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A 10 000-fold Nuclear Hyperpolarization of a Membrane Protein in the Liquid Phase via a Solid-State Mechanism

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S *Supporting Information*

ABSTRACT: Several techniques rely on electron−nuclear interactions to boost the polarization of nuclear spins in the solid phase. Averaging out of anisotropic interactions as a result of molecular tumbling strongly reduces the applicability of such hyperpolarization approaches in liquids. Here we show for the first time that anisotropic electron−nuclear interactions in solution can survive sufficiently long to generate nuclear spin polarization by the solid-state photo-CIDNP mechanism. A 10 000-fold NMR signal increase in solution was observed for a giant biomolecular complex of a photosynthetic membrane protein with a tumbling correlation time in the submicrosecond regime, corresponding to a molecular weight close to 1 MDa.

Nuclear spin hyperpolarization, i.e., the creation of large, nonequilibrium population differences between nuclear spin states, allows for a significant enhancement of signal intensities in NMR experiments. This signal increase is crucial for NMR investigations where sensitivity represents a limiting factor, such as the characterization of large (bio)molecular systems. Several hyperpolarization methods, including spin-exchange optical pumping,[1] dynamic nuclear polarization (DNP),[2,3] and photochemically induced dynamic nuclear polarization (photo-CIDNP),[4,5] realize this process via a polarization transfer from electron spins to nuclear spins. In solution, electron−nuclear anisotropic interactions are generally averaged out, and nuclear spin polarization transfer depends on the efficiency of incoherent processes, such as relaxation (Overhauser effect)[6] for DNP, or on the presence of isotropic electron−nuclear interactions that are not affected by the molecular tumbling, as for photo-CIDNP.[7−10] Here we report on a coherent mechanism for hyperpolarization in the liquid phase that is applicable to very large molecular complexes in solution. In the slow motional regime pertaining to these systems, the large anisotropic electron−nuclear interactions are conserved over the time required for polarization transfer, as in solids, while all other anisotropic nuclear interactions are averaged out by molecular tumbling, as in liquids. This approach was tested on the molecular complex formed by the photosynthetic reaction centers (RCs) of wild-type *Rhodobacter sphaeroides (Rb.)* and lauryldimethylamine oxide (LDAO) detergent using photo-CIDNP as the hyperpolarization technique. Photo-CIDNP in the solid state depends on electron−nuclear spin anisotropic interactions[11] and until now has been observed exclusively on frozen samples of electron-transfer proteins as photosynthetic reaction centers[11,12] and blue-light photoreceptors.[13] In contrast to liquid-state photo-CIDNP, the anisotropic part of the hyperfine interaction does not induce significant nuclear relaxation on the slower recombination product[14,15] but rather mixes the spin states, promoting polarization transfer from the electrons to the nuclei. The hyperpolarization mechanism for RCs of wild-type *Rb. sphaeroides* in the solid phase is here briefly summarized: The rotating-frame Hamiltonian governing the spin dynamics in a model system consisting of two electron spins ($\mathbf{S}_1$ and $\mathbf{S}_2$) and one nuclear spin ($\mathbf{I}$) coupled to the electron spin $\mathbf{S}_1$ can be written in angular frequency units as:

$$\begin{aligned}\hat{H} = {} & \frac{\Delta\Omega}{2}\hat{S}_{1z} - \frac{\Delta\Omega}{2}\hat{S}_{2z} + \omega_I\hat{I}_z \\ & + [A_{\mathrm{iso}} + \Delta A(3\cos^2\theta_{\mathrm{en}} - 1)]\hat{S}_{1z}\hat{I}_z \\ & + (3\Delta A\sin\theta_{\mathrm{en}}\cos\theta_{\mathrm{en}})\hat{S}_{1z}\hat{I}_x \\ & + \frac{D}{2}(1 - 3\cos^2\theta_{\mathrm{ee}})(3\hat{S}_{1z}\hat{S}_{2z} - \hat{\mathbf{S}}_1\cdot\hat{\mathbf{S}}_2) - J\left(2\hat{\mathbf{S}}_1\cdot\hat{\mathbf{S}}_2 + \frac{1}{2}\right)\end{aligned} \tag{1}$$

where the parameters $\Delta\Omega$, ω_I, A_{iso}, ΔA, D, and J characterize the difference of the electron Zeeman interactions, the nuclear Zeeman interaction, the isotropic and anisotropic parts of hyperfine interaction, the electron dipole−dipole interaction, and the exchange interaction, respectively, and θ_{ee} (θ_{en}) denotes the angle between the symmetry axis of the electron−electron (electron−nuclear) dipole−dipole interaction and the external field axis.

