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Dynamic nuclear polarisation of biological matter

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Abstract. Polarised targets as used in high energy physics experiments may be of considerable interest in biological structure research using polarized neutrons. So far, this promising method has been facing difficulties in getting reasonable polarization of the target nuclei. We report on a polarized "frozen spin" target which has been prepared from an enzyme dissolved in a mixture of heavy water and deuterated propanediol doped with a completely deuterated paramagnetic radical. Clusters of 700 protons defined by the structure of lysozyme embedded in a fully deuterated matrix were polarized to 75% within an hour by 4 mm microwave irradiation in a magnetic field of 2.5 tesla at a temperature of 0.3 K. The polarisation behaviour of biological targets can be compared to the best frozen spin target materials in high energy physics research.

Key words: Polarised targets, spin diffusion, cryobiochemistry of proteins, polarised neutron scattering

Introduction

The interest in polarized targets originates from the spin dependence of the interaction of thermal neutrons with protons (Abragam et al. 1982). It is convenient to express the scattering amplitude, a, of the proton in an operator form as

$$a = b + 2B\,\boldsymbol{I}\cdot\boldsymbol{s}. \qquad (1)$$

The second term describes the interaction of a neutron carrying a spin $\boldsymbol{s}$, $|\boldsymbol{s}| = 1/2$, and the nucleus carrying a spin $\boldsymbol{I}$. The constants b and B are determined by the two eigenvalues $b_{(+)}$ and $b_{(-)}$ of the operator $(\boldsymbol{I}\cdot\boldsymbol{s})$, depending on whether $\boldsymbol{I}$ and $\boldsymbol{s}$ couple in the channels $I + 1/2$ or $I - 1/2$.

For the proton with $I = 1/2$ one obtains

$$b = [(I+1)\,b_{(+)} + I\,b_{(-)}]/(2I+1) = -0.375 \cdot 10^{-12}\,\mathrm{cm}$$

and

$$B = (b_{(+)} - b_{(-)})/(2I+1) = 2.91 \cdot 10^{-12}\,\mathrm{cm}\,. \qquad (2)$$

To show the essential features of spin dependent nuclear neutron scattering from a macroscopic target, we refer to neutron diffraction from a crystal built of identical atoms of only one nuclear species with non zero spin, this crystal having only one atom per unit cell. We assume a polarized target with a polarisation, $P(H)$, along an axis and the neutron beam polarized along the same axis with polarisation $P(n)$. Furthermore, there are no correlations between the relative orientations of two nuclear spins and their relative positions in an unpolarized target or in a polarized target with Zeeman order only. Under these conditions neutron differential scattering cross section of the N unit cells of the crystal is given by the following expression (Abragam and Goldmann 1982):

$$\begin{aligned}\frac{d\sigma}{d\Omega} &= \sum_i \left(\langle |a_i|^2\rangle - \langle a_i\,a_j\rangle_{i\neq j}\right.\\ &\quad \left. + \langle a_i\,a_j^*\rangle_{i\neq j}\exp\left(-i\,\boldsymbol{Q}\cdot(\boldsymbol{r}_i - \boldsymbol{r}_j)\right)\right)\\ &= N\left(\frac{d\sigma}{d\Omega}\right)_{\mathrm{inc.}} + N\left(\frac{d\sigma}{d\Omega}\right)_{\mathrm{coh.}}\sum_i \exp\left(-\boldsymbol{Q}\cdot\boldsymbol{r}_i\right)\end{aligned}$$

with

$$\left(\frac{d\sigma}{d\Omega}\right)_{\mathrm{inc.}} = B^2\,[I(I+1) - P(n)\,P(H)\,I - P^2(H)\,I^2]$$

$$\left(\frac{d\sigma}{d\Omega}\right)_{\mathrm{coh.}} = b^2 + 2\,b\,BIP(n)\,P(H) + B^2 I^2 P^2(H), \qquad (3)$$

where $P(H)$ and $P(n)$ are assumed to have values between -1 and $+1$. $\boldsymbol{Q}$ is the momentum transfer.

