Detection of Stereoselective Interaction of D- and L-Tryptophan with Human Serum Albumin by Selective Relaxation Rate Measurements

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Mono- and bi-selective proton spin-lattice relaxation rates of L- and D-tryptophan have been measured in the free state and in the presence of human serum albumin; the selective relaxation rates of the amino acid are strongly enhanced in the presence of albumin and different values for the protons of the two antipodes in the bound state are observed.

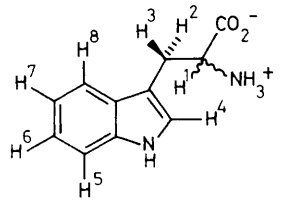
N.m.r. methods based on the determination of the relaxation rate of selectively excited protons[1,2,3] have been extensively employed in detecting binding interactions between small molecules and biological macromolecules.[4,5] Indeed, selective relaxation rates (R^s) show a remarkable and sharp increase in the region of slow molecular motions ($\omega\tau_c \gg 1$) in which the small molecule is forced when interacting with the macromolecule. Therefore, in the fast exchange limit, changes in R^s values (related to motional and conformational features of the substrate in the bound state) also can be detected in the presence of a large excess of free ligand.[4,5] Non-selective relaxation rates (R^{ns}) are much less sensitive to the presence of the macromolecule.[5]

To date, no cases have been reported in which selective relaxation rate methods have been used to detect differences in the interaction of two enantiomers of a chiral molecule with a biological macromolecule. This can be of interest to investigate the relationship between the biological activity of chiral substrates and their absolute configuration.[6]

With the aim of exploring the usefulness of proton selective relaxation rate measurements in providing evidence for differences in the conformational or dynamic features of two enantiomers, due to the interaction with a macromolecule, we measured the spin–lattice relaxation rates (R^s) of L- and D-tryptophan (L- and D-Trp) in the free state and in the presence of human serum albumin (HSA). For this system, the occurrence of stereospecific interactions has been already established by other techniques.[7,8,9]

Selective relaxation rates were measured in the initial rate approximation[1] on a Varian VXR-300 MHz instrument by applying a selective 180° pulse with the proton decoupler at the selected frequency for 20 ms. After time t, a nonselective 90° pulse was applied to detect the longitudinal magnetization. Measurements were carried out for all protons of L- and D-Trp in the free state and in the presence of HSA (Trp 0.03 M, molar ratios Trp/HSA 140, 70, 35, pH 7.4 in phosphate buffer, D_2O solutions, 22 °C). HSA (fatty acid free) was purchased from Sigma; oligomerized HSA was removed by gel filtration on a Sephacril S-300 column, 50 × 2.5 cm; monomeric HSA was lyophilized and then dissolved in the deuteriated buffer.

We found that the proton selective relaxation rates of Trp are strongly enhanced in the presence of HSA, particularly for the indole moiety and, more importantly, the R^s values of the D-form are increased to a larger extent than those of the L-form. For example, the selective relaxation rate of proton H-5 (Scheme 1) is 0.14 s^{-1} in the free state and, in the presence of HSA (molar ratio 140), increases to 0.75 s^{-1} for L-Trp and 0.92 s^{-1} for D-Trp. In the fast exchange limit, the observed value of R^s is the weighted mean of the values in the free and bound states: $R^s = (\chi_{free} \times R_{free}) + (\chi_{bound} \times R_{bound})$. The R^s values for L- and D-Trp in the bound state can be easily calculated, there being a large excess of Trp with respect to



Trp

Scheme 1

HSA ($\chi_{free} \simeq 1$). Assuming a 1 : 1 interaction, $R_{5\ bound}$ for H-5 was 86.2 s^{-1} for the L-form and 109.6 s^{-1} for the D-form. Analogous results were obtained starting from molar ratios Trp/HSA of 70 and 35.

Therefore, the measured differences in the relaxation parameters of the D- and L-forms reflect differences in their dipolar interaction; D-Trp is exposed to dipolar interactions with protein protons near the binding site to a greater extent than is L-Trp. It is notable that, at the high value used for the molar ratio between the amino acid and the protein, the observed effects cannot be due to different degrees of dissociation for the two complexes L-Trp/HSA and D-Trp/HSA. Combination of bi-selective relaxation rate[3] measurements for the proton H-5 under simultaneous irradiation of the proton H-6 (R_{56}) and mono-selective relaxation rate measurements (R_5) allowed determination of the cross-relaxation term $\sigma_{56} = R_{56} - R_5$. This term was 0.06 s^{-1} in the free state, -27.9 s^{-1} for L-Trp in the bound state, and -35.9 s^{-1} for D-Trp in the bound state (the negative value is indicative of the slowing down of molecular motions to the $\omega\tau_c \gg 1$ region). The cross-relaxation terms were used to calculate the correlation times τ_{c56} for the vector $\overline{56}$,[3,5] the value of the r_{56} distance (2.44 Å) being known.[10] The values obtained for free Trp, bound L-Trp, and bound D-Trp are 4.25×10^{-11}, 9.6×10^{-8}, and 1.2×10^{-7} s, respectively.

Hence, the molecular motion of both D- and L-forms is strongly slowed by the interaction with HSA; however, the reorientation time of the D-form is different from that of the L-form.

It should be noted that the differences between the selective relaxation parameters of the two isomers in the bound state are small when compared to the differences observed between the R^s values of Trp in the free and bound state; nevertheless, these differences are completely reproducible.

On the basis of the above results it can be concluded that the selective relaxation rates are not only a suitable probe of the occurrence of interaction between the protein and the amino acid, but they are also signficantly affected by the differences in the conformational or dynamic features of the two diastereoisomeric adducts formed by HSA and L- or D-Trp.

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