , is catalytically added across an unsaturated bond in the substrate in such a way that the symmetry of the singlet state is broken and polarization is transferred to other nuclei by means of coupling [14,15]. In comparison to DNP, PHIP is relatively inexpensive, requiring only a catalyst, an unsaturated substrate and a source of $p\text{-H}_2$. The drawback of PHIP is the necessity for a precursor molecule having an unsaturated bond. Over the past decade, only a few unsaturated substrates have been used with PHIP to obtain metabolic data in animals [16–20].

In 2009, an alternative method known as signal amplification by reversible exchange (SABRE) was introduced whereby polarization is transferred from $p\text{-H}_2$ via a metal bound

hydride to an exchanging substrate [21–25]. The efficiency of polarization transfer reportedly depends on the frequency difference between protons on the substrate and the hydride relative to their coupling constant and the lifetime of the substrate-bound catalytic intermediate [24]. Initially, various forms of $[\text{Ir}(\text{COD})(\text{PR}_3)]\text{Cl}$ (COD = cyclooctadiene, PR_3 = PPh_3 = triphenylphosphine; PCy_3 = tricyclohexylphosphine) were used as catalytic templates. Following formation of an Ir-dihydride complex, the $p\text{-H}_2$ singlet state is destroyed and polarization is transferred to the trans, equatorial exchanging substrates via spin coupling at low magnetic fields [22,24–26] or by nuclear spin cross relaxation at high magnetic fields [27]. Ortho-hydrogen ($o\text{-H}_2$) and the hyperpolarized substrates then exchange off the complex and the cycle is repeated (Scheme 1). More recently it has been shown that iridium N-heterocyclic carbene (NHC) complexes have greater electron donating ability and optimum lifetimes, increasing the efficiency of polarization transfer [28–30]. With few exceptions, most SABRE substrates are aromatic nitrogen-containing compounds where the basic nitrogen acts as the electron donor to the metal, likely reflecting the preference of third row metal ions such as platinum and iridium for somewhat softer N-based ligands [21–23,25–35]. The SABRE experiment is carried out by dissolving the complex and substrate in a methanolic solution and bubbling or shaking the sample with several atmospheres of $p\text{-H}_2$ in the fringe field of the NMR magnet or in a polarizer equipped to generate fields on the order of several mT [36,37]. Recently, it has been shown that substrates can be enhanced using a catalytic complex soluble in an alcoholic water solution or attached to a solid support [26,34,38,39].

An initial goal of our investigations was to determine whether substrates more commonly used in *in vivo* MR metabolic studies (i.e., pyruvate, lactate, and TCA cycle intermediates fumarate and succinate) could be polarized using the SABRE method. While we found no examples of non-aromatic nitrogen containing metabolites that could be polarized using SABRE, it occurred to us that substrates excluded from the catalytic complex might be polarized indirectly by proton chemical exchange. In fact this same strategy has recently been used to transfer polarization from water hyperpolarized by DNP to amide protons and ^{15}N of arginine and urea [40]. Several reports of the participation of methanol (MeOH) in the catalytic complex have been reported. Fekete *et al.* [29] confirmed the presence of $[\text{Ir}(\text{H})_2(\text{MeOH})(\text{py})(\text{IMes})(\text{PPh}_3)]\text{Cl}$ (py = pyridine; IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene) by multinuclear NMR. Eshius *et al.* [32] proposed the presence of $[\text{Ir}(\text{IMes})(\text{H})_2(\text{py})_2(\text{MeOH})]\text{Cl}$ to account for the reduced enhancement observed at trace concentrations while Lloyd *et al.* [36] confirmed the presence of this complex as well as $[\text{Ir}(\text{IMes})(\text{H})_2(\text{py})_2(\text{H}_2\text{O})]\text{Cl}$ at low temperatures. Drücker *et al.* [35] observed a 2 to 5-fold enhancement of the methanol OH proton using the $[\text{Ir}(\text{COD})(\text{PCy}_3)(\text{py})]^+$ catalyst and a pyrazole substrate while others have observed a NMR signal from H-D, likely produced by reaction of an Ir-hydride with an exchangeable deuterium ion donated to the catalytic complex by the solvent [31]. Here, we report that one can amplify solvent enhancement up to 40-fold using $[\text{Ir}(\text{COD})(\text{IMes})]\text{Cl}$ (Ir-IMes) or $[\text{Ir}(\text{COD})(\text{SIMes})]\text{Cl}$ (Ir-SIMes) as catalysts under slightly acidic conditions in the presence of an aromatic nitrogen-containing substrate. No solvent enhancement was observed in the absence of substrate or absence of $p\text{-H}_2$. These conditions seem to work with a variety of structurally similar substrates, even those such as pyridine containing only a single aromatic ring nitrogen.

