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# Proton Spin-lattice Relaxation in Thylakoid Membranes of Chloroplasts
## Proton Dilution Studies

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SUMMARY

Measurements of proton spin-lattice relaxation time $T_1$ for thylakoid membranes of wheat chloroplast suspensions as a function of proton concentration in solvent are performed. The solvent was a mixture of $H_2O$ and $D_2O$. Different contributions to the proton relaxation process due to dipolar interactions are considered. The dipolar interactions p-p′ and p-i between exchangeable protons /p/ of membrane or solvent and non-exchangeable ones of membrane /p′/ and paramagnetic ions /i/ give predominant contribution to the relaxation process.

## INTRODUCTION

Previous investigations of thylakoid membranes of chloroplasts using proton nuclear magnetic relaxation method were concerned on: loosely bound manganese effect on relaxation process /WYDRZYNSKI et al., 1975, 1978/, phase dynamics investigations /BLICHARSKI et al., 1980/, membrane illumination observations /GOVINDJEE, 1978, WYDRZYNSKI et al., 1976, ROBINSON et al., 1980/ or magnetic relaxation in thylakoid membranes deprived of the loosely bound manganese fraction /BLICHARSKI et al./.

Our paper is a continuation of research in this field. The aim of this paper is to examine dipolar interactions p-p between solvent protons /p/ bound on membrane surface or exchangeable protons /p/ of membrane, and dipolar interaction p-i and p-p′ between proton of solvent /p/ or exchangeable one /p/ of membrane and paramagnetic ion /i/ or non-exchangeable proton /p′/ of membrane which can play an important role in the relaxation process. The loosely bound manganese fraction was removed in all investigated samples of thylakoid membranes.

## EXPERIMENTAL

Materials: E class chloroplasts /lamellae purified from stroma proteins/ were obtained from the wheat variety "Kolibri" as previously described /BLICHARSKI et al., 1980/. This procedure is more efficient in removing loosely bound manganese /longer relaxation time $T_1$/ than the usual procedure /WYDRZYNSKI et al., 1975, 1978/ and moreover it avoids using acetone which can disturb the membrane structure. The thylakoid membranes were suspended in mixture of $D_2O$ and $H_2O$ made in several proportions and incubated for about 24 hours to achieve the equilibrium state of the isotope exchange in the sample /KOENIG and SCHILLINGER, 1969/. The above suspensions were

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centrifuged at 16000xg for 1 hour and the sediment was resuspended in the suitable solvent. The final centrifugation was performed in glass tubes designed for NMR experiments. The supernatant was carefully removed and the sediment was used to the $T_1$ measurements. The dry weight of membranes was about 7%. In preparation we used $D_2O$ enriched to 99.75% obtained from the Institute of Nuclear Research in Świerk.

Apparatus: Proton spin-lattice relaxation times $T_1$ were measured on a spin echo spectrometer type SES-26, working at the resonance frequency 26 MHz, constructed by the Spin Echo Group from the Institute of Nuclear Physics in Cracow. The Carr-Purcell sequence of pulses was used, i.e. $\pi - t - \pi/2 - \pi$. The data were calculated fitting the exponential function with the three-parameter procedure using the Minuits program. The temperature of the sample was stabilized using a Unipan temperature stabilizer type 650 with an accuracy of about 0.1°C. The measurements were performed at the temperature 25°C.

## THEORY

If in the sample containing water solution of biological substance one can observe single exponential relaxation function, i.e. there is one mean relaxation time $T_1$, it permits assuming that the fast proton exchange between the free solvent and substance takes place /ZIMMERMAN and BRITTIN, 1957/. In such a case the average relaxation rate $T_1^{-1}$ can be described using a modified equation /DASZKIEWICZ et al., 1963/ provided that one does not observe the signal from non-exchangeable protons. In the case of thylakoid membrane suspensions without loosely bound paramagnetic manganese the exact formula for $1/T_1$ /BLICHARSKI et al./ has a simplified form:

