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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 113–121
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Synthetic polymers as biomacromolecular models for studying ligand–protein interactions: A nuclear spin relaxation approach

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Received 14 February 2005; received in revised form 15 June 2005; accepted 15 June 2005
Available online 18 August 2005

Abstract

In this paper, we applied an NMR methodology based on the analysis of selective spin–lattice relaxation rate enhancements of ligand protons induced by interaction processes between prednisolone and a synthetic copolymer, namely poly(*N*-isopropylacrylamide-*co*-*N*-acryloil-L-phenylalanine), in order to investigate this system as a model for studying drug–biomacromolecules interactions. The contribution from the bound ligand fraction to the observed relaxation rate in relation to macromolecule concentration allowed the calculation of the normalized affinity index $[A_I^N]_L^T$, in which the effects of motional anisotropies and different proton densities have been removed. This parameter, which represents the global affinity of the ligand towards the macromolecule, isolates the contribution due to a decrease in the ligand dynamics caused by the binding with the copolymer. The affinity index calculated for prednisolone–copolymer complex compared to that obtained for prednisolone–albumin system, suggested that synthetic polymers as models of biomacromolecules can play an important role in drug–protein interaction studies.

Keywords: ^{1}H spin–lattice relaxation rate; Ligand–macromolecules interaction; Affinity index; Prednisolone; Synthetic polymers

1. Introduction

Several stimuli-responsive polymers were synthesized in view of their potential application in the biotechnological field [1–3]. These compounds are called "intelligent polymers" [4].

Intelligent polymers are soluble, surface-coated or crosslinked polymeric materials capable of undergoing phase separation in response to external stimuli such as temperature, pH, ions or other chemical species, electric or magnetic fields [5]. A thermosensitive polymer is highly hydrated, water-soluble, extended chain below its lower critical solution temperature (LCST) in water, but becomes hydrophobic and an insoluble aggregate due to rapid dehydration above the LCST [6,7].

Many applications have also been designed for these polymers: for instance, they are being studied in drug delivery [8–10], solute separation [11] and solvent extraction [12]; furthermore, they can be grafted onto membranes to be used as "chemical valves" [13,14].

Poly(*N*-isopropylacrylamide) (PNIPAAm), with its LCST of 32 °C is the most extensively used polymer since its conformational changes can be conveniently examined by adjusting the solvent quality via temperature [15]. Furthermore, polymerizing *N*-isopropylacrylamide (NIPAAm) with weakly ionizable comonomers allows to obtain intelligent polymers capable of responding to both temperature and pH variations.

In particular, in this work the copolymers are synthetized using the *N*-isopropylacrylamide (NIPAAm) and *N*-acryloyl-L-phenylalanine (PHE). In relation to the molar ratio between the two reagents the obtained copolymers are defined as coPHE *x*/*y*, having the general structure, reported in Fig. 1. In

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doi:10.1016/j.jpba.2005.06.019

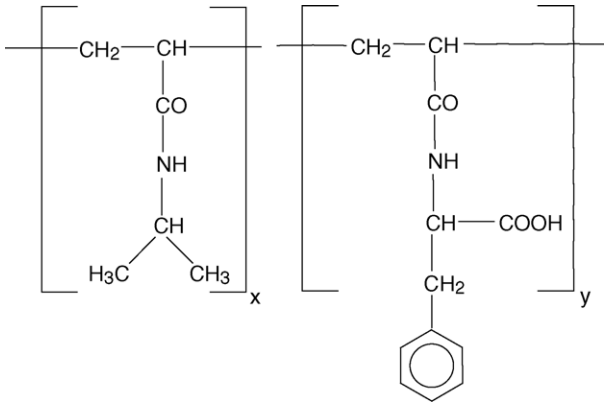

Fig. 1. Structure of (coPHE *x*/*y*) copolymer.

this paper, coPHE *x*/*y* was used as macromolecular receptor to test the interaction processes with glucocorticoid drugs.

Glucocorticoids are potent anti-inflammatory drugs and exert their anti-inflammatory action through inhibition of lymphocyte proliferation and synthesis of proinflammatory cytokines as well as by down-regulating specific adhesion molecules resulting in redistribution of lymphocyte traffic [16]. The broad effects of glucocorticoids are generally mediated through binding of glucocorticoids to cytoplasmic receptors (GRs).

Presently, in the clinic practice prednisolone and prednisone are widely used due to their pharmacologic activity four times greater than cortisol.

In particular, prednisolone (11,17,21-tridihydroxy-pregna-1,4-diene-3,20-dione) is a synthetic corticosteroid that is used to decrease inflammation in various different diseases and conditions [17].