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The cross section of incoherent neutron scattering $(d\sigma/d\Omega)_{\mathrm{inc.}}$ reduces to zero for $P(n)\,P(H) = 1$ but not for $P(n)\,P(H) = -1$. In the latter case an incoming neutron can exchange its spin state with that of an individual nucleus.

For $P(n) = 1$ the coherent neutron scattering length assumes the form

$$\langle a \rangle = b + BIP(H)\,, \tag{4}$$

which coincides with the formula used with isotopic substitution. Using polarized neutrons, the scattering length of protons will therefore vary with the proton spin polarisation, $P(H)$, as follows

$$\langle a \rangle_{\mathrm{proton}} = (-\,0.374 + 1.455\,P(H))\,10^{-12}\,\mathrm{cm}\,. \tag{5}$$

As the target polarisation $P(H)$ may assume values between -1 and $+1$, the effective coherent scattering length of the protons varies between $-1.83 \cdot 10^{-12}$ cm and $+1.08 \cdot 10^{-12}$ cm. On the other hand, the often used isotopic substitution of ^{1}H by its heavier isotope ^{2}H, deuterium (D), will increase the scattering length of hydrogen by $1.04 \cdot 10^{-12}$ cm only. Polarisation variation changes the scattering length of hydrogen over an interval which is about three times wider than with this isotopic substitution.

For a lattice with more than one atom per unit cell, the coherent cross section is obtained as follows. Define in the unit cell the structure factors

$$F_0 = \underbrace{\sum_n b_n \exp(-\,i\,\boldsymbol{Q} \cdot \boldsymbol{r}_n)}_{\text{spin-independent}}\,;$$

$$F = \underbrace{\sum_n P_n I_n B_n \exp(-\,i\,\boldsymbol{Q} \cdot \boldsymbol{r}_n)}_{\text{spin-dependent}}\,, \tag{6}$$

where $b_n + B_n(\boldsymbol{s} \cdot \boldsymbol{I}_n)$ is the scattering amplitude for the nucleus in $\boldsymbol{r}_n$, whose spin is I_n and the polarisation P_n. The coherent neutron scattering cross section is

$$\left(\frac{d\sigma}{d\Omega}\right)_{\mathrm{coh}} = |F_0|^2 + P(n)\,(F_0F^* + FF_0^*) + |F|^2\,. \tag{7}$$

With $P(n) = 1$ and assuming homogeneous polarisation P of one nuclear species, coherent neutron scattering will depend on P as follows

$$S(\boldsymbol{Q}) = S_0(\boldsymbol{Q}) + PS_{0P}(\boldsymbol{Q}) + P^2 S_P(\boldsymbol{Q})\,. \tag{8}$$

$S_0(\boldsymbol{Q})$, $S_{0P}(\boldsymbol{Q})$ and $S_P(\boldsymbol{Q})$ are basic (or fundamental, or characteristic) scattering functions known from contrast variation (Stuhrmann 1985).

Polarized neutron beams can be produced most efficiently by total reflection from magnetized mirrors (Schärpf 1982). The polarisation of the neutron beam is then nearly $P(n) = 1$. It can be changed to $P(n) = -1$ by inversion of the spin orientation of the neutrons. This is done most conveniently by changing the current of a flat coil spin flipper (Mezei coil) (Schärpf 1982). With the neutron polarisation parallel or antiparallel to the target polarisation, we obtain from Eq. (7):

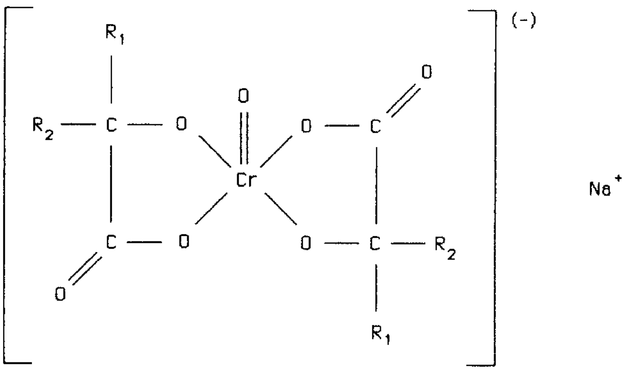
$$\begin{aligned} S_{\uparrow\uparrow}(Q) &= S_0(Q) + P(H)\;S_{0P}(Q) + P^2(H)\;S_P(Q) \\ S_{\downarrow\uparrow}(Q) &= S_0(Q) - P(H)\;S_{0P}(Q) + P^2\,S_P(Q)\,. \end{aligned} \tag{9}$$

The cross term $S_{0P}(Q)$ is twice the difference between $S_{\uparrow\uparrow}(Q)$ and $S_{\downarrow\uparrow}(Q)$. The scattering from the polarized protons, $S_P(Q)$, can also be obtained in an unpolarized neutron beam.

