APPLICATION OF NOE AND SELECTIVE T_1 FOR THE INVESTIGATION OF BINDING OF LIGANDS

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I. INTRODUCTION

One of the drawbacks of NMR is its low sensitivity relative to other spectroscopic methods. However, taking advantage of the averaging property of NMR of exchanging systems, one may obtain information concerning minute amounts of bound ligands from the signals of the excess free ligands. This was successfully used in systems of paramagnetically labelled enzymes (1). The present work deals with diamagnetic systems.

II. RESULTS AND DISCUSSION

Selective T_1 was shown to be a sensitive method for detecting binding of ligands to macromolecules (2). The dependence of selective T_1 on the concentration of the ligand which follows an equation analogous to that of T_1 and non selective T_1 (see e.g. ref.3) is a very convenient way to measure binding constants. In Fig.1, the determination of the binding constant of the activator carbobenzoxyglycine (Z-Gly) to Zn(II) carboxypeptidase A (CPA) is described.

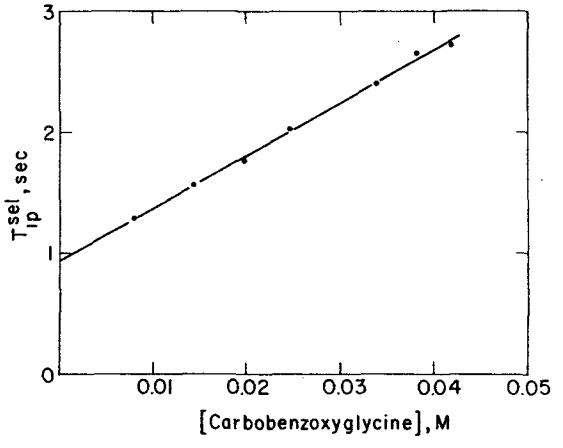

Fig. 1

The result K_d=21.2 mM at 28°C agrees with the dissociation constant found for the activation of the hydrolysis of dipeptides (3), inhibition of hydrolysis of esters (4) and tripeptides (5), and is similar also to K_d for Mn(II) CPA (3). The addition of substrates abolishes the relaxation effects of CPA on Z-Gly indicating competition for the same site, thus supporting the hypothesis of activation through productive and non productive binding sites (6).

Transferred NOE. Another sensitive method for detecting binding of ligands is the measurement of NOE which is transferred from bound to free ligand by chemical exchange (7-9). The expression for the overall NOE ($\eta_I(S)$) in the presence of exchange (7) may be written in the following simplified form:

$$\eta_I(S) = \frac{\langle I_z \rangle - I_o}{I_o} = \eta^b \frac{bT_{1f}+bf\tau_b}{fT_{1b}+f\tau_b+bT_{1f}} \quad (1)$$

where f and b are the fractions of free and bound ligand and τ_{1f} and T_{1b} are their selective T_1. We can see that in the case $bT_{1f} \gg f\tau_b$, fT_{1b} one obtains $\eta_I(S)=\eta^b$. For ligands bound to macromolecules where $\omega\tau_c \gg 1$, η^b may reach the value of -1. Therefore this is a very sensitive method for detecting minute amounts of binding to macromolecules or receptors.

In Fig. 2 the NOE of phenyl protons of Z-Gly bound to CPA was measured as a function of the bound fraction. The points are experimental results while the theoretical plot was not based on fitted parameters but was calculated using Eq.(1) with b, T_{1f} and T_{1b} independently measured as described in the first section.