The aim of this paper is the characterization of the interaction between coPHE 1/2 and prednisolone by nuclear spin relaxation analysis, in order to check the ability of the copolymer to mime biomacromolecular structures as receptors for studying drug–protein interaction processes. In fact, the synthesis of these polymers can be driven in order to obtain different protein-like structures to mime membrane and/or transport proteins and other receptor systems as well as in drug delivery. Moreover, these polymers may be chemically conjugated to biomolecules to achieve polymer–biomolecule systems that can respond to biological as well as to physical and chemical stimuli [18]. The conjugation may be used in order to control protein–ligand recognition and binding properties.

NMR investigation is based on the comparison of selective (R_1^{SE}) and non-selective (R_1^{NS}) proton spin–lattice relaxation rate of the ligand in the presence and absence of the macromolecule [19,20]. The formation of ligand–macromolecule complexes affect R_1^{NS} and R_1^{SE} to different extents, depending on the dynamical parameters (i.e. the correlation time τ_c), assuming fast chemical exchange between the bound and the free environments with respect to both chemical shift difference and proton relaxation rate. In particular, the slower ligand dynamics in the ligand–macromolecule complex mostly affects R_1^{SE}. In the presence of well-resolved proton resonances R_1^{SE} can be easily determined in different systems. The contributions arising from the fraction of the ligand bound to the protein allowed the calculation of the "affinity index" $[A]_L^T$, a useful parameter to attain information about the strength of non-specific and/or specific interactions occurring within the systems [21]. Since ligand motional anisotropies and different proton densities may affect the relaxation rates, $[A]_L^T$ has been normalized to the proton selective relaxation rate of the free ligand. The new calculated parameter, $[A_I^N]_L^T$, the normalized affinity index, appears to be totally independent from the intrinsic relaxation properties of any proton nuclei and can be proposed as a more suited parameter to compare the recognition processes between a protein and different ligands. The affinity index represents the global affinity between the ligand and the macromolecule, and its calculation does not require an a priori knowledge of the number of ligand coordination sites present at the macromolecule surface or their specific kinetics constant values. In particular, this methodology allows to compare the strength of the interaction processes involving the same protein and different ligands [22,23].

2. Theory

For multispin interaction as it occurs in complex systems of biomolecules, the "non-selective" spin–lattice relaxation rate R_1^{NS} of an *i* nucleus interacting with neighbouring *j* nuclei and the selective R_1^{SE} obtained by excitation of the *i* nucleus, while the *j* nuclei are at thermal equilibrium [24–27] are as follows [26,28,29]:

$$R_I^{NS} = \frac{1}{10}\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left[\frac{3\tau_c}{1+\omega_H^2\tau_c^2} + \frac{12\tau_c}{1+4\omega_H^2\tau_c^2}\right] \quad (1)$$

$$R_1^{SE} = \frac{1}{10}\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left[\frac{3\tau_c}{1+\omega_H^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_H^2\tau_c^2} + \tau_c\right] \quad (2)$$

The spin–lattice relaxation rate of a ligand under conditions of fast chemical exchange between the free and bound states is described by:

$$R_{1obs} = \chi_B R_{1B} + \chi_F R_{1F} \quad (3)$$

where R_{1obs} is the relaxation rate of the ligand in the presence of the macromolecule, R_{1B} and R_{1F} are the relaxation rates of the pure bound and free environments, and χ_B and χ_F are the molar fractions of the ligand in bound and free conditions.

If we consider the ligand–macromolecule equilibrium:

$$M + L \leftrightarrows ML \quad (4)$$

with an equilibrium constant $K = \frac{[ML]}{[M][L]}$, assuming $[L] \gg [M_0]$, it has been shown that:

$$\Delta R_1 = \frac{K R_{1B}}{1 + K[L]}[M_0] \quad (5)$$

where $\Delta R_1 = R_{1obs} - R_{1F}$, K is the thermodynamic equilibrium constant, and $[M_0]$ is the initial macromolecule concentration.As suggested by equation [5], the plot ΔR_1 versus $[M_0]$ would have a straight line through the origin, with slope:

$$[A]_L^T = \left(\frac{K R_{1B}}{1 + K[L]}\right) \quad (6)$$

which was defined as "affinity index" ($l\,mol^{-1}\,s^{-1}$) [21]. The affinity index is a constant if temperature and ligand concentration are specified, as suggested by the T and L subscripts in the affinity index symbol.

The recognition process between small ligands and biomacromolecules can be studied using the proposed approach if the following conditions hold:

(i) the ligand must experience a fast chemical exchange between the free and bound environments with respect to the NMR timescale. In these conditions the NMR parameters have a weighted means between the values assumed in each environment;
(ii) the total bound ligand must be small compared to that of the free ligand;
(iii) the observed NMR parameters (i.e. in this case, the proton spin–lattice relaxation rates), must be heavily affected by the presence of the macromolecules.