Polarization variation is particularly suitable for in situ structure determination of subunits in quaternary structures, e.g. of ribosomal proteins in ribosomes. The scattering of a single ribosomal protein can be rendered visible as $S_P(Q)$ if the proteins of all other constituents of the ribosome molecule and those of the solvent have been exchanged by deuterium (Stuhrmann 1985). – We note, in passing, that the polarization of the deuterons also changes the neutron scattering length of deuterium, though to a much smaller extent. This will be discussed later. Spin dependent neutron scattering from other nuclei occurring in organic matter is negligibly small.

Experimentals

We therefore prepared samples, where a major part of the protons were concentrated in the molecular structure of a protein. Lysozyme was dissolved in various mixtures of heavy water and deuterated 1,2-propanediol containing about 2% of an organic chromium-(V) complex (Table 1). The structure of this metallo-organic compound is



The addition of this paramagnetic compound is essential for dynamic nuclear polarisation (DNP). We used a fully deuterated Cr(V) complex, called Cr(V)-HMPA complex, as its synthesis starts from 2-*h*ydroxy-2-*m*ethyl*p*ropanoic *a*cid. The reduction of dried sodium dichromate by this tertiary 2-hydroxy acid is carried out in acetone, yielding nearly quantitatively the water soluble, crystalline Cr(V) complex

(Krumpolc and Rocek 1979). The solubility of the product can be tailored to the experimental requirements by a proper choice of R_1 and R_2, all of them supporting dynamic nuclear polarisation (Niinikoski 1978). In this case, the ligands R_1 and R_2 were CD_3 (Krumpolc and Rocek 1979).

Two lysozyme solutions were prepared:

(*1*) Hen eggwhite lysozyme from SERVA Feinbiochemica, Heidelberg, was dissolved in heavy water and dialysed against D_2O. The lysozyme concentration after dialysis was 40 mg/ml.

(*2*) A concentrated solution of 270 mg lysozyme/ml D_2O was used as such. These solutions were added in various quantities to

(*3*) a 2% solution of the Cr(V)-HMBA complex[5] in deuterated 1,2-propanediol (98% D) (from Merck, Sharp & Dohme, Montreal Canada, lot nr. B-383) yielding the samples

LYS 1: [1]/[3] = 1/3
LYS 2: [1]/[3] = 1/1
LYS 3: [2]/[3] = 1/2.

A schematic cross section of the sample LYS 3 on a molecular level is given in Fig. 1.

Two reference samples were used, REF 1 and REF 2:

REF 1: The paramagnetic centres were created by reduction of sodium dichromate by propanediol (PD6), in which the two hydrogens in the OH-groups were unsubstituted and the six others deuterated. The product was mixed with 98% perdeuterated propanediol PD8 (solvent [3]) in volume proportion PD6/PD8 = 1/2. The preparation has been described in more detail by de Boer et al. (1974). This is the so far best polarized deuteron target.

REF 2: This is the above pure solvent [3] in which the deuterated Cr(V) complex was dissolved.

It is very fortunate that the requirements of dynamic nuclear polarisation agree very well with the practice of cryobiochemistry (Douzou 1977). One of them is that the solution should remain in the glass state. This also keeps small-angle scattering from the solvent very low. Another requirement is the compatibility of the organic Cr(V) complex with the dissolved protein. The solutions are clear and give transparent beads after freezing in liquid nitrogen. Studies with a microcalorimeter showed that the samples were stable agains nucleation of crystal growth and formed a good glass with no evidence for microcrystals.