2. Experimental Section

Materials

[Ir-(μ -OMe)(COD)]₂ was obtained from Strem Chemicals (Newburyport, MA). Methanol-d₄ (99.97% enriched in ²H) was obtained from Cambridge Isotope Laboratories. All other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO) and were used without further purification.

Preparation of [Ir (COD)(IMes)]Cl (Ir-IMes)

Ir-IMes was prepared from [Ir-(μ -OMe)(COD)]₂ and 1,3-Bis(2,4,6-trimethylphenyl)imidazolium chloride (IMes•HCl) according to published procedures [41]. Exactly the same procedure was used for the synthesis of [Ir(COD)(SIMes)]Cl (Ir-SIMes) using 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazolium chloride (SIMes•HCl) in place of IMes•HCl. The crude product was then purified first by column chromatography (8:1 DCM/Acetone, R_f = 0.95) followed by a recrystallization in EtOH/hexanes mixture to yield 800 mg of product (74 % yield). Synthesis of 1*H*-[2-¹³C]Imidazolium-1,3-bis(2,4,6-trimethylphenyl) HCl salt was taken from the literature [42] and is described in detail in the section on Supplemental Information. [Ir(COD)([2-¹³C]IMes)]Cl was prepared using the same procedure as the Ir-IMes catalyst [41].

NMR Sample Preparation

Samples for ¹H NMR SABRE experiments were prepared by dissolving Ir-IMes or Ir-SIMes in 3 mL of methanol-d₄ to 4 or 5 mM. Particularly in the case of the SIMes catalyst, dissolution required gentle warming and stirring. Once the catalyst was dissolved the substrate was added. Samples were prepared fresh daily. In some samples, 15 μ L of 1 M aqueous HCl was added to the 3 mL sample to yield a solution 5 mM in HCl and 0.27 M in H₂O (acidic conditions). For samples in which the concentration of HCl was varied, the initial stock HCl solution was diluted so the H₂O concentration remained constant.

SABRE ¹H NMR Experiments

p-H₂ used for SABRE NMR experiments was generated by passing hydrogen over a metal catalyst at low temperature (36K) (Bruker Instruments, Billerica, MA) [36,37]. This procedure yielded 92.5% p-H₂. SABRE experiments were carried out in a mixing chamber holding 3 mL of solution containing the substrate and catalyst. The mixing chamber was located directly below the 14.1 T NMR magnet such that there was a fringe field of 15 mT from the spectrometer magnet. The mixing chamber was surrounded by a solenoid which was computer controlled to yield fields from -14 mT to 14 mT. When added to the fringe field of the spectrometer magnet the net field could be varied from 1 mT to 29 mT. For conventional SABRE experiments substrates were polarized by bubbling p-H₂ enriched gas through the mixing chamber for 8 s at slightly more than 1 atm. Following the polarization period, the sample was pneumatically driven to the coil of an NMR flow probe installed in a Bruker Avance II 600 MHz spectrometer and a spectrum was acquired (at 14.1 T). Studies were carried out under both neutral and acidic conditions at 21 °C using methanol-d₄ as a solvent. SABRE enhancements were measured using pyridine, pyrazole, pyridazine and