The spin–lattice relaxation rate R_1^{SE} appears to be the best experimental parameter for obtaining information about ligand–macromolecule interactions.

A significant contribution from the second term of equation [3] is possible only if $R_{1B}^{SE} \gg R_{1F}^{SE}$. These conditions apply when an interaction between the ligand and the macromolecule occurs.

A temperature dependency analysis of R_1^{SE} and R_1^{NS} is also required to test whether $R_1^{SE} > R_1^{NS}$ conditions are really due to a large $\chi_B R_{1B}$ term to R_1^{SE}; in fact, $R_1^{SE} > R_1^{NS}$ could also be the result of a reduction in molecular tumbling due to an increase in viscosity caused by the presence of a macromolecule in the solution. A reduction in both R_1^{SE} and R_1^{NS} with an increasing temperature demonstrates that the ligand fast motion condition $\omega_0 \tau_c \ll 1$ holds in the solution. This allows the effects on R_1^{SE} to be attributed to the formation of the ligand–macromolecular complex.

In previous studies performed using this methodology, the affinity index was mainly calculated from selective relaxation rate enhancements calculated for a single proton, assuming an isotropic motion for the ligand molecule. However, even for small ligands, there can be differences in the dynamics of different portions of the molecule, leading to effects on the selective relaxation rates and as a consequence, on the affinity index value) due to different correlation times modulating the dipolar interactions between protons at different positions. The normalization of $\Delta R_1 = R_{1obs} - R_{1F}$ to R_{1F} removes the effects of different correlation times and isolates the effects of restricted motions due to the interaction of the ligand with the macromolecule:

$$\frac{R_{1obs}^{SE} - R_{1F}^{SE}}{R_{1F}^{SE}} = \frac{K R_{1B}^{SE}[M_0]}{(1 + K\,[L])\,R_{1F}^{SE}} \quad (7)$$

Let us put the normalized ratio as:

$$\frac{R_{1obs}^{SE} - R_{1F}^{SE}}{R_F^{SE}} = \Delta R_{1N}^{SE} \quad (8)$$

obtaining:

$$\Delta R_{1N}^{SE} = \frac{K R_{1B}^{SE}[M_0]}{(1 + K[L])R_{1F}^{SE}} \quad (9)$$

The dependence of the normalized relaxation rate enhancements ΔR_{1N}^{SE} from the concentration of the macromolecole $[M_0]$ is represented by a straight line passing through the origin of the axes with slope:

$$[A_I^N]_L^T = \frac{K R_{1B}^{SE}}{(1 + K[L])R_{1F}^{SE}} \quad (10)$$

$[A_I^N]_L^T$ is still a constant at fixed temperature and ligand concentration and it is defined as "normalized affinity index" ($dm^3\,mol^{-1}$).

3. Experimental

3.1. Materials

Prednisolone (11,17,21-tridihydroxy-pregna-1,4-diene-3,20-dione) (Fig. 2) was purchased from Sigma Chemical Co. and used without any further purification.

3.2. Methods

3.2.1. Synthesis of monoPHE

The monomer *N*-acryloyl-L-phenylalanine was synthesized according to a previously reported procedure [30]. Briefly, to a well-stirred aqueous solution of L-phenylalanine

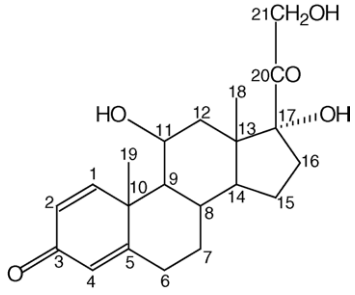

Fig. 2. Structure and numbering of prednisolone.

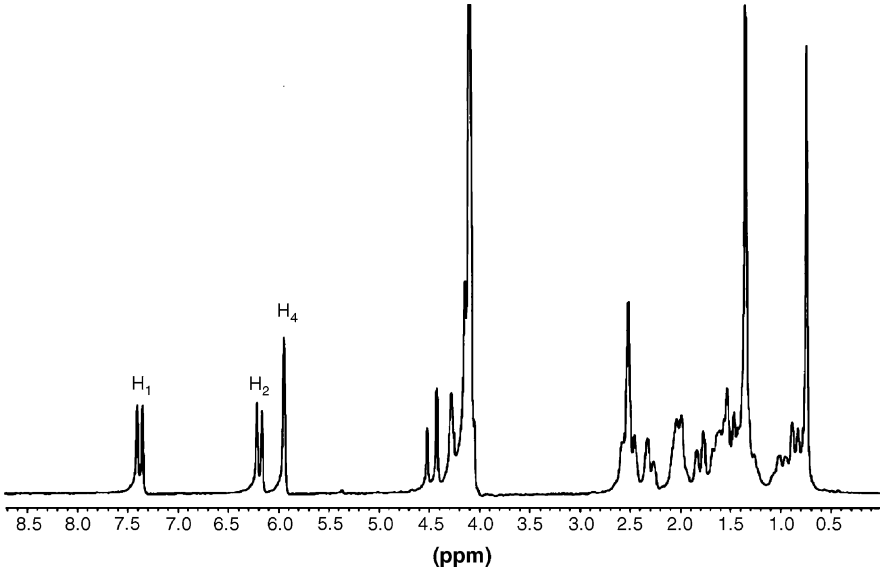

Fig. 3. Proton spectrum of prednisolone recorded at 200 MHz.