The samples were prepared in the form of 1.5 mm diameter glassy beads by letting droplets of the sample liquid freeze on the meniscus of liquid nitrogen. The beads were loaded onto a perforated FEP cartridge located in the mixing chamber of a fast-access dilution refrigerator (Niinikoski and Rieubland 1978). The DNP was achieved by irradiating the sample by 4 mm microwaves at helium bath temperatures between 0.28 and 0.35 K in a magnetic field of 2.5 tesla. The polarisations of the protons, $P(H)$, and the deuterons, $P(D)$, were measured by continuous-wave nuclear magnetic resonance (NMR) (Niinikoski and Rijllart 1982), calibrated by the measurement of the signal surfaces in thermal equilibrium with the helium bath around 1 K. For the deuterons also the NMR line asymmetry (Borghini and Scheffler 1970) was used for determining the spin temperature and therefore polarisation.

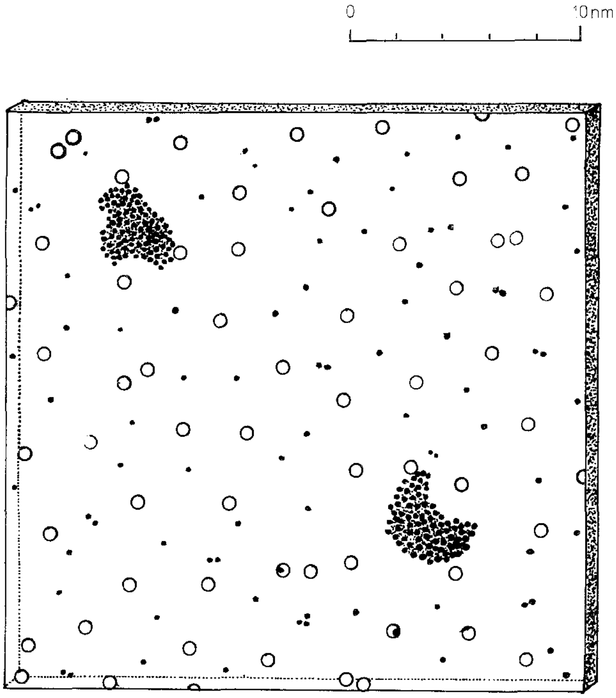


Fig. 1. Schematic representation of the proton density distribution of a frozen lysozyme solution in a slice of 1 nm thickness. The protons are marked by *dots*. Each lysozyme molecule contains 700 non-dissociating hydrogens. About 40% of the protons belong to the solvent molecules, which are not fully deuterated. This corresponds to the composition of the sample LYS3. The deuterated organic Cr(V) complexes are marked by *open circles*. 93% of the hydrogens are of the heavier isotope ^2H not shown here

Results and discussion

After switching on the microwave $P(H) = 50\%$ was reached for the samples REF 1 and LYS 3 in about ten minutes (Fig. 2) at 0.28 K bath temperature. For the other samples the increase of polarisation was about three times slower. Polarisations beyond 50% were easily reached by reducing the microwave power; consequently the polarisation growth was

Table 1. Concentrations and proton densities of the samples and their constituents

Sample	Protons from				Fraction of protons from		Concentration of		$P(H)$
	Propanediol [1/nm]	Water [1/nm]	Lysozyme [1/nm]	Total [1/nm]	Solvent	Lysozyme	Lysozyme [mmol/l]	Cr (V) [mmol/l]	[%]
REF1	5.26	0	0	5.26	1	0	0	116	80
REF2	1.31	0	0	1.3	1	0	0	88	67
LYS1	0.985	0.03	0.287	1.30	0.78	0.22	0.68	66	58
LYS2	0.675	0.06	0.557	1.29	0.57	0.43	1.3	44	61
LYS3	0.894	0.914	2.56	4.37	0.41	0.59	5.9	60	77