$$T_1^{-1} = T_{1,s}^{-1} + (W_1 + W_2)c + q \qquad /1/$$

where $T_1$ is a measured mean spin-lattice relaxation time, $T_{1,s}$ is a relaxation time for a free solvent /water/, c is weight concentration of membranes, q is the relaxation contribution due to paramagnetic oxygen in the solvent, $W_i$ is given by:

$$W_i = (\alpha_b T_{1,b}^{-1})_i \qquad (i=1,2) \qquad /2/$$

where $\alpha_b$ is the ratio of the sum of the numbers of the bound solvent protons and exchangeable protons of membrane to the number of protons in the whole membrane, $T_{1,b}$ is the proton spin-lattice relaxation time for exchangeable protons of the membrane and solvent protons bound on the membrane surface and index i=1 refers to the dipolar interaction p-p between two protons of solvent bound on membrane surface or exchangeable ones of membrane, and i=2 refers to the dipolar interactions p-p and p-i between proton of solvent and paramagnetic ion of the membrane or non-exchangeable proton of the membrane. In the case of solvent being a mixture of $H_2O$ and $D_2O$, i.e. isotope dilution of protons the average value of $1/T_1$ can be presented in the following form /ANDERSON and ARNOLD, 1956, HILTON and BRYANT, 1976/:

$$T_1^{-1} = u_p T_{1,s}^{-1} + c u_p W_1 + c W_2 + q \qquad /3/$$

where $u_p$ is a proton concentration in the solvent.

RESULTS AND DISCUSSION

The measurements of proton spin-lattice relaxation time $T_1$ were performed for mixtures of $H_2O$ and $D_2O$ in several proportions. Figure 1 shows the relaxation rate $T_1^{-1}$ versus proton concentration $u_p$ /full circles/ for the above mixtures. From this dependence one can calculate the paramagnetic contribution $q=/0.178\pm0.03/s^{-1}$ which is comparable with that obtained previously /ANDERSON and ARNOLD, 1956, HILTON and BRYANT, 1976/. The results of proton spin-lattice relaxation time $T_1$ for thylakoid membrane suspensions in $H_2O/D_2O$ mixtures as a function of proton concentration $u_p$ are shown on Figure 1 /open circles/. The relaxation functions for the thylakoid membrane suspensions have single exponential form because of the relatively long period between pulse and sampling point of the spin echo signal /1ms/, which permits avoiding an additional contribution to the signal due to non-exchangeable