(PHE) (54.51 g, 0.33 mol), sodium hydroxide (26.67 g, 0.67 mol), and 2,6-di-*tert*-butyl-*p*-cresol (0.02 g) in twice distilled water (100 ml) was added dropwise acryloyl chloride (29.26 g, 0.32 mol) over a 30 min period. The reaction mixture was kept at 0 °C by external ice-bath cooling, and then the temperature was raised to room temperature for 60 min more. The mixture was acidified to pH 2 with concentrated hydrochloric acid (27.6 ml, 37%). The white voluminous product was separated by filtration and recrystallized from water. The reaction yield was 34 wt.%. Spectroscopy (^{1}H NMR, IR), elemental analysis and potentiometry showed a product of analytical grade.

3.2.2. Synthesis of coPHE 1/2 and polyPHE

The poly(*N*-acryloyl-L-phenylalanine) and its copolymers with NIPAAm were obtained by a radical polymerization of the corrisponding monomers [30]. The homopolymer was obtained as follows. To a well-degassed and nitrogen-purged solution of 2.00 g of monoPHE in 20 ml of ethanol/benzene (1:1) solution was added 30 mg of recrystallized (from methanol) α,α'-azobisisobutyronitrile (AIBN). The mixture was purged with nitrogen and allowed to stand in a thermostated water-bath at 60 °C for 24 h. The yield was 87%.

The coPHE was obtained with a similar procedure by using 2.04 g of monoPHE dissolved in 25 ml of ethanol/benzene (1:1) solution together with 0.55 g of NIPAAm. To this mixture was added 43 mg of AIBN. The reaction yield was 60 wt.%. The amount of COOH groups incorporated into the compounds, determined by acid-base titration, was in agreement with expected. The used HC-MALS (hydrodynamic chromatography multi-angle laser light scattering) method was able to recover a rielable weight-average molar mass Mw of 47.6 and 146.1 kDa for polyPHE and coPHE, respectively.

3.2.3. NMR measurements

The solutions for the NMR experiments were obtained by dissolving the appropriate amounts of ligand and polymers in DMSO-d_6:D_2O (3:1). The solvent mixture was required due to the low solubility of the two antibiotics in D_2O. In all the experiments prednisolone concentration was 4×10^{-2} mol dm^{-3}.

^{1}H NMR spectra were obtained on a Bruker AC 200 spectrometer, operating at 200.13 MHz. The spin–lattice relaxation rates were measured using the (180°-τ-90°-t)$_n$ sequence. The τ values used for the selective and

Table 1
R_1^{SE} and R_1^{NS} values calculated for H_1, H_2, and H_4 protons of prednisolone (4×10^{-2} mol dm^{-3}) in the presence of variable concentrations of coPHE 1/2 at 298 K

coPHE 1/2 concentration (mg/ml)	coPHE 1/2 concentration (mol dm^{-3})	R_{11}^{SE} (s^{-1})	R_{11}^{NS} (s^{-1})	R_{12}^{SE} (s^{-1})	R_{12}^{NS} (s^{-1})	R_{14}^{SE} (s^{-1})	R_{14}^{NS} (s^{-1})
0	0	2.14	2.86	0.79	0.87	0.98	1.09
2	1.37×10^{-5}	2.29	2.83	0.84	0.92	1.06	1.12
4	2.74×10^{-5}	2.67	2.79	1.03	0.99	1.42	1.29
5	3.42×10^{-5}	3.09	2.86	1.19	1.05	1.57	1.32
6	4.11×10^{-5}	3.00	2.92	1.06	1.04	1.42	1.26
8	5.48×10^{-5}	3.59	2.93	1.41	1.15	1.80	1.54

non-selective experiments were: 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.8, 1, 1.5, 2, 3, 4, 5, 7, 10 s, respectively, and the delay time t in this case is 10 s. The 180° selective inversion of the proton spin population was obtained by a selective soft perturbation pulse, generated by the decoupler channel [31]. All the selective and non-selective spin–lattice relaxation rates refer to the H_1, H_2, and H_4 protons of prednisolone. Since in general the recovery of proton longitudinal magnetization after a 180° pulse is not a single exponential, due to the sum of different relaxation terms, the selective spin–lattice relaxation rates were calculated using the initial slope approximation and subsequent three-parameter exponential regression analysis of the longitudinal recovery curves. The maximum experimental error in the relaxation rate measurements was 5%. The affinity index was calculated by linear regression analysis of the experimental data.

