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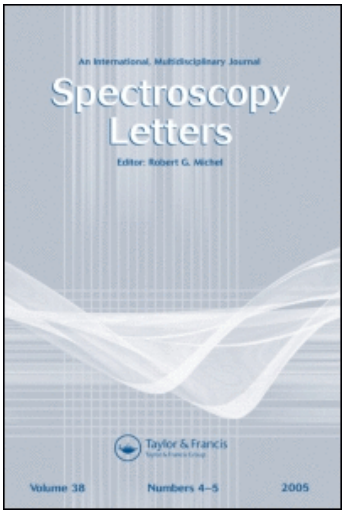

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Nuclear Relaxation Analysis of the Xenobiotic- Receptor (DNA Or Plasmatic Protein) Recognition Process

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NUCLEAR RELAXATION ANALYSIS OF THE XENOBIOTIC-RECEPTOR (DNA OR PLASMATIC PROTEIN) RECOGNITION PROCESS

Key words: DNA , NMR, relaxation rate, interaction, affinity index.

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ABSTRACT

The study of interactions of xenobiotics with macromolecular receptors is very important for understanding the chemical behaviour of xenobiotic compounds in the biological organisms.

The xenobiotic molecules are able to affect the natural activities of biological receptor such as DNA or plasmatic protein. In fact the modification of the conformation of DNA or plasmatic protein, induced by interaction with xenobiotic molecules, can determine profound alterations of the normal biochemical activity.

In this study a method based on proton NMR selective and non-selective spin-lattice relaxation rate measurements and their dependence on

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temperature for analyzing the ability of ligand to interact with receptor is used. The NMR parameters are a weighted average between the free and bound to xenobiotic environments.

INTRODUCTION

The ligand-macromolecule interaction is related to the surface properties of the macromolecule. Biological macromolecules, the surface conformational features of which are widely modulated, have considerable flexibility and ability in forming complexes of different chemical and biological significance with small ligands[1]. In large organized systems like cells and organisms, specific classes of biomacromolecules are involved in interaction, uptake and transport processes with small bio-ligands. In any biological reaction involving two or more chemical structures, the recognition step, which is related to the surface properties of the interating molecules[2], is crucial.

Interactions with bioactive molecules are of primary interest for defining the biological role of large biopolymers and for the activation of specific chemical processes. Effector molecules such as hormones, drugs, pollutants and synthetic molecules can induce different chemical responses in biopolymers.

In order to have a better description of the ligand-biopolymer interaction, techniques are needed for studying the chemical events of the interaction process. Here we report a method developed for investigating the biopolymers-effector recognition process. It is based on temperature-dependent NMR proton selective and non-selective spin-lattice relaxation rate experiments[3-6] and assumes fast chemical exchange conditions of the effector molecules between the free and bound sites. Since the selective R_1^{SE} and non-selective R_1^{NS} spin-lattice relaxation rates depend on the dynamical parameter (i.e. the correlation time τ_c) in different ways, the behaviour of R_1^{SE} and R_1^{NS} in the presence and absence of the biopolymer provides information on the ability of the effector molecule to interact with the biopolymer[7,9].

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To demonstrate the feasibility of this method, the interaction psoralen-DNA as well as lamotrigine-bovin albumin were analyzed.

EXPERIMENTAL

Calf thymus DNA sodium salt and psoralen (FIG. 1) were purchased from Sigma Chemical Co. and used without further purification. The 50% DMSO-d_6/50% D_2O solutions of DNA were in the range 0.1-1.2 mg/ml. Lamotrigine (FIG.2) was a gift from Wellcome, Bovine Albumin (molecular weight 67,000) was purchased from Sigma Chemical Co. Solutions were prepared dissolving appropriate amounts of protein and drug in 75% DMSO-d_6 and 25% D_2O mixture. This mixture was necessary because of low solubility of lamotrigine in D_2O.

NMR spectra were recorded on a Varian XL-200 and on a Bruker AC200 spectrometers operating at 200.058 MHz for ^1H. Spin lattice relaxation rates were measured using a $(180°\text{-}\tau\text{-}90°\text{-}t)_n$ pulse sequence and subsequent three parameter exponential regression analysis of the longitudinal magnetization recovery curve. The selective inversion square pulse was generated by the decoupler channel. The maximum experimental error was ±5%. The affinity index was calculated by a linear regression analysis. The fitting of the experimental data was obtained by the least square method.