Upon light-induced electron transfer, a spin-correlated radical pair is born in a pure singlet state. During the lifetime of the radical pair ($\tau_{\mathrm{RP}} \sim 20$ ns), the electron-spin system oscillates between the singlet state and a triplet state because of the difference in the electron Zeeman interactions (the first two terms on the right-hand side of eq 1) and the secular part of the hyperfine interaction (the fourth term). Concomitant with this intersystem crossing, nuclear spin hyperpolarization is coherently generated by the anisotropic components of the electron−nuclear hyperfine interaction (the fifth term) as well as the electron−electron coupling (the last two terms) via the three-spin mixing (TSM)[12,16] mechanism. Additionally, the differential

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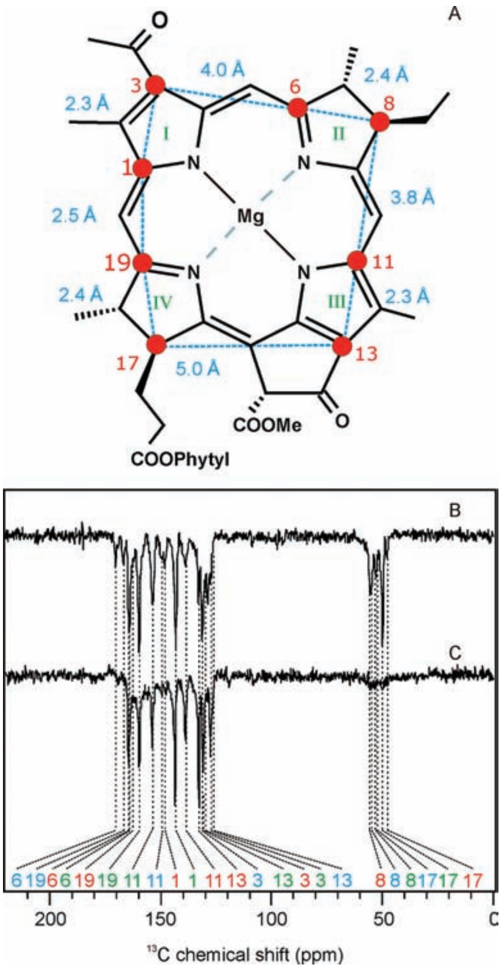

Figure 1. (A) Structure of the primary donor (bacteriochlorophylls *a*) in the 4-ALA-labeled RCs of wild-type *Rb. sphaeroides*. The ^{13}C labeling pattern and the ^{13}C$-$^{13}C internuclear distances are indicated. The labeled positions are the same for the primary acceptor, a bacteriopheophytin *a* (Φ). (B, C) ^{13}C photo-CIDNP NMR spectra of 4-ALA-labeled RCs of wild-type *Rb. sphaeroides* acquired at 4.7 T under continuous illumination (B) in the solid phase at 233 K and (C) in the liquid phase at 283 K. The assignments follow the IUPAC nomenclature; green, red, and light-blue refer to the two bacteriochlorophylls *a* of the donor (P_L, P_M) and Φ, respectively.

decay rate of the radical pair in the triplet and singlet state together with the anisotropic components of the electron−nuclear hyperfine interaction can generate nuclear spin hyperpolarization via the differential decay (DD)[12,17] mechanism. Provided that the tumbling correlation time of the RCs in solution is longer than the lifetime of the radical pair, θ_{en} and θ_{ee} are essentially time-independent, as in the solid state. We therefore expect these coherent hyperpolarization mechanisms to be effective also in the liquid phase, where the condition to observe enhanced signals should be expressed as:

$$\frac{1}{\Delta A}, \tau_{RP} < \tau_C < \frac{1}{d_{nn}}, \frac{1}{\Delta \delta} \qquad (2)$$

in which d_{nn} and $\Delta\delta$ denote, respectively, the nuclear−nuclear dipolar interaction and the chemical shift anisotropy in angular frequency units.