All the spectra were processed using the Bruker Software XWINNMR, version 2.5 on Silicon Graphics O_2 equipped with RISC R5000 processor, working under the IRIX 6.3 operating system.

4. Results and discussion

Fig. 3 shows the proton spectrum of prednisolone. Prednisolone proton chemical shift values are in agreement with those found in the literature [32–34].

Table 1 reports the values of R_1^{SE} and R_1^{NS} obtained for H_1, H_2, and H_4 protons of prednisolone in relation to coPHE 1/2 concentration. Experimental proton spectra used for R_1^{SE} measurements are shown in Fig. 4. The results show that in the absence of the polymer, $R_1^{NS} > R_1^{SE}$ while increasing polymer concentration R_1^{SE} becomes greater than R_1^{NS}. As reported in Section 2, this represents the main indication of the existence of interaction processes between the prednisolone and coPHE 1/2. In fact, selective relaxation rate enhancements reflect a large contribution from the bound ligand fraction to the experimentally calculated relaxation rate. However, systems containing a relatively high concentration of macromolecules, as in this case, may be subject to an increase in viscosity, which can cause a decrease in the ligand dynamics independently from the existence of interaction processes. For this reason, the analysis of the behaviour of the non-selective relaxation rates with changing temperature in the presence of the copolymer has been carried out. As explained in Section 2, if the ligand in the bulk experiences fast motion conditions in the presence of the macromolecule, an increase in temperature should cause a decrease in R_1^{NS}. Table 2 reports the values of R_1^{NS} in relation to temperature measured for H_1, H_2, and H_4 protons of prednisolone in the presence of 5 mg/ml of coPHE 1/2. The observed decrease of R_1^{NS} with increasing temperature indicates that the presence

Table 2
R_1^{NS} values calculated for H_1, H_2, and H_4 protons of prednisolone (4×10^{-2} mol dm^{-3}) in relation to temperature in the presence of 5 mg/ml of coPHE 1/2

Temperature (K)	R_{11}^{NS} (s^{-1})	R_{12}^{NS} (s^{-1})	R_{14}^{NS} (s^{-1})
300	2.82	1.09	1.42
308	2.42	0.75	0.95
316	1.93	0.59	0.72
323	1.52	0.48	0.57

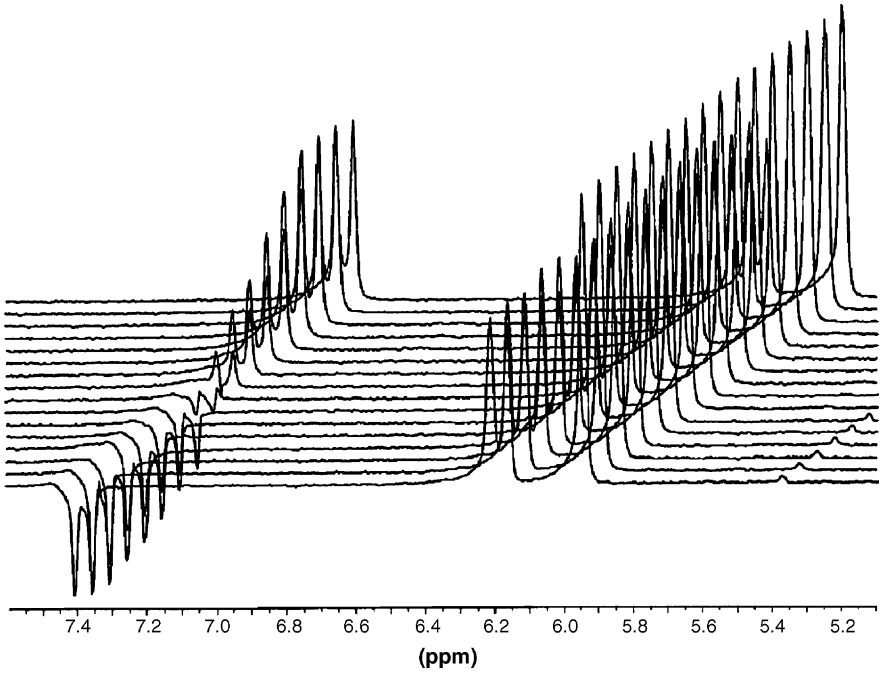

Fig. 4. Selective partially relaxed aromatic proton spectra of a 4×10^{-2} mol dm^{-3} prednisolone solution. The selective measurements refer to the prednisolone H_1 proton.