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THEORY

For a homonuclear two-spin system four energy levels are necessary to describe the magnetic interaction between the two spins. Zero, single and double quantum transition probabilities W_i, refer to the change in magnetic spin quantum numbers during both the excitation and relaxation processes. In the presence of a short excitation pulse at the proton resonance frequency, the non-selective spin-lattice relaxation rate, R_1^{NS}, which gives the rate of change of the longitudinal magnetization, $M_z(t)$ is[10]:

$$R_1^{NS} = (W_0 + 2W_1 + W_2) + (W_2 - W_0) = \rho + \sigma \qquad (1)$$

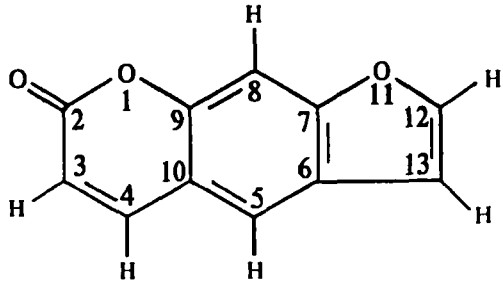

FIG. 1 Structure and numbering of psoralen.

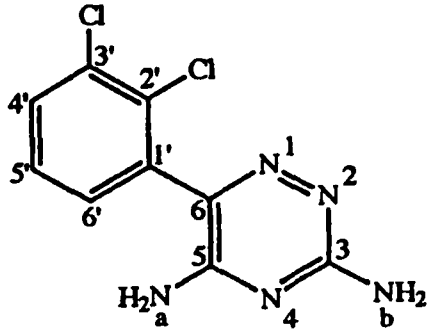

FIG. 2 Structure and numbering of lamotrigin.

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where ρ and σ are the direct and "cross-relaxation" terms respectively. The inversion of the spin population of a selected proton nucleus by a selective perturbation pulse cancels the cross-relaxation contribution and the selective spin-lattice relaxation rate is given by[11,13]:

$$R_1^{SE} = W_0 + 2W_1 + W_2 = \rho \qquad (2)$$

The transition probabilities are expressed by:

$$W_0 = \frac{1}{10} K^2 \tau_c \tag{3}$$

$$W_1 = \frac{1}{10} K^2 \frac{\tau_c}{1+\omega_H^2 \tau_c^2} \tag{4}$$

$$W_2 = \frac{1}{10} K^2 \frac{\tau_c}{1+4\omega_H^2 \tau_c^2} \tag{5}$$

in which $K = \hbar \gamma_H^2 / r_{H-H}^3$. In the above equation $\hbar$ is Planck's constant, ω_H and γ_H are the proton Larmor frequency and the magnetogyric ratio respectively, τ_c is the correlation time and r_{H-H} the internuclear proton-proton distance. The cross-relaxation term r_{i-j} may be calculated as a difference between eqs. (1) and (2). For a multi-spin system the inversion of all proton spin populations induces a cross-relaxation contribution on the observed signal that is the sum of all effective cross-relaxation terms. In this case:

$$R_1^{NS} = \sum \rho_{ij} + \sum \sigma_{ij} \tag{6}$$

$$R_1^{SE} = \sum \rho_{ij} \tag{7}$$

Fig. 3 shows the dependence of R_1^{SE} and R_1^{NS} on the molecular-motion conditions.

Considering the different dependence of R_1^{NS} and R_1^{SE} on the $\omega_H \tau_C$ term, it is possible to identify two different dynamical regions. When $\omega_H \tau_c << 1$, $R_1^{NS} \cong R_1^{SE}$ and the rate R_1^{NS}/R_1^{SE} assumes the constant value of 1.5. In slow motion conditions $\omega_H \tau_c >> 1$, $R_1^{NS} << R_1^{SE}$ and the ratio R_1^{NS}/R_1^{SE} assumes values close to zero.