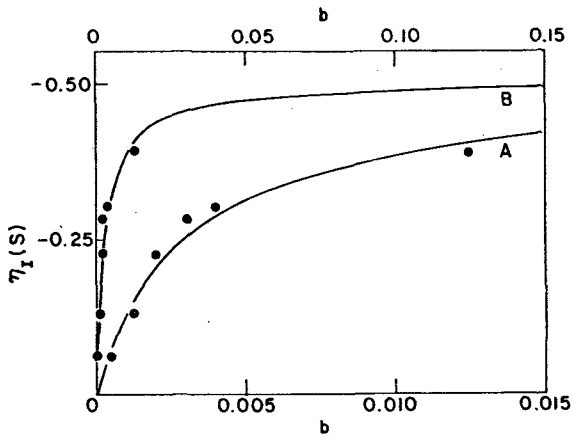

Fig. 2

The assignment of the functional groups on the receptor which interact with the ligand is hindered by spin diffusion. This can be demonstrated by the fact that a plot of the transferred NOE, $\eta_I(S)$, as a function of the irradiation frequency, is very similar to the spectrum of the enzyme itself. In Fig.3, the "NOE spectrum" is given for the phenyl protons of two activators: cinnamic acid (71 mM) in the presence of CPA (0.33 mM) (A) and Z-Gly (57 mM) in the presence of CPA (0.3 mM) (B). The NMR spectrum of the enzyme itself (1mM) is given in the upper plot of the Figure. Note that in the "NOE spectrum" the region usually obscured by the water signal is also observable.

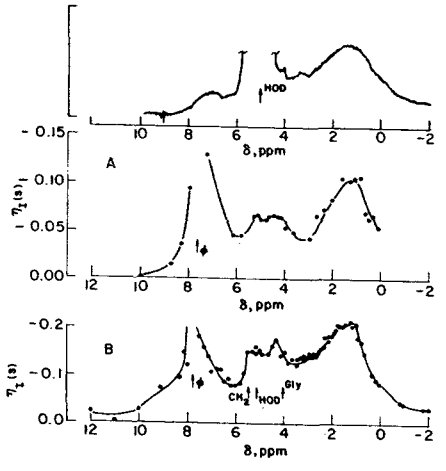

Fig. 3

Transient NOE. To avoid the effect of spin diffusion, the transient NOE methods were suggested by several authors (10-13). The equations of the time dependence of the transient NOE were developed for the case of a single species. Here we deal with exchanging ligands. There are two possible methods for measuring transient NOE (see Fig.4).

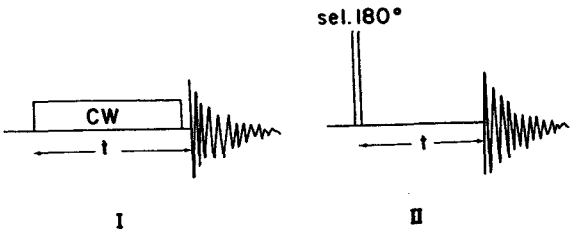

Fig. 4

I. Progressive saturation. In this method $\langle S_z \rangle$ does not reach a steady state level:

$$\langle S_z \rangle - S_o = S_o(e^{-Ct} - 1)$$

where C is the saturation rate of the irradiated spin. By introducing this expression into the general rate equations:

$$\frac{d\langle I_z \rangle^f}{dt} = -\rho^f(\langle I_z \rangle^f - I_o^f) - \frac{\langle I_z \rangle^f}{\tau_f} + \frac{\langle I_z \rangle^b}{\tau_b}$$

$$\frac{d\langle I_t \rangle^b}{dt} = -\rho^b(\langle I_t \rangle^b - I_o^b) \qquad (2)$$

$$-\sigma(\langle S_z \rangle - S_o) - \frac{\langle I_z \rangle^b}{\tau_b} + \frac{\langle I_z \rangle^f}{\tau_f}$$

where ρ^f and ρ^b are the relaxation rates of the free and bound ligand, respectively and τ_f, τ_b are their exchange lifetimes, we obtain for the case of fast exchange and b<<1:

$$\eta_T = \eta_I(S)\left[1 + \frac{1}{(C-\alpha)}(\alpha e^{-Ct} - Ce^{-\alpha t})\right]$$

where: $\alpha = \rho^f + b\rho^b$, and $\eta_I(S)$

is given by Eq.(1). Calculated values of observed NOE for several values of b are given in Fig. 5.

