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POLARISED NEUTRON SCATTERING FROM DYNAMIC POLARISED TARGETS OF BIOLOGICAL ORIGIN

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Abstract

Polarised targets as used in high-energy physics experiments may be of considerable interest in biological structure research using polarised neutrons. So far, this promising type of macromolecular structure research has been facing difficulties in getting reasonable polarisation of the target nuclei. We report on various polarized targets which have been prepared from proteins (lysozyme, bovine serum albumin, urease), transfer ribonucleic acid and the large subunit of E.coli ribosomes dissolved in mixtures of heavy water with deuterated ethylene glycol, propylene glycol or glycerol doped with a paramagnetic radical, sodium bis[2-deuteroxy-2-methylpropanoato(2-)] oxochromate(V)-d_{12}. Clusters of 700 to 6000 protons defined by the structure of the dissolved particles embedded in a fully deuterated matrix were polarised 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. Polarised neutron scattering from these targets changed by factors up to four when the polarisation direction of the neutron beam was inverted. The results agree with the predicted values.

1. Introduction

The interaction between an atomic nucleus and a thermal neutron can be described by a scattering amplitude operator [1] .

$$A = b + 2 B \vec{I} \cdot \vec{s} \qquad (1)$$

b is the isotropic nuclear scattering amplitude. The second term describes the interaction of the neutron spin $\vec{s}$ with the nuclear spin I. - For the pourpose of this presentation, we only need to remember that for

protons: $b = -0.374\ 10^{-12}$cm $B = 2.9\ 10^{-12}$cm, and for

deuterons: $b = +0.66\ 10^{-12}$cm $B = 0.3\ 10^{-12}$cm (2)

The spin dependence as expressed by B is small compared with other nuclei usually occuring in biomolecules.

For a system of many atoms the cross section of coherent scattering as a function of momentum transfer $\vec{Q}$ is

$$\left(\frac{d\sigma}{d\Omega}\right)_{coh} = |U|^2 + 2\,p\,\mathrm{Re}[\,U\,V\,] + |V|^2 \qquad (3)$$

where $U = \sum_j b_j \exp(-i\vec{Q}\cdot\vec{r}_j)$ and $V = \sum_j P_j\, I_j\, B_j \exp(-i\vec{Q}\cdot\vec{r}_j)$

$b_j + B_j(\vec{s}\cdot\vec{I}_j)$ is the scattering amplitude for the nucleus of the atom which has the position $\vec{r}_j$ whose spin is I_j and the polarisation P_j.

As the polarisation p of the neutrons varies from -1 to +1 inversion of the neutron allows the direct determination of the product UV in Eq. 3 provided the target is polarised. This cross term is just the difference between a measurement of the scattering intensity S(↑ ↑) with neutron spins parallel to those of the protons and S(↓ ↑) where the relative spin orientation is antiparallel. - V depends on the target polarisation only and U is strictly independent of any polarisation. These three terms are also called the basic scattering functions of contrast variation .

We studied frozen dilute solutions of macromolecules. These give rise to strong forward scattering which van be analysed in terms of

$$U(Q) = \varrho\, v\, (1 - Q^2R^2/6 + -\ldots) \quad \text{and} \quad V(Q) = \varrho'\, v'\, (1 - Q^2R'^2/6 + -\ldots)$$

Here we have introduced the idea that the dissolved particle is characterised by its volume v, its radius of gyration R and its excess scattering density (or contrast) ϱ with respect to the solvent. The scattering density is just the sum of the scattering lengths of atoms in a given unit volume.

Example: In 1 cm^3 heavy water there are $1.1 * 6\cdot10^{23} / (2 + 2 + 16)$ D_2O molecules. Each of them has the scattering length $(2 * 0.667 + 0.580)\cdot10^{-12}$ cm. The scattering length (in cm) per cm^3 is $6.34\ 10^{10}$ cm^{-2}, the scattering density.