The spin-lattice relaxation rate of a ligand in conditions of fast chemical exchange between the free state and a state in which it is bound to a

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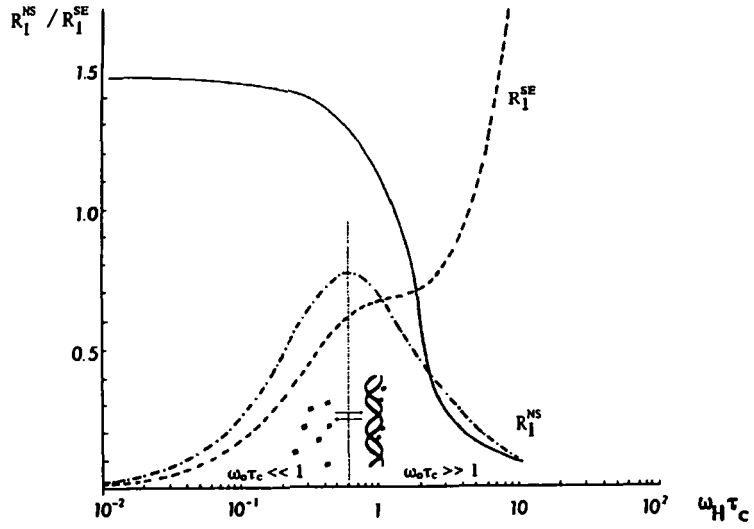

FIG. 3 R_1^{NS}, R_1^{SE} and R_1^{NS}/R_1^{SE} ratio of a proton pair in relation to $\omega_0\tau_c$, calculated from of equations (6) and (7) respectively, assuming a constant $r_{i\text{-}j}$ distanxe for the i-j dipolar interaction. The equilibrium between the ligand molecules in the bulk ($\omega_0\tau_c << 1$) and DNA-bonded ($\omega_0\tau_c >> 1$) is also shown.

biomacromolecule, is[4,14]:

$$R_{1exp} = \chi_B R_{1B} + \chi_F R_{1F} \tag{8}$$

where R_{1exp} is the relaxation rate of the ligand in the presence of a macromolecule, R_{1B} and R_{1F} are the relaxation rates of the pure bound and free environments respectively, and χ_B and χ_F are the molar fractions of the ligand in the bound and bulk conditions. As the concentration of the receptor is much smaller than that of the ligand, $\chi_F \approx 1$. It follows that:

$$R_{1exp} - R_{1F} = \chi_B R_{1B} = \Delta R \tag{9}$$

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If we consider the ligand macromolecule equilibrium:

$$M + L \underset{\leftarrow}{\overset{\rightarrow}{}} ML \tag{10}$$

the thermodynamic equilibrium constant is:

$$K = \frac{[ML]}{[M][L]} = \frac{[ML]}{\{[M_0]-[ML]\}[L]} \tag{11}$$

where $[M_0]$ is the initial concentration value of the macromolecule. From Eq. (11) we can derive:

$$[ML] = \frac{K[L][M_0]}{1+K[L]} \tag{12}$$

Since:

$$\chi_B = \frac{[ML]}{[ML]+[L]} \cong \frac{[ML]}{[L]} \tag{13}$$

using Eq. [12] we have:

$$\chi_B = \frac{K[ML][M_0]}{[L](1+K[L])} = \frac{K[M_0]}{1+K[L]} \tag{14}$$

Substituting Eq. (14) in Eq. (9) yields:

$$\Delta R = \frac{KR_{1B}[M_0]}{1+K[L]} \tag{15}$$

or:

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$$\frac{1}{\Delta R} = \left(\frac{1}{K} + [L]\right)\frac{1}{R_{1B}[M_0]} \tag{16}$$

from which the value of K is obtained by extrapolating the plot of $1/\Delta R$ vs [L] to zero. This analysis is based on the case of a single receptor site on the macromolecule. For n sites and assuming equivalent and non-interacting sites, Eq. (16) becomes:

$$\frac{1}{\Delta R} = \left(\frac{1}{K[L]^{n-1}} + [L]\right)\frac{1}{R_{1B}[M_0]} \tag{17}$$

In this case the calculation of K could be difficult because of uncertainty on the number of macromolecular receptor sites. Moreover, the calculation of K by extrapolating the $1/\Delta R$ vs [L] plot to zero could be subject to large errors.