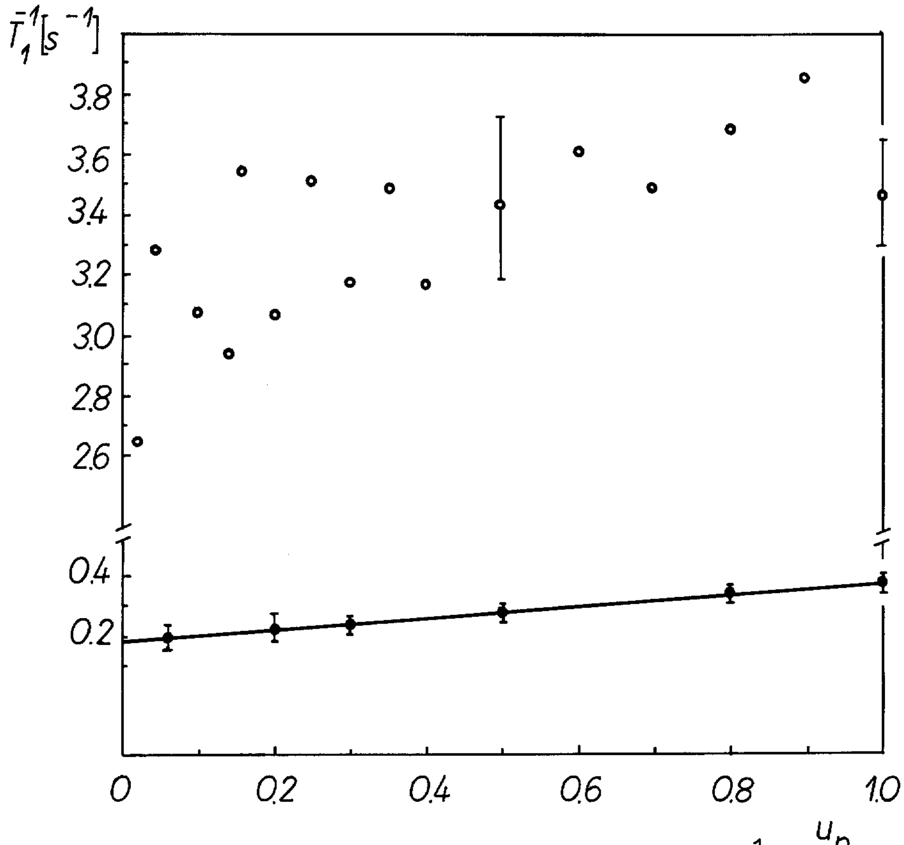


Fig. 1: The proton concentration dependence of $T_1^{-1}$ for $H_2O$ and $D_2O$ mixtures /full circles/ and for thylakoid membranes of wheat chloroplasts suspended in the above solvents /open circles/.

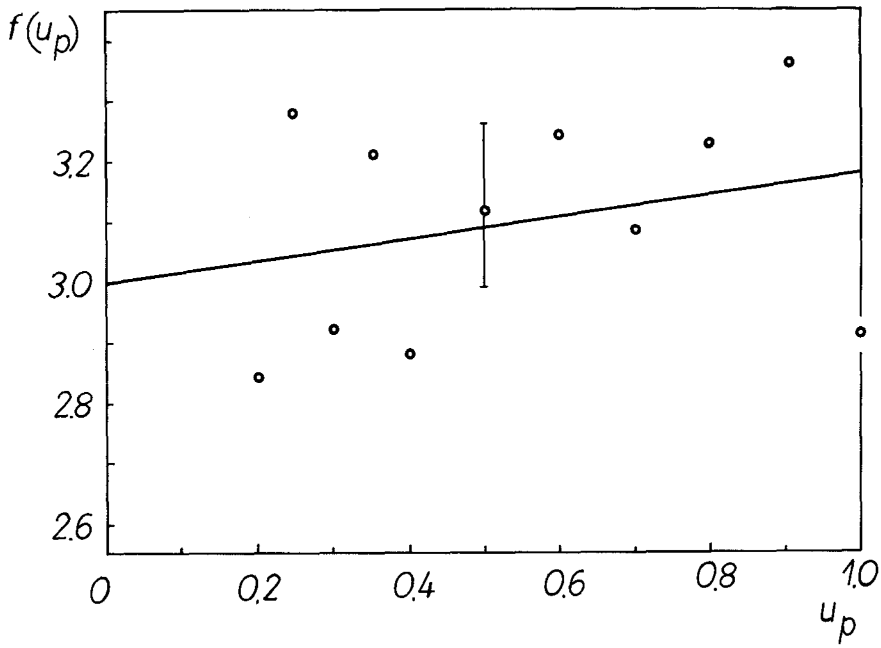


Fig. 2: The proton concentration dependence of $f/u_p/=cu_pW_1+cW_2$ for thylakoid membranes of wheat chloroplasts.

protons of skeleton of the membrane /BLICHARSKI et al./. From the experimental data one can calculate values of the function $f/u_p/=cu_pW_1+cW_2$ presented in Figure 2. The fitting of the straight line to these experimental results allows us to calculate the relative relaxation contributions of $W_1$ and $W_2$ which are equal to /6±4/% and /94±4/% respectively. The relatively large contribution of $W_2$ compared with similar results for water solutions of proteins /HILTON and BRYANT, 1976/ suggests that in thylakoid membranes of chloroplasts without loosely bound manganese /BLICHARSKI et al./ exist probably other paramagnetic species contributing to the spin--lattice relaxation process. Further investigations of this problem will be presented in the next papers.

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