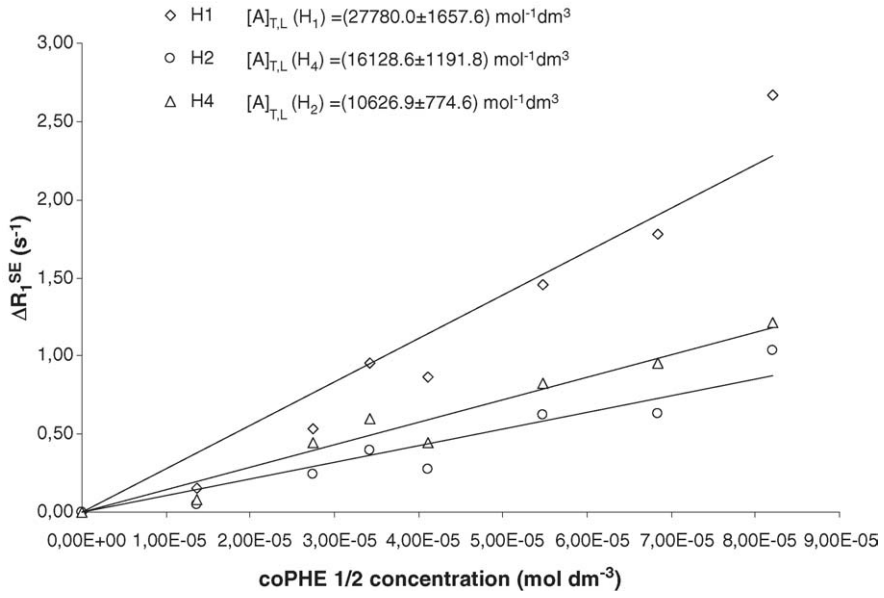

Fig. 5. Comparison of the linear regression analysis of the H_1, H_2, and H_4 selective relaxation enhancement, ΔR_1^{SE}, as a function of coPHE 1/2 concentration of a solution of prednisolone (4×10^{-2} mol dm^{-3} at 298 K). The values of the affinity indexes $[A]_L^T$ are also reported with the corresponding errors.

of the polymer does not affect the dynamics of the free ligand and confirms the occurring of ligand–macromolecule interaction between coPHE 1/2 and prednisolone.

As shown in Fig. 5, the affinity indexes $[A]_L^T$ for prednisolone–coPHE 1/2 system referring to H_1, H_2, and H_4 protons, were calculated from the slope of the straight line describing the dependence of proton ligand selective relaxation rate enhancements on polymer concentration. As can be deduced analysing the values of $[A]_L^T$ calculated for different protons, the affinity indexes show appreciable changes at different proton positions. This behaviour reflects the effects of motional anisotropies and differences in the magnetic environment of the nuclei of the ligand molecule on the observed spin–lattice relaxation rates. In order to remove these effects, $[A]_L^T$ was normalized to the selective spin–lattice relaxation rate of the free ligand and a "normalized affinity index" $[A^N]_L^T$ was calculated. Fig. 6 shows the effect of the normalization on $[A]_L^T$, leading to very close values of the normalized affinity indexes $[A^N]_L^T$ for all the observed spins. The average value of $[A^N]_L^T$ was 13,700 dm^3 mol^{-1}.

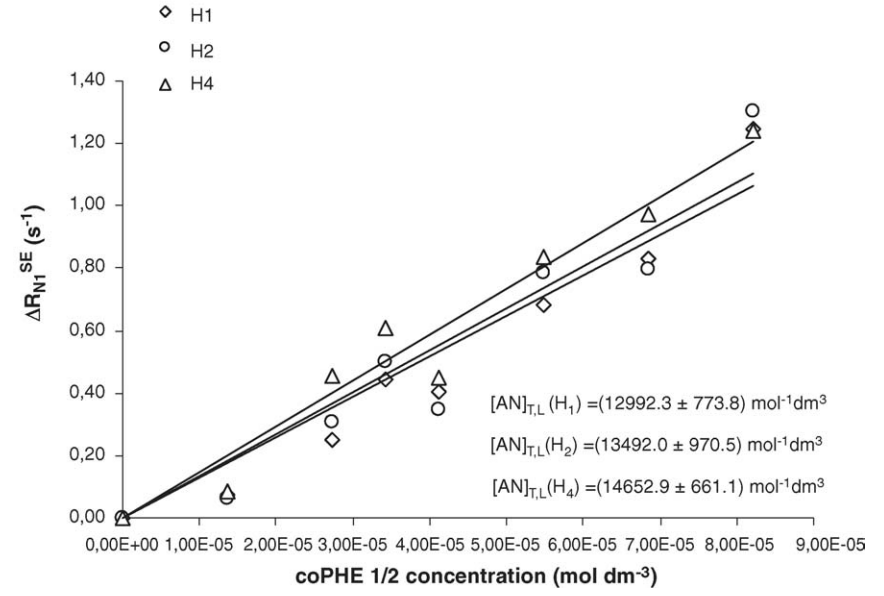

Fig. 6. Comparison of the linear regression analysis of the H_1, H_2, and H_4 normalized selective relaxation enhancement, ΔR_{N1}^{SE}, as a function of coPHE 1/2 concentration of a solution of prednisolone (4×10^{-2} mol dm^{-3} at 298 K). The values of the normalized affinity indexes $[A_N]_L^T$ are also reported with the corresponding errors.