The approach we propose is based on a simplified procedure. If we plot ΔR versus the macromolecular concentration $[M_0]$ (Eq. (15)) we obtain a straight line passing through the origin, with a slope:

$$[A]_L^T = \left(\frac{KR_{1B}}{1+K[L]}\right) \tag{18}$$

which we define the "affinity index". The dimensions of the affinity index are $[mol^{-1}\ s^{-1}\ l]$. For n receptor sites of similar strenght:

$$\Delta R = \frac{KR_{1B}[L]^{n-1}}{1+K[L]^n}[M_0] \tag{19}$$

The plot of ΔR versus $[M_0]$ is still linear. The general form of the affinity index is therefore:

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$$[A]_L^T = \left(\frac{K[L]^{n-1} R_{1B}}{1+K[L]^n} \right) \quad (20)$$

In order to compare the value of $[A]_L^T$ determined in different systems, the temperature and the constant ligand concentration must be specified. This is the meaning of T and L subscripts in the affinity index symbol.

The comparison of eqs. (15) and (18) shows that the "affinity index" can be determined independently from the stoichiometry of the interaction. This parameter is related to the global affinity between the ligand and the receptor molecule. The presence in the macromolecule of binding sites with different affinity toward the ligand do not alter significantly the form of the equation (18).

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RESULTS AND DISCUSSION

The study of ligand-macromolecule interactions by nuclear relaxation analysis is based on the fast chemical exchange conditions between the free and bound forms of the ligand in respect to their relaxation rates.

Two experimentally determined parameters are required for analysis of the interaction, the selective (R_1^{SE}) and the non-selective (R_1^{NS}) spin-lattice relaxation rates.

The dependence of R_1^{SE} and R_1^{NS} on fast and slow reorientational molecular dynamics is shown in FIG. 3 . For the free ligand in the fast motion limit, $R_1^{NS} > R_1^{SE}$. For ligand-biomacromolecule complexes, in slow motion conditions a relevant contribution to R_1^{SE} appears, and $R_1^{SE} > R_1^{NS}$.

In order to calculate the affinity index the relationship between ΔR_1^{SE} and the protein concentration $[M_0]$ was studied (FIG. 4). As suggested by Eqs. (15) and (19) the relationship is linear and passes through the origin. The slope of the line obtained by linear regression analysis of the experimental results gives the value of the affinity index.

Here we report the results obtained studying the interaction between Lamotrigine and Bovine Albumine. The affinity index calculated by

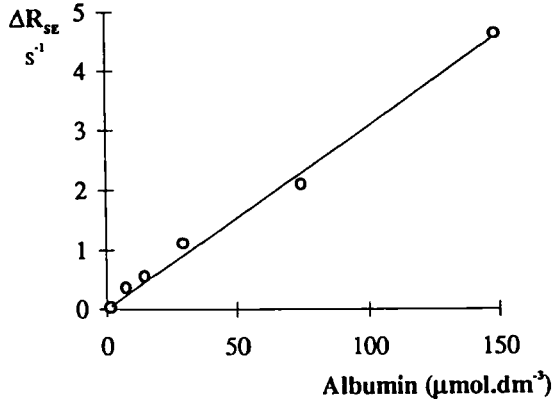

FIG. 4 Linear dependence of H_4' selective relaxation enhancements, ΔR_1^{SE}, versus bovine albumin concentration. The affinity index was calculated from the slope of this plot.

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studying the ΔR_1^{SE} of H_4' proton of Lamotrigine at 300K and 7.5×10^{-2} mol.dm^{-3} lamotrigine is 3.11×10^4 mol^{-1} s^{-1}·dm^3

The affinity index offers several advantages. It is calculated as the slope of a straight line passing through the origin and is less affected by intrinsic errors than intercept calculation. It represent the global affinity between the ligand and the macromolecules and can be calculated without knowledge of the stoichiometry of the interaction, i.e. the number of binding sites is not required for affinity index calculation. The affinity index enables comparison of the binding capacity of a specific macromolecular structure toward several ligands.

A further consideration is required. The method is based on the assumption that the dipolar is the main relaxation machanism. In case of protons were the dipolar is not the main relaxation mechanism, the calculation of the affinity index is not suggested. The importance of the dipolar machanism can be evaluated by the analysis of R_1^{NS}/R_1^{SE} ratios.