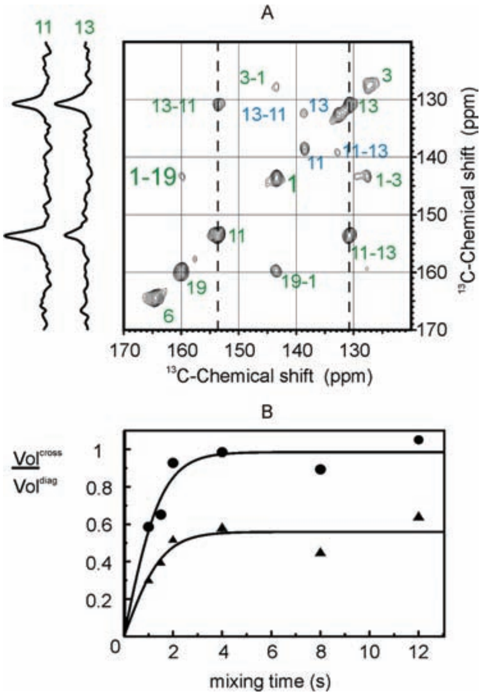

Figure 2. (A) Photo-CIDNP 2D (^{13}C,^{13}C) NOESY spectrum of 4-ALA-labeled RCs of wild-type *Rb. sphaeroides* recorded at 283 K with a mixing time of 4 s. The two vertical 1D traces show diagonal and cross-peaks of the isolated spin pair formed by C11_L and C13_L. (B) Plot of the integral ratios $V(\mathrm{C}13_L{-}\mathrm{C}11_L)/V(\mathrm{C}11_L)$ (●) and $V(\mathrm{C}11_L{-}\mathrm{C}13_L)/V(\mathrm{C}13_L)$ (▲) as functions of the NOESY mixing time. The solid curves were obtained by independent fits of the two buildups.

To confirm this prediction, we acquired and compared ^{13}C photo-CIDNP spectra of the molecular complex of the RCs and LDAO detergent in the solid phase and in the liquid state. Figure 1A shows the specific ^{13}C isotope enrichment pattern on the bacteriochlorophylls *a* and bacteriopheophytin *a* cofactors employed in this study [see the Supporting Information (SI)]: for every five-membered ring, ^{13}C isotope labeling occurs in pair with a separation of two C−C bonds, which corresponds to an internuclear distance of 2.30−2.50 Å. The ^{13}C photo-CIDNP NMR spectra of 4-ALA-labeled RCs acquired under continuous illumination at 4.7 T in the solid phase at 233 K and in solution at 283 K are shown in Figure 1B,C, respectively. The spectra display the enhanced signals of the selectively ^{13}C-labeled cofactors in the RCs, together with the peak assignments from previous studies in the solid phase.[12,18] The two spectra were acquired with identical parameters and present very similar patterns of emissive signals, confirming our prediction. A 10 000-fold increase in the ^{13}C signal by photo-CIDNP was previously estimated for the solid-state spectrum.[19] A comparison between the signal intensities in the two spectra in Figure 1B,C indicates a similar sensitivity enhancement in the liquid phase. To the best of our knowledge, this result exceeds by far any signal enhancement previously reported for a biological macromolecule in solution.

Most of the strong signals in the solid-state spectrum can be recognized in the solution spectrum with comparable intensities and chemical shifts; these peaks originate from the P_L bacteriochlorophyll *a* component of the electron donor, which carries most of the electron spin density.[18]

Whereas the aromatic regions of the spectra are very similar, there is a clear difference in the appearance of the aliphatic signals (spectral region at ~50 ppm). In solution, signals from aliphatic ^{13}C (i.e., C8$_L$ and C17$_L$) display much larger line widths (several hundred Hz) because of the line broadening caused by ^{13}C−^{1}H dipolar relaxation. In contrast, the aromatic ^{13}C signals in solution are much sharper (line width 35−40 Hz) because of the absence of directly attached protons and the modest strength of the external magnetic field, which guarantees a longer transverse relaxation time T_2.