Table 3a
R_1^{SE} and R_1^{NS} values calculated for H_1, H_2, and H_4 protons of prednisolone (4×10^{-2} mol dm^{-3}) in the presence of variable concentrations of PolyPHE at 298 K

PolyPHE concentration (mg/ml)	PolyPHE concentration (mol dm^{-3})	R_{11}^{SE} (s^{-1})	R_{11}^{NS} (s^{-1})	R_{12}^{SE} (s^{-1})	R_{12}^{NS} (s^{-1})	R_{14}^{SE} (s^{-1})	R_{14}^{NS} (s^{-1})
0	0	2.14	2.86	0.79	0.87	0.98	1.09
2	4.20×10^{-5}	2.31	2.90	0.86	0.92	1.05	1.15
5	1.05×10^{-4}	2.58	2.90	0.95	0.96	1.23	1.20
8	1.68×10^{-4}	2.82	2.92	1.13	0.98	1.53	1.27
10	2.10×10^{-4}	2.92	2.91	1.16	0.99	1.54	1.24

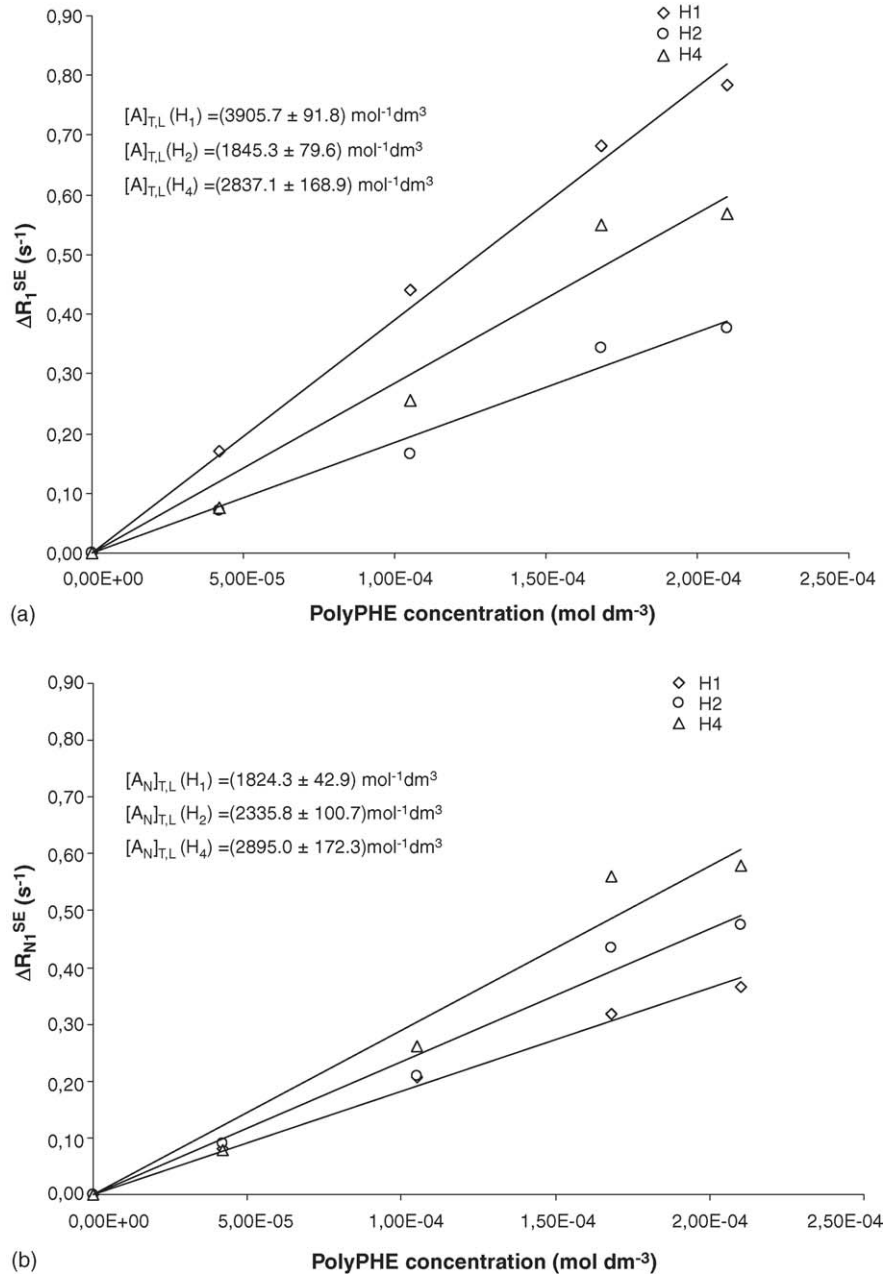

Fig. 7. Comparison of the linear regression analysis of the H_1, H_2, and H_4 of: (a) selective relaxation enhancement; and (b) normalized selective relaxation enhancement, as a function of PolyPHE concentration of a solution of prednisolone (4×10^{-2} mol dm^{-3} at 298 K). The values of the affinity indexes are also reported with the corresponding errors.

Table 3b
R_1^{NS} values in relation to temperature

Temperature (K)	R_{11}^{NS} (s^{-1})	R_{12}^{NS} (s^{-1})	R_{14}^{NS} (s^{-1})
300	2.88	1.07	1.38
308	2.46	0.75	0.96
316	1.99	0.59	0.72
323	1.53	0.47	0.57

In order to evaluate the contribution of the *N*-isopropylacrylamide fraction of coPHE to the interaction process analysed above, prednisolone–polyPHE system (in which NIPAAm is absent) was studied.

Table 3a shows the values of R_1^{SE} and R_1^{NS} calculated for H_1, H_2, and H_4 protons of prednisolone in relation to PolyPHE concentration. The observed R_1^{SE} values show significant enhancements with increasing polymer concentration, while R_1^{NS} did not change considerably. Table 3b reports the temperature dependent analysis of R_1^{NS} in order to establish if the high concentration of the polymer did affect the viscosity of the solution or not. Data show a decrease of R_1^{NS} with increasing temperature, which suggests that the free ligand experiences fast motion conditions even in the presence of the macromolecule. These evidences indicate that interaction processes between prednisolone and PolyPHE occurred at solvent–polymer interface. Fig. 7a shows the plot of selective relaxation rate enhancements in relation to PolyPHE concentration for H_1, H_2, and H_4 protons of prednisolone, with the calculated affinity index for each proton. Fig. 7b reports the normalised relaxation rate changes versus polymer concentration, which gave the values of the normalised affinity index. The average value of $[A^N]_L^T$ calculated for the three protons was 2500 $dm^3\,mol^{-1}$.