imidazole as substrates with both the Ir-IMes and Ir-SIMes catalysts. Thermal spectra were acquired by destroying the magnetization of the substrate with a 90° pulse and waiting 5 min for the sample to re-equilibrate inside the spectrometer before acquiring a thermal spectrum. Enhancements were calculated as the ratio of peak areas from the SABRE and thermal spectra. Exchange rates for substrates were measured using 1D EXSY experiments, as previously described [22,28,30].

SABRE Decay

We measured the time it took SABRE enhanced resonances to decay to equilibrium. Following the bubbling of p-H₂ for 60 s, the catalyst containing sample was immediately transferred to the flow probe (at 14.1 T) where we introduced a variable wait time, τ , before applying the 90° read pulse and acquisition. Signals were then integrated and processed to yield the percent of the initial signal intensity, α , where

$$\alpha(\tau) = Abs \left(100 \bullet \frac{[M(\tau) - M(\infty)]}{[M(0) - M(\infty)]} \right) \quad (1)$$

Here $M(t)$ is the signal integral at wait time τ and $M(0)$ and $M(\infty)$ are signal integrals at the shortest and longest values of τ (0.5 s and 256 s). $\alpha(\tau)$ was then fit to a single exponential decay using a two parameter non-linear fitting routine

$$\alpha(\tau) = \alpha(0)e^{-\tau/T^*} \quad (2)$$

to yield $\alpha(0)$ and T^* , the characteristic decay rate in the presence of the SABRE catalyst. Proton T₁s were measured by inversion recovery on the same samples under identical conditions without p-H₂ bubbling.

3. Results and Discussion

Optimization of Catalyst

Previous studies have reported that the magnitude of SABRE enhancements depend upon the dissociation rate of the substrate from the Ir-complex [28,30,36]. In the case of N-heterocyclic-iridium (NHC-Ir) catalysts, these dissociation rates depend on the buried volume and electron donating ability of the NHC ligand and the substrate structure [30]. In light of these results, we anticipated that the optimum enhancements for the substrates and solvent would vary with the structure of the Ir-catalyst and for this reason we chose to compare the ability of Ir-IMes and Ir-SIMes to polarize 4 target substrates; pyridine, pyridazine, imidazole and pyrazole (Chart 1). Table 1 summarizes the SABRE enhancement results for substrate protons. For enhancements of pyridine protons, Ir-IMes was superior to Ir-SIMes by approximately a factor of 2, supporting previous results [30]. For other substrates, Ir-SIMes was equal to or better than Ir-IMes for signal enhancement of most protons, exceptions being H4/H5 of imidazole and H4/H5 of pyrazole. Pyrazole was 1.2–1.7-fold better enhanced with Ir-SIMes while imidazole-H2 improved by ~ 5-fold with Ir-SIMes compared to Ir-IMes. Given these observations, most SABRE data were collected for pyridine using the Ir-IMes catalyst and for the remaining substrates using the Ir-SIMes catalyst.

SABRE Hyperpolarization of Substrate and Solvent

Previous results suggest two mechanisms for transfer of polarization from p-H₂ to substrate in SABRE experiments [24,27]. For both mechanisms, p-H₂ displaces the COD on the Ir-catalyst and the symmetry of p-H₂ is broken (Scheme 1). At low but non-zero field, such as the polarizing field used in these studies, the interplay between chemical shift evolution and scalar coupling between the hydride protons and the substrate protons create single spin longitudinal z-magnetization [21–25,28,29]. The chemical shift interaction between this z-magnetization and zero quantum states introduces a complex field dependency in the polarization of protons of the substrate. Figure 1A shows the SABRE effect for the ortho-, meta- and para-protons of pyridine as the polarizing field is changed from 1 to 29 mT in 2 mT increments. Maximum polarization is observed for these protons between 19 and 27 mT. Also visible in the spectra is o-H₂ (~4.55 ppm) and the residual hydroxyl protons (OH) of the deuterated solvent (CD₃OH/HOD at ~ 4.9 ppm).