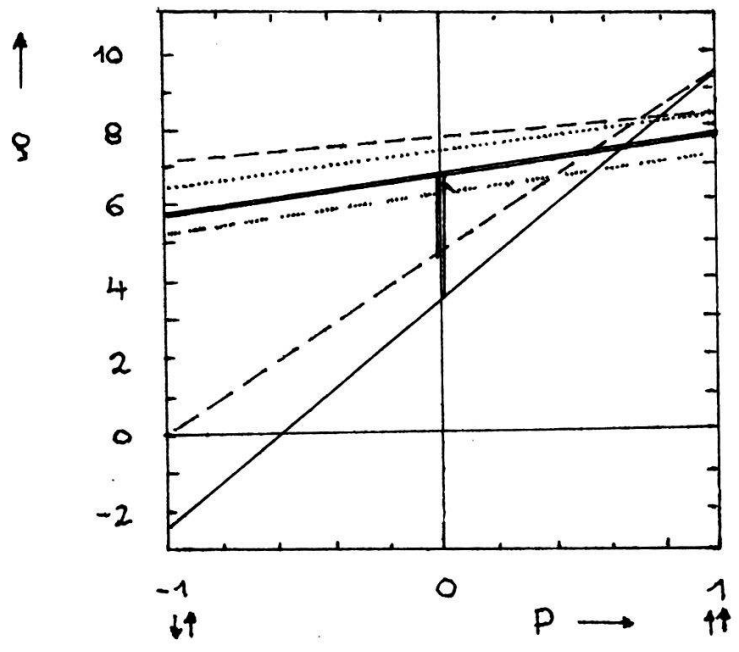


Fig. 1

Scattering density in units of 10^{-12} cm

——— proteins
– – – – – RNA

– – – – deuterated ribosome

solvents :
..... glycerol
— ·· — ·· heavy water
═══ 1 : 1 mixture of both

Spin dependence of polarized neutron scattering is considerably enhanced when the structures in question are embedded in a deuterated matrix. The protons of the dissolved particles act as markers which can be switched on/off by changing the mutual orientation of the neutron spin with respect to the proton spin direction. The variation of scattering density of some components of living cells and of deuterated solvents as a function of proton spin polarisation is shown in Fig. 1. The difference between the scattering density of the solute and that of the solvent will enter into forward scattering by its square. Spin dependent neutron scattering will enhance the contrast of proteins by a factor 3 and that of RNA by a factor 5 with respect to the widely used techniques of isotopic substitution at P = 0, provided a deuterated solvent is used.

Both the variation and the increase of contrast are most welcome in the investigation of large composite macromolecules. The variation of the target polarisation P allows the determination of the three basic scattering functions in eq.(3) A high contrast is needed whenever the proton rich label of a macromolecule becomes very small ($v' \ll v$), as this is the case with the proteins of ribosomes. These protein synthesizing organellae consist of more than 50 different proteins interwoven with two larger ribonucleic acid chains. The strategy of structure research relies on labelling one (or two) ribosomal proteins. In order to learn about the shape if each ribosomal protein in situ and its position and orientation within the ribosome many different samples have to be (and are being) prepared from cell cultures which are grown in heavy water and in light water. - The relative increase in contrast with proton polarisation is particularly high in the case of RNA. This is of interest for tracing the path of the messenger RNA and the role of transfer RNA during a cycle of amino acid binding to a nascent protein chain.

2. Experimental

The choice of the first experiments of polarised neutron scattering by dynamic polarised targets was ment as a basis for the detailed investigation of structure and function of ribosomes. The following samples were studied:

- Proteins of different molecular weight:

lysozyme	M = 14 000	R = 14 Å
bovine serum albumin	M = 70 000	R = 29 Å
urease	M = 500 000	R = 50 Å

- As a very handy RNA species we took tranfer ribonucleic acid

tRNA (bulk)	M = 27 000	R = 23 Å

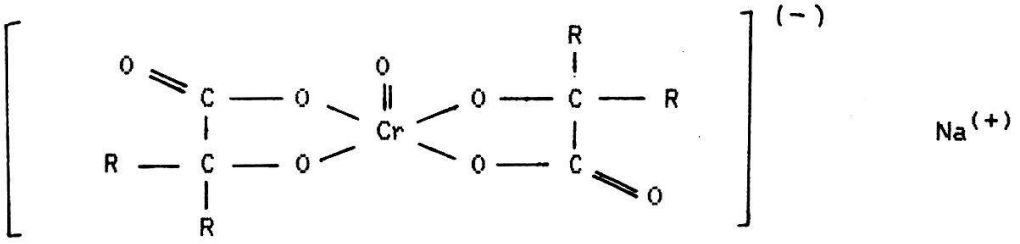
- The large subunit of E.coli ribosomes

50 S subunit	M = 1 700 000	R = 72 Å

As the ribosomal proteins have to be investigated in situ all unlabelled parts of the 50S subunit have to be deuterated as well. We therefore measured spin dependent neutron scattering of this particle as well, as it will serve as a reference for the study of the differently labelled large ribosomal subunits.