The comparison of the results obtained for prednisolone–coPHE 1/2 and prednisolone–PolyPHE systems suggests that the ligand interacts with the two macromolecular systems with different strengths, indicating a greater affinity towards the synthetic copolymer with respect to the polyaminoacid.

Moreover, $[A^N]_L^T$ value for prednisolone–coPHE 1/2 was greater than that found for prednisolone–albumin interaction (7694 $dm^3\,mol^{-1}$) in the same experimental conditions [35]. This constitutes an interesting result, which underlines the ability of the synthetic copolymer to mime biomacromolecular structures. This characteristic makes this copolymer a suitable model for studying drug–macromolecule recognition processes. The use of synthetic copolymers instead of proteins as albumin, allows the investigation of interaction processes in relation to specific chemical modifications in order to optimize the efficacy of drug–macromolecule recognition.

5. Conclusions

The determination of the affinity index from nuclear spin relaxation analysis as a measure of the overall complexing behaviour of different ligands towards macromolecules, constitutes a useful approach in order to evaluate the strength of all specific and non-specific binding phenomena occurring at macromolecule–solvent interface. Moreover, as the contributions of anisotropic dynamics and different proton densities to the nuclear relaxation rates have been normalized, the calculated values of $[A_I^N]_L^T$ should be the same when determined for any ligand proton nuclei. In case where the normalized affinity index calculated for different ligand protons still presents different values, these should be attributed to the specificity of the ligand–receptor interactions. The ligand–receptor complexing in favourable cases may re-introduce a difference in the $[A_I^N]_L^T$ values as a consequence of anisotropic contributions of the complex to the ligand proton relaxation properties. This effect could be of interest for the identification of the ligand moiety directly involved in the recognition step.

In this paper, we applied this methodology to the study of a system composed by a synthetic copolymer and a corticosteroid. The normalized affinity index calculated for this complex was found to be greater than the one obtained for the interaction between the same ligand and bovine serum albumin. These results indicate that the co-polymer was able to mime the structure of natural polymers such as proteins.

Acknowledgement

The authors would like to thank the Italian Interuniversities Consortium CSGI for financial support.

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