Since the p-H₂ used in these experiments contained 7.5% o-H₂ and the hydrogen concentration in methanol was about 4 mM (at 1 atm) [43], o-H₂ could be detected even in the absence of SABRE catalyst. Close inspection reveals that the intensity of the solvent OH protons changes with polarizing field so that the signal is undetectable in spectra acquired at polarizing fields less than 11 mT but is quite visible at higher polarizing fields. The intensity of this signal is never greater than its intensity in the spectrum acquired under thermal conditions, so there is no overall enhancement of the signal at any polarizing field strength. Slight net polarization of the solvent OH has been previously observed in SABRE experiments [35].

Figure 1B shows a similar field sweep SABRE experiment for pyridine following the addition of aqueous HCl to a concentration of 5 mM. While under these conditions we obtained slightly less polarization of the pyridine protons, we obtained a nearly 40-fold enhancement of the solvent protons (0.2% polarization). Figure 2 summarizes the enhancement for substrate and solvent OH protons as a function of polarizing field strength for 4 different substrates.

Little solvent enhancement was observed using imidazole as the substrate. For the other substrates the maximum enhancement of the solvent OH appears to correspond to the field at which the maximum enhancement is also observed for the substrate protons, suggesting that the mechanism of SABRE polarization is similar. No enhancement was observed for the residual solvent OH in the absence of substrate or p-H₂.

In an effort to maximize solvent enhancement under acidic conditions, field sweep experiments were run at different substrate:catalyst ratios. These experiments were run by holding the concentration of catalyst constant at 4 mM while varying the substrate concentration. Figure 3 summarizes the maximum enhancements observed under acidic conditions. Little solvent OH enhancement was observed for imidazole/Ir-SIMes at any substrate concentration. A maximum was observed in substrate and solvent OH enhancement as a function of substrate concentration for pyridine and pyrazole between 80 and 100 mM. In contrast, maximum enhancement of pyridazine protons was observed near 40 mM and the enhancement of the solvent OH decreased between 20 and 100 mM. In

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general these experiments suggest that SABRE polarization of the solvent OH and the substrate protons reach a maximum at about the same substrate:catalyst ratio. Others have shown that the enhancement of the substrate protons decreases at high catalyst:substrate ratios as a result of a decrease in the T_1 of the catalyst while holding the concentration of the substrate constant have shown that the polarization transferred to the substrate increases linearly as the ratio of catalyst:substrate increases [22,28,36]. Our results are in reasonable agreement with these results under neutral conditions with imidazole or pyridazine as substrates (Fig. S2 and S4). In contrast, under acidic conditions, our results suggest a maximum enhancement is reached at an optimum catalyst:substrate ratio such that enhancement is reduced when this ratio is very high or very low. Quenching of the SABRE enhancement at high bound substrate, thus effectively shortening the time allowed for polarization transfer [32]. Conversely, at low substrate:catalyst ratios, the solvent competes for one of the substrate sites in the metal complex leading to less effective enhancement of the substrate [32,36]. The conditions under which the residual solvent OH polarization is maximized for different substrates are summarized in Table 2.

In addition to solvent OH protons, enhancements were also observed for the methyl protons of CD_2HOD and CH_3OD contained in the 99.97% enriched CD_3OD . These enhancements as well as enhancements for the pyrazole substrate protons are shown as a function of hydrogen ion concentration in Figure 4. Enhancements of -1 to -2 were observed from the solvent methyl protons and enhancements of up to -3 were observed for solvent OH protons at H^+ :catalyst ratios less than unity. This result agrees with results previously observed by Drücker *et al.* [35]. When a H^+ :catalyst ratio of ~ 1 is reached, the enhancement of the substrate protons ortho to the nitrogen (peak 3,5) changes sign and the enhancement of the solvent OH protons dramatically increases. This effect was found to be independent of overall substrate concentration. No enhancements were observed for the methyl protons of residual CD_2HOD and CH_3OD when substrates other than pyrazole were used.