3. Solvents, paramagnetic centres and dynamic polarisation

The solvents were completely deuterated. They were mixtures of heavy water with 1,2-propanediol, ethylene glycol or glycerol in the ratio 1 : 1 all of them supporting dynamic polarisation in the presence of an organic chromium(V) compound given by the following structural formula:



We used a species of the above complex where R_1 and R_2 were methyl groups both as CD_3 and CH_3. The fully deuterated complex with $R_1 = R_2 = CD_3$ was preferred whenever a high deuteration of the solvent was considered to be essential.

The samples were prepared in pieces of 20 * 5 * 4 mm^3 by letting droplets of sample liquid freeze in a copper mould held at liquid nitrogen temperature. Three prisms were mounted vertically at a distance of 4 mm between two quartz blocks fitting with the walls of the aluminium mixing chamber of a fast access dilution refigerator. The DNP was achieved by irradiating the sample with 4 mm microwaves at helium bath temperatures at 0.3 K in a magnetic field of 2.5 T. The polarisation of the protons was measured by continuous-wave nuclear magnetic resonance (NMR), calibrated by measurements of the NMR signal in thermal equilibrium with the helium bath around 1 K

The proton spins of the above biomolecules can be nearly as easily polarized as the best frozen spin target materials in high-energy physics research. Lysozyme and bovine serum albumin require around 1% of the Cr(V) complex for polarisation to more than 60% after one one hour microwave irradiation at 0.3 K bath temperature of the dilution refrigerator whereas for the same result with urease the concentration of paramagnetic centres has to be doubled in order to obtain the same result. With tRNA a considerable increase of the polarisation rate with the electrolyte concentration of the solvent has been observed. tRNA is a poly-anion which is repulsive to the negatively charged Cr(V) complex. The proton spins of ribosomes in the reconstition buffer are easily polarized in the presence of 2% Cr(V) complex.

4. Polarised Neutron Scattering Experiments

These were carried out at the research reactor FRG1 of the GKSS Forschungszentrum at Geesthacht using a polarized target station of CERN.
The experimental set up (Fig. 2) consists of a neutron diffractometer with the following additional equipment:

- neutron polariser (broad band type, [2])
- neutron spin flipper and magnetic guide fields
- polarised target station [3]

The neutron velocity selector cuts out of the spectrum of thermal neutrons a wavelength band of 20% width centered at 5 Å. After a collimation length of 3 m

about 5000 polarised neutrons per second arrive at the 20 * 18 mm^2 cross section of the sample. Sometimes it is advantageous to work without a velocity selector as there is a gain in flux by a factor 30 using a spectrum of 50% fwhm at 4 Å.

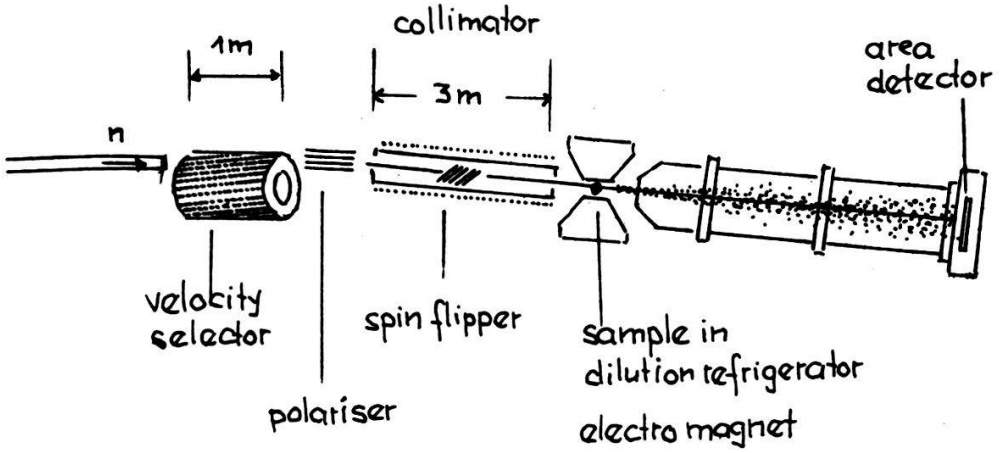


Fig. 2

Set up for polarised neutron scattering by a dynamic polarised target

As an example we show the results obtained from a 13% solution of bovine serum albumin in a solvent mixture containing 63% deuterated glycerol and 37% heavy water. Only 0.53% of the Cr(V) complex were needed in order to obtain a proton spin polarisation of 66% after 2 hours microwave irradiation. The polarisation was still increasing though at a slower rate as the microwave power had to be reduced. Drastic changes of neutron scattering - typically by a factor of four - are observed when the polarisation direction of the neutrons is inverted.