To estimate the actual tumbling correlation time of the RC−detergent complex in solution and verify the condition given in eq 2, a series of 2D (^{13}C,^{13}C) nuclear Overhauser effect spectroscopy (NOESY) spectra were acquired at different mixing times, exploiting the signal enhancement provided by photo-CIDNP. A 2D (^{13}C,^{13}C) NOESY spectrum recorded with a mixing time of 4 s is shown in Figure 2A. According to the structure of the cofactor P$_L$ (Figure 1A), ^{13}C11 and ^{13}C13 form an isolated spin pair with an internuclear distance of 2.3 Å. In Figure 2B, the values of the integral ratios of the cross- and diagonal peaks (i.e., $V(\mathrm{C13_L{-}C11_L})/V(\mathrm{C11_L})$ and $V(\mathrm{C11_L{-}C13_L})/V(\mathrm{C13_L})$) are plotted as functions of the mixing time of the 2D NOESY spectra. The solid curves were obtained by fitting the experimental values with the theoretical expression describing the dependence of the integral ratios on the mixing time (see the SI). The separate fittings of the two buildups provide very close estimates of the cross-relaxation rate $\sigma_{11,13}$ (0.65 and 0.63 s^{-1}, respectively).

From the standard expression for the cross-relaxation rate[20] with the assumption of isotropic rigid-body diffusion in the slow tumbling regime for the RCs, a correlation time τ_C of 420 ns was obtained (see the SI), which greatly exceeds the lifetime of the radical pair in agreement with eq 2. This value of τ_C is consistent with the line widths of the quaternary ^{13}C aromatic signals estimated under the assumption that chemical shift anisotropy is the dominating relaxation mechanism. Using a typical chemical shift tensor for aromatic[21] carbon nuclei with $\sigma_{11} = 225$ ppm, $\sigma_{22} = 149$ ppm, and $\sigma_{33} = 15$ ppm yielded an estimate of ~40 Hz for the ^{13}C line widths, which is in good agreement with the experimental values. Our data are consistent with previous small-angle neutron scattering studies on this system[22] that demonstrated the presence of large aggregates of RC units and detergent in solution. From the calculated value of τ_C, a molecular size of ~1 MDa was estimated for the RC−LDAO complex using the Einstein−Stokes equation under the assumption of an approximately spherical shape (see the SI).

While liquid-state DNP is usually applied in the extreme narrowing regime ($\omega\tau_C \ll 1$), which is fulfilled for small molecules at moderately high fields, our case is exactly the opposite: we observed enhanced signals in the slow tumbling regime ($\omega\tau_C > 1$), typical of macromolecules in the liquid phase. In the slow motional regime pertaining to photosynthetic RCs in solution, the large anisotropic electron−nuclear interactions are conserved over the time needed for polarization transfer, as in solids, while all other anisotropic nuclear interactions are averaged out by molecular tumbling, as in liquids. In general, the conditions for realizing this situation in solution and coherently transferring polarization to nuclear spins via photo-CIDNP are expressed by eq 2: the tumbling correlation time τ_C must be longer than or comparable to the time scale of electron−nuclear interactions relevant for polarization buildup ($1/\Delta A \sim 100$ ns) and of the radical pair lifetime (τ_{RP}); at the same time, it should be much shorter than the time scale of the nuclear interactions relevant during NMR detection.

Other electron−nuclear polarization transfer mechanisms in the solid state rely on electron−nuclear anisotropic interactions as essential mixing elements. Future work will show whether the solid-state approach here exploited for photo-CIDNP can be transferred to other hyperpolarization techniques in the liquid phase. We believe this could propel the field of nuclear polarization to a new level, opening the door to an entirely new range of applications concerning large biomacromolecular systems in solution.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental details, materials and methods, spectral deconvolution, and correlation time. This material is available free of charge via the Internet at http://pubs.acs.org.

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