Mechanism of Solvent Proton Enhancement Using SABRE

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In the past, 1D EXSY experiments have been used to measure exchange rates of the substrate and attempts have been made to correlate these to observed enhancements. In some cases these exchange rates have been quite successfully used as a guide to design better SABRE catalysts [22,28,30,32,36]. In light of these experiments, we attempted to correlate substrate exchange rates measured using 1D EXSY experiments with observed substrate and solvent enhancements. The results of these experiments are presented graphically in Figure 5 (and tabulated in Table S1 of the Supplementary Information). Figure 5A shows that the measured k_{diss} for all substrates was greater using the Ir-SIMes catalyst compared to the Ir-IMes catalyst under both neutral and acidic conditions. Furthermore k_{diss} was much greater for pyridine than for other substrates. Values obtained for pyridine using the Ir-IMes or Ir-SIMes catalyst are in reasonable agreement with those previously reported. Disappointingly, there seems to be no obvious correlation between k_{diss} and enhancement values measured for the substrate protons or the solvent OH protons. In summary, neither the substrate nor solvent enhancement seem to be determined only by the substrate exchange rate.

Drücker *et al.* previously observed slight enhancement of the residual solvent OH under neutral conditions in SABRE experiments with pyrazole but failed to see any enhancement when pyridine was used as a substrate [35]. They suggested a mechanism where the polarization of the solvent was acquired by exchange of the proton onto the nitrogen not participating in bonding to the metal center of the Ir-IMes complex. Presumably the residence time of this exchangeable proton on pyrazole would be long enough to acquire SABRE polarization. In contrast to their results, our data show nearly a 40-fold solvent OH enhancement with pyridine as a substrate under mildly acidic conditions. Since pyridine has only one nitrogen available to coordinate to iridium, a similar mechanism would require chemical exchange to take place after the pyridine had dissociated from the metal complex. In this case hydrogen ion available in acidic solution (from added aqueous HCl) would be transferred to free pyridine from CD_3ODH^+ (and other protonated solvent species including H_3O^+) and be hyperpolarized via spin coupling to other pyridine ring protons (Scheme 2). In order to test this mechanism we carried out SABRE using pyridine- d_5 as a substrate with added 5 mM aqueous HCl. Figure 6 shows thermal and SABRE hyperpolarized spectra of pyridine (A & B) and pyridine- d_5 (C & D). We observed a 21-fold enhancement of the solvent OH in the SABRE spectrum of pyridine but only a 3.6-fold enhancement of the solvent OH in SABRE spectrum of pyridine- d_5 suggesting that this mechanism makes a 6-fold contribution to solvent hyperpolarization. However, the observation of a non-zero enhancement of the solvent when pyridine- d_5 is used as the Ir-IMes substrate suggest that other mechanisms may also contribute to the polarization of the solvent.

Recently others have developed methods for the observation of SABRE enhancements at high magnetic field either through cross relaxation [27], the transfer of hydride singlet spin order into bulk magnetization of the substrate using a sequence of low power pulses [44] or by Level Anti-Crossing using high-power spin-locking fields [45]. Knowing this, we hypothesized that it might be possible to transfer hyperpolarization of the solvent protons to biomolecules such as labile NH protons by chemical exchange, should the hyperpolarization of the solvent be sufficiently long lived. To this end we measured decay times of the hyperpolarized substrate protons and the solvent OH protons in solutions containing pyridine and Ir-IMes catalyst in methanol- d_4 under acidic conditions. Following p-H_2 bubbling at low magnetic field the sample was transferred to high magnetic field where we imposed a variable wait time (τ) before applying the 90° read pulse. Figure 7A shows the SABRE spectra obtained and Figure 7B shows a single exponential fit to the data for pyridine substrate protons and solvent OH protons.