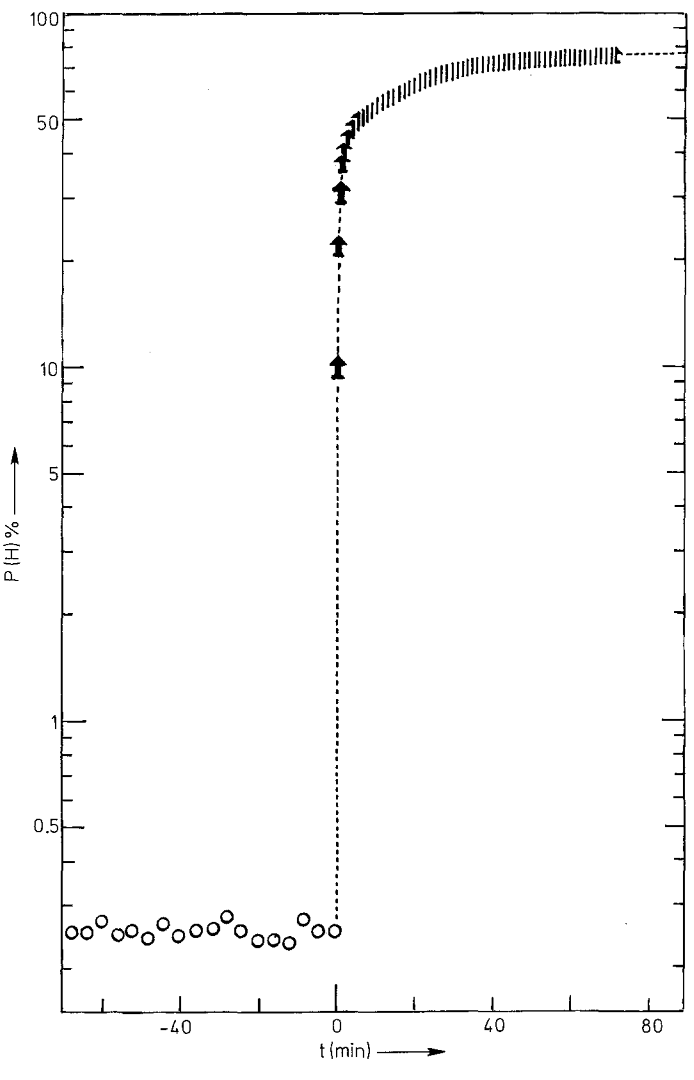


Fig. 2. Proton polarisation of the sample LYS3 before and during microwave irradiation. Before irradiation ($t < 0$) the sample was kept at $T = 1$ K in a magnetic field of 2.5 tesla. The NMR signal (○) of the polarised protons was taken every 4 min over a period of 2 h. Then the temperature of the helium bath was lowered to 0.28 K in about half an hour. This time interval is not shown here. At $t = 0$ microwave irradiation started. Polarisation of the proton spins then was measured every 40 s indicated by *arrows* (↑)

slower, so that after one hour values between 60% and 80% were reached (Table 1).

Similar trends were observed for the growth of the deuteron polarisation (Fig. 3). The polarisation of the deuterons is lower because of the small magnetic moment. The nuclear spin temperatures of both hydrogen isotopes were found within experimental uncertainty to become equal in steady state, particularly when the microwave frequency was optimal for the best growth of the proton polarisation (Niinikoski 1976). The curve in Fig. 3 shows the predicted equilibrium of deuteron polarisation, as a function of proton polarisation, as well as the experimental results.

In addition to the proton NMR signal surface area, the second moment, M_2, of the signal was also recorded. The line width, defined as $(M_2/M_0)^{1/2}$, where M_0 is the zeroth moment or signal surface, is 58 kHz in LYS3 at low polarisation and decreases to about 56 kHz for the maximum polarisations, both positive and negative. The line width is probably predominantly determined by the dipolar interactions of the proton spins with the electron and deuteron spins rather than with each other, judging from the weak influence of their polarisation upon the linewidth.

This can be expected for the unsubstituted solvent protons, but is somewhat less straightforward in the case of the lysozyme molecules, where the proton spin density is about one half of that in the usual polarised target materials. As the dipolar linewidth scales with the spin density (for one spin species), the lysozyme proton dipolar linewidth should be about 30–40 kHz in the absence of other broadening mechanisms, at low polarisation.

The linewidth of the REF2 matrix protons is about 30 kHz (as found by earlier measurements), with a proton dipolar component of 1 kHz.