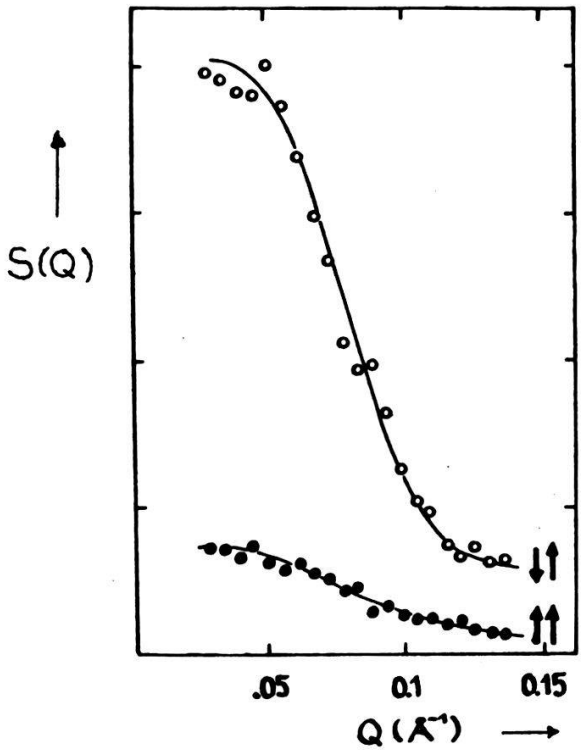


Fig. 3

Nuclear spin dependent polarised neutron small-angle of bovine serum albumin

The lower curve is obtained with neutron spins parallel to proton spins.

The proton spins of nucleic acids are polarised equally well as those of proteins provided the salt concentration is not too low. The results of polarised neutron scattering are rather similar to those obtained from the proteins.

A new pheonomenon was observed with the study of ribosomes. As these particles are larger than the previous ones the measurement had to be extended to lower momentum transfer. And here we hit upon the fact that the solvent too may show spin dependent neutron scattering starting typically at $Q < 0.03\ A^{-1}$. Spin dependent neutron scattering of ribosomes has to be corrected for this contribution from the solvent.

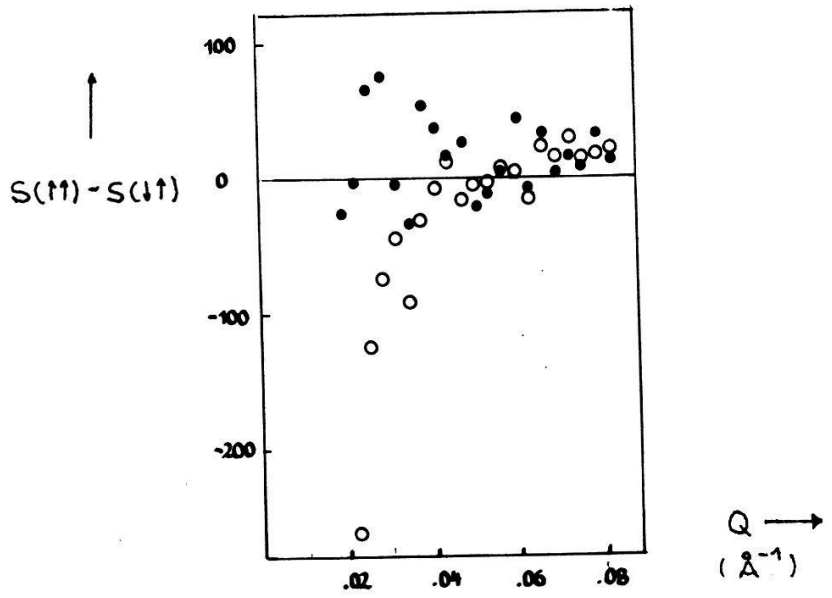


Fig. 4

Nuclear spin dependent neutron scattering $S(\uparrow\uparrow) - S(\downarrow\uparrow)$ of a 0.5 % solution of the large subunit of E.coli ribosomes. in a 1:1 D_2O - glycerol-d mixture.

● solution
o solvent

From spin dependent neutron scattering of the large ribosomal subunit we deduce R = 80 Å. This is the expected value from the protons of this particle.

Neutron scattering from the deuterated subunit is much weaker as the contrast is lower (Fig. 1). The sign of $S(\uparrow\uparrow) - S(\downarrow\uparrow)$ however remains the same as with the native ribosomes indicating a change of sign of both V(0) and V(0) in Eq.3 . Although there are still about 5% of the hydrogens of the deuterated subunit present as protons, the density of deuterons in this particle is so much lower than that of the solvent, that the net spin dependence as given by V(0) gets inverted.

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