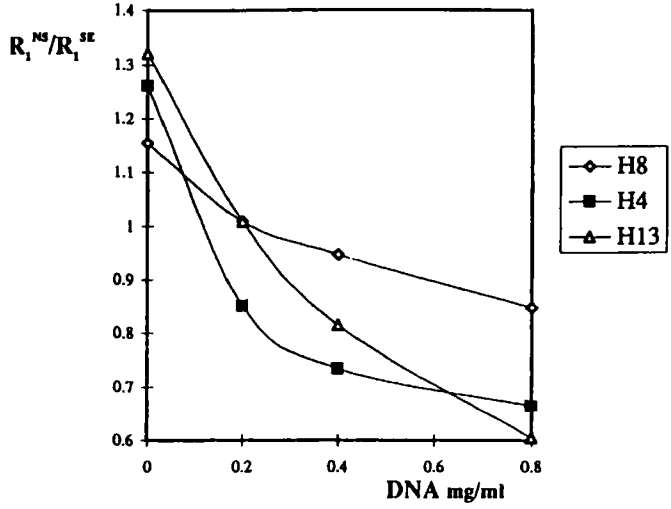

FIG. 5 Plot of R_1^{NS}/R_1^{SE} ratios against the DNA concentration. (Psolaren, 0.1 mol.dm^{-3}; T=298 K).

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In FIG. 5 is shown the behaviour of R_1^{NS}/R_1^{SE} for three psoralen protons at different DNA concentrations. The H_4 and H_{13} protons had higher values of the R_1^{NS}/R_1^{SE} ratio than the H_8 proton in the absence of DNA. This can be explained by the fact that the H_8 nucleus does not have a high proton density in its environment. This may reduce the efficiency of the dipolar relaxation term and may also account for the slower increase in R_1^{SE} of the H_8 proton on addition of DNA. This suggests that only H_4 and H_{13} protons could be used for the affinity index calculation.

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REFERENCES

[1] Nelson J.W, "Chemistry and Physics of DNA-Ligand Interactions" (N.R.Kallenbach, ed.), Adenine Press, Schenectady, New York, (1990), p.65.

[2] Pullman B.and Jortner J(eds.), "Molecular Basis of Specificity in Nucleic Acid-Drug Interactions". Kluwer Academic Publ., Dordrecht, The Netherlands, (1990).

[3] Rossi C., Donati A. and Sansoni M. R.; Nuclear Magnetic Resonance as a tool for the identification of specific DNA-Ligand interaction, Chem. Phys. Letters, 189, (1992), 278 .

[4] Rossi C., Sansoni M. R. and Donati A., "Proton Selective and Non-Selective Spin-Lattice Relaxation Rates Analysis as a Tool for Studying the Interaction of DNA with Biomolecules and Pollutants", Trends in Ecological Physical Chemistry. D. Pitea and A. Schiraldi eds. (Elsevier Applied Science Publishers, Amsterdam) 57 (1993).

[5] Valensin G., Kushnir T. and Navon G.; "Selective and nonselective proton spin-lattice relaxation studies of enzyme-substrate interactions," J. Magn. Reson., 46, (1982), 23.

[6] Bonechi C., Donati A., Picchi M.P., Rossi C.and Tiezzi E.; "DNA-ligand interaction detected by proton selective and non-selective spin-lattice relaxation rate analysis". Colloids and Surfaces A, 115, (1996), 89-95.

[7] Uccello-Baretta G., Bertucci C., E. Domenici and P. Salvadori; J. Am. Chem. Soc., 113, 1991, 7017.

[8] Gaggelli E., Valensin G., Tamir K. and Navon G., Magn. Reson. Chem.; 30, 1992, 461.

[9] Rossi C.: "Selective relaxation techniques in biological NMR", Encyclopedia of NMR, D.M.Grant and R.K.Harris eds.J.Wiley & Sons Publishers, 7, 1995, 4237.

[10] Freeman R., Hill D.W., Tomlinson B.L. and Hall L.D.; J.Am.Chem.Soc., 61, 1974, 4466,.

[11] Noggle J.H. and Shirmer R.E., "The Nuclear Overhauser Effect", Academic Press, New York, 1971.

[12] Neuhaus D. and Williamson M., "The nuclear overhauser effect in structural and conformational analysis", VCH, New York, 1989.

[13] Campbell I.D. and Freeman R. ; J. Magn. Reson. 11, 1973, 143.

[14] C.Rossi, G.Valensin, A.Prugnola and N.Niccolai: "Structure and dynamics of biomolecules in solution: nuclear magnetic relaxation studies", Steric Aspects of Biomolecular Interactions. G.Náray-Szabó and K.Simon editors. CRC Press Inc. Boca Raton (USA). (1987), 123-141 .

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