Table 3 summarizes the fitted decay times (T^*) and compares them to spin-lattice relaxation times at high field in a sample identical to that used for SABRE measurements.

T^* values for the ortho- (H2, H6) and meta-(H3,H5) aromatic ring protons on pyridine are about 20% larger than their T_1 s while the T^* of the para-proton (H4) is nearly 60% larger than its T_1 . It may be that differences between T_1 and T^* arise from the continued exchange of dissolved p-H_2 on the catalytic complex at high field and the enhancement of the pyridine protons from a cross relaxation mechanism as previously described [27]. It is unclear why the difference between T_1 and T^* should be greatest for the H4 proton since this proton is farther away from the hydride protons than are the ortho- or meta-protons. Surprisingly, the

T^* of the solvent OH protons are nearly 3 times their T_1 and 1.6 times as long as the longest T^* of the ring protons (20.2 s for the solvent protons compared to 12.2 s for H4). The large difference between T^* of the pyridine protons and the T^* of the solvent protons suggests that the solvent protons are at least partially enhanced by a mechanism other than by the proton exchange mechanism described in Scheme 2. A plausible alternative mechanism is by direct coordination of the solvent to the catalytic complex allowing for the same high field NOE effect as was described for the pyridine protons. Others have confirmed the presence of a MeOH substrate as a SABRE catalyst by proton and multinuclear NMR and by theoretical calculations, supporting the hypothesis that the solvent may be polarized in a manner similar to the pyridine substrate [29,32,36]. In summary, our experiments with pyridine- d_5 suggest that the solvent is hyperpolarized by a SABRE mechanism involving the exchange of the solvent OH proton with the labile proton on the pyridine nitrogen (Scheme 2) while our decay studies suggest a complimentary mechanism involving formation of a catalytic complex involving the direct coordination of the solvent. We suggest that both mechanisms contribute to the solvent enhancement, explaining the residual hyperpolarization of the solvent OH when pyridine- d_5 acts as a substrate in place of pyridine. Furthermore, we have discovered that under the SABRE conditions used in these experiments the hyperpolarization of the solvent is long lived whose decay time (T^*) is roughly 3 times longer than its T_1 (~20 s compared to ~7 s). This should allow for hyperpolarization of biomolecules having exchangeable protons.

Magnitude of Solvent Enhancement

We also did experiments where the concentration of water added to our SABRE solutions was increased. When the water concentration of a solution containing 80 mM pyridine, 4 mM Ir-IMes, 5 mM HCl(aq) was increased from 0.27 M to 3 M the solvent OH enhancement dropped from nearly 40-fold (Table 2) to only about 3-fold (data not shown). Clearly hyperpolarization of the solvent is limited by the concentration of exchangeable protons relative to the concentration of catalyst and hyperpolarized pyridine. As expected the magnitude of the enhancement was also limited by the concentration of residual solvent protons in the SABRE system before experiments. Typically we dried our system with nitrogen for at least 45 min before measurements.

4. Conclusions

We have discovered that under slightly acidic conditions solvent protons can be polarized up to nearly 40-fold using SABRE. No such effect was observed in the absence of substrate, $p\text{-H}_2$ or catalyst. The difference in the solvent enhancement between neutral and acidic conditions was marked where the enhancement seen under acidic conditions was at least an order of magnitude greater than that previously seen by others under neutral conditions [35]. Furthermore, the extent of solvent enhancement was found to depend on the substrate and catalyst used and correlated roughly with the enhancement of the substrate protons as a function of the polarizing field and catalyst:substrate ratio. Although our EXSY data supported a faster exchange rate of the substrate on the Ir-catalyst under acidic conditions, we found no obvious correlation between this exchange rate and the magnitude of the solvent enhancement (Fig. 5). Experiments where pyridine- d_5 was used as a SABRE