The usual test of the polarized target material and magnetic field homogeneity consists of observing the shape of the proton NMR line during polari-

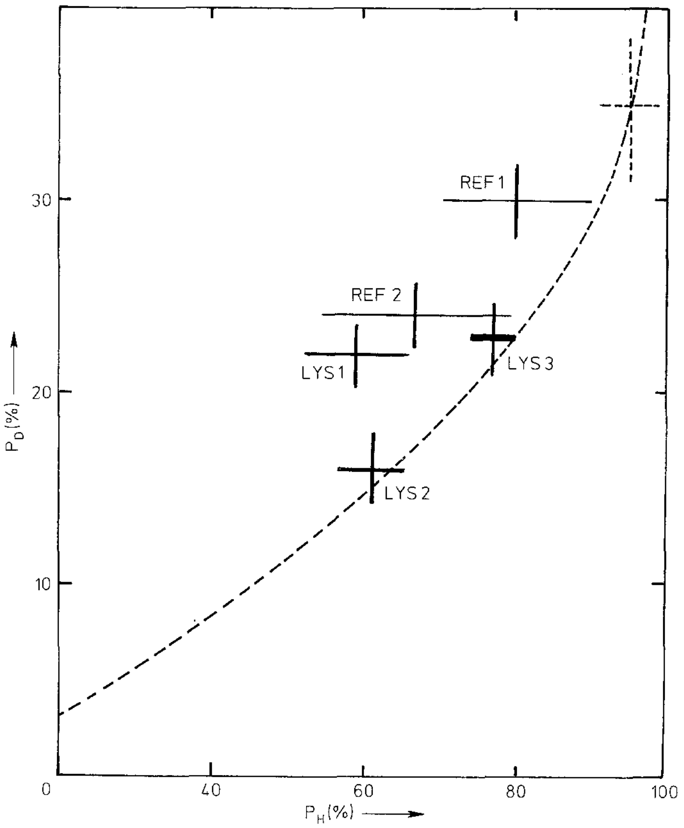


Fig. 3. Polarisation of the deuterons versus the polarisation of the protons: the crosses of the various samples follow roughly the equilibrium polarisation of the protons and deuterons (*broken line*)

sation reversal, at zero crossing. Serious material inhomogeneity problems due to precipitation of microcrystals, for instance, are observed qualitatively by recording the reversal speed of the various components of the NMR line. A microcrystal of lysozyme with slow reversal speed due to limited spin diffusion would display a shifted NMR line with one sign of polarisation, while the fast reversing solvent with paramagnetic impurities well dispersed would already show a high degree of opposite polarisation. The signals can be shifted up to 10 kHz at zero crossing, owing to opposite signs of internal magnetisations. No such phenomena were observed in any of the samples, but it was found that unlike the usual target materials, the wings of the proton NMR signal reversed somewhat faster than the narrow centre of the line. We interpret this by noting that the protons (solvent or lysozyme) near the electronic spins experience a stronger dipolar field and therefore have a broader NMR line; these protons are reversed fast because of this stronger dipolar field. This phenomenon cannot be seen in the usual target materials, where the dipolar interactions among the protons themselves dominate. This observation agrees with the above explanation for the relatively small narrowing of the proton NMR line at high polarisation.

The polarisation decay time in the magnetic field of 2.5 tesla varies strongly with the temperature and behaves as expected from samples where the dipolar coupling to electron spins provides the dominant relaxation mechanism (de Boer and Niinikoski 1974). At $T = 1$ K a depolarisation time constant of 7 min was measured, whereas at 0.35 K the time constant has already increased to 20 hours. The spin lattice relaxation was found to be exponential within experimental accuracy at all temperatures and for all polarisations, indicating rapid spin diffusion in the proton and deuteron spin systems. This confirms the conclusions, drawn from the study of the proton line shape and its transient behaviour, of the absence of nucleation of crystal growth or any other phase separation phenomena, which could isolate part of the sample from the electronic impurities.

Polarized neutron small-angle scattering is very sensitive to the polarisation of proton clusters (Stuhrmann 1985). More definite statements on the mechanism of dynamic nuclear polarisation can be made once the spatial distribution of the paramagnetic centres is known. Resonant X-ray scattering near the K-absorption edge of chromium would show to what extent this organic complex might be bound to the enzyme molecule (Stuhrmann 1985). This type of experiment is best done with synchrotron radiation.

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