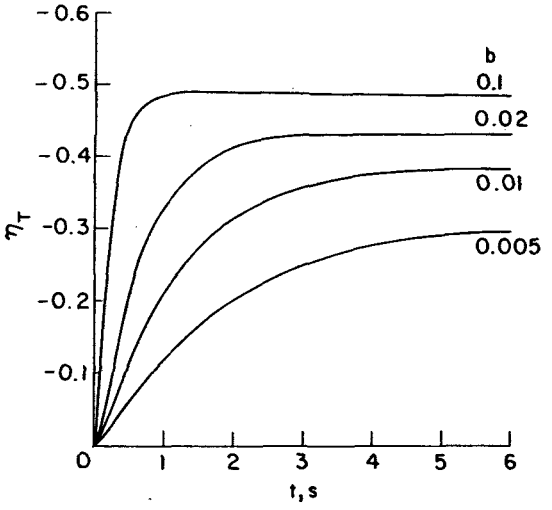

Fig. 5

II. Selective inversion. In this case the time dependence of the z component of the irradiated spins is given by the equation $(\langle S_z \rangle - S_o) = -2S_o e^{-Ct}$. Again, by introducing this expression into Eqs.(2) we obtain for the case of fast exchange:

$$\eta_p = 2\eta^b{}_{b\rho}{}^b \; (e^{-\alpha t} - e^{-Ct})/(C-\alpha)$$

In this case we get a maximum in the time dependence function, at a point $t_{(max)} = \ln(C/\alpha)/(C-\alpha)$, as can be seen in Fig.6.

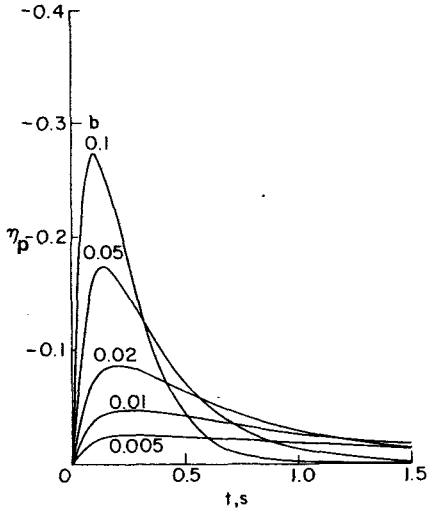

Fig. 6

By comparing the two methods for two values of b (Fig.7), it is seen that the second method is the more sensitive one for the NOE obtained within short time durations. However, the requirement of a selective inversion of magnetization is sometimes difficult to achieve in macromolecular systems, since the pulse has to be short enough to avoid relaxation during the pulse and long enough to be selective.

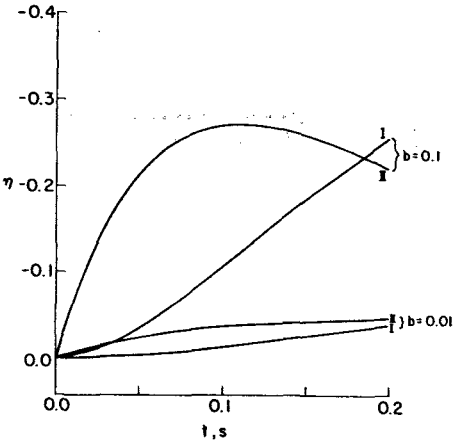

Fig. 7

In conclusion we see that selective T_1 and the transferred NOE are sensitive methods for the detection of binding of ligands to macromolecules. While selective T_1 is a convenient method for the determination of binding constants, the transferred NOE may give information concerning the mode of binding. However, in order to avoid effects of spin diffusion the transient methods of truncated or selective inversion should be used and may be analyzed according to the explicit expressions given above.

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