substrate in place of pyridine showed the solvent OH enhancement was reduced by nearly 6-fold, suggesting a mechanism where polarization is transferred from the free substrate to the solvent by chemical exchange (Scheme 2). We found the decay time of the solvent hyperpolarization was nearly 3 times its T_1 and at least 1.6 times the hyperpolarization decay time for the pyridine protons, suggesting an alternative mechanism for polarization to occur by direct coordination of the solvent in the SABRE catalytic complex. Experiments at high field suggest that in the latter case the solvent is enhanced by cross relaxation to the hyperpolarized hydride protons [27]. Our studies suggest the hyperpolarization of the solvent may be sufficiently long lived to polarize biomolecules having exchangeable protons using recently developed high field SABRE polarization methods [44,45]. Transfer of hyperpolarization by chemical exchange has already been demonstrated in the case of water hyperpolarized by DNP to ^{15}N -labeled biomolecules and this route may offer future opportunities for SABRE to impact metabolic imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A: Supplementary Material

Supplementary material associated with this article may be found in the online version

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Highlights

- Under acidic conditions the solvent can be hyperpolarized using SABRE
- Solvent hyperpolarization takes place by chemical exchange
- The solvent hyperpolarization is longer lived its T_1
- Method shows potential for hyperpolarizing biomolecules with exchangeable protons

Figure 1.

(A) Thermal ^1H NMR spectrum (bottom trace) and SABRE spectra at 1 mT - 29 mT of 80 mM pyridine in 99.97% enriched methanol- d_4 using 4 mM Ir-IMes catalyst. (B) Series of thermal and SABRE spectra acquired as in (A) after addition of HCl(aq) to 5 mM. The final concentration of H_2O in the sample was 0.27 M.

Figure 2.

Observed ^1H SABRE enhancements for substrate and solvent OH protons as a function of polarizing field for (A) 80 mM pyridine, 4 mM Ir-IMes catalyst, (B) 64 mM pyridazine, 4 mM Ir-SIMes catalyst, (C) 64 mM pyrazole, 4 mM Ir-SIMes catalyst and (D) 64 mM imidazole, 4 mM Ir-SIMes catalyst. All solutions contain 5 mM HCl, 0.27 M H_2O in methanol- d_4 .

Figure 3.

Variation of maximum ^1H SABRE enhancements with substrate concentration for pyridine (A), pyridazine (B), pyrazole (C), and imidazole (D). SABRE data was acquired using 4 mM Ir-IMes (pyridine) or Ir-SIMes catalyst in a solution containing 5 mM HCl and 0.27 M H_2O in methanol- d_4 . Maximum enhancements for protons of the substrate or solvent OH were measured at 23 or 25 mT (whichever field gave the greatest enhancement).

Figure 4.

Variation of 1H SABRRE enhancements with HCl concentration using 50 mM pyrazole with 4 mM Ir-SIMes catalyst. Data was acquired in solutions containing 0.27 M H_2O in methanol- d_4 . Maximum enhancements of the solvent OH were measured at 23 mT or 27 mT (whichever field gave the greatest enhancements).

Figure 5.

(A) Dissociation rate constant ($k_{\text{diss}}(\text{s}^{-1})$) measured from 1D EXSY SABRE enhanced spectra at 40 mM substrate with 4 mM Ir-catalyst at 294 K. Spectra under neutral conditions were acquired using methanol- d_4 solvent while those acquired under acidic conditions used the same solvent containing 5 mM HCl and 0.27 M H_2O . (B) The maximum solvent OH enhancement using conditions under which maximum enhancement was observed (Table 2). (C) Maximum substrate proton enhancements using conditions described in (B).

Figure 6.

(A) Thermal spectrum of 80 mM pyridine in methanol- d_4 containing 0.27 M H_2O and 5 mM HCl (B) SABRE spectrum of 80 mM pyridine and 8 mM IMes. Enhancement was carried out at 23 mT with 8 s of $p-H_2$ bubbling. (C) Thermal spectrum of 80 mM pyridine- d_5 under the same conditions as in spectrum A. (D) SABRE spectrum of 80 mM pyridine- d_5 under the same conditions as as in spectrum B.

Figure 7.

(A) SABRE spectra of 80 mM pyridine, 8 mM Ir-IMes in methanol- d_4 under acidic conditions (5 mM HCl(aq)) collected at 14.1 T using a variable wait time (τ) prior to the 90° read pulse. Initial polarization was at 23 mT using a 60 s p- H_2 bubbling time. (B) fit of the resonance intensity to a single exponential (Eqn. [1] and [2]).

Scheme 1.

The low transfer of hyperpolarization from hydride protons to trans equatorial pyridine substrate protons on Ir-IMes catalyst [21,22,24,26]. Hyperpolarization is transferred between hydride and substrate and between protons in the substrate via spin-spin coupling. IMes structure is shown in Fig. 1. Arrows on $p\text{-H}_2$, $o\text{-H}_2$ illustrate singlet and triplet nuclear spin states. Protons on all but one pyridine have been removed for clarity. Magenta colored hydrogens represent those which are hyperpolarized.

Scheme 2.

Proposed mechanism for SABRE hyperpolarization of solvent protons from free hyperpolarized pyridine. In the mechanism pyridine is protonated in acidic solution and polarization is transferred to the labile proton via coupling to hyperpolarized ring protons. Thermal and hyperpolarized hydrogens are represented in white and magenta, oxygens in red, nitrogens in dark blue, deuterium in light blue and carbon in gray.

pyridine

pyridazine

imidazole

pyrazole

SIMes

IMes

Chart 1.
Structures of substrates and the IMes and SIMes ligands used in this study.

Table 1Comparison of SABRE Catalysts^a

Compound	¹ H	Ir-IMes ϵ^b	Ir-SIMes ϵ^b
Pyridine	2,6	−279	−153
	3,5	−218	−125
	4	−264	−158
Pyridazine	3,6	−79	−156
	4,5	−59	−140
Imidazole	2	−51	−249
	4,5	−42	−27
Pyrazole	3	−337	−345
	4,5	−369	−211

^a All studies were performed at 21 °C and 2.3 mT in methanol-d₄ with concentrations of 40 mM substrate and 4 mM catalyst

^b The signal enhancement was determined as the ratio of hyperpolarized to thermal signal at 14.1 T.

Table 2

Conditions under which Maximum Polarization of Solvent was Observed

substrate	catalyst	[substrate]	max ϵ^a	field ^b
imidazole	4 mM SIMes	64 mM	−0.9	25 mT
pyrazole	4 mM SIMes	64 mM	30.2	23 mT
pyridazine	4 mM SIMes	64 mM	15.2	23 mT
pyridine	4 mM Imes	80 mM	37.0	25 mT

^aEnhancements were measured at 14.1 T and 21 °C.^bPolarizing field. Samples were exposed to p-H₂ for 8 s prior to measurement

Table 3Spin-Lattice Relaxation Times (T_1) and SABRE Decay Times (T^*) for Pyridine^a

Resonance	T^*	T_1
Solvent OH	20.2	7.3
H3,H5	8.2	6.9
H4	12.2	7.7
H2,H6	7.6	6.5

^aEstimated error ± 0.2 s for all measurements. T_1 measurements were carried out at 14.1 T on solutions containing 80 mM pyridine, 8 mM Ir-IMes and 5 mM HCl(aq) in methanol-d₄ at 21 °C.

T^* measurements were carried out on identical solutions with 60 s of p-H₂ bubbling.