A macromolecular Gd(III) complex as pH-responsive relaxometric probe for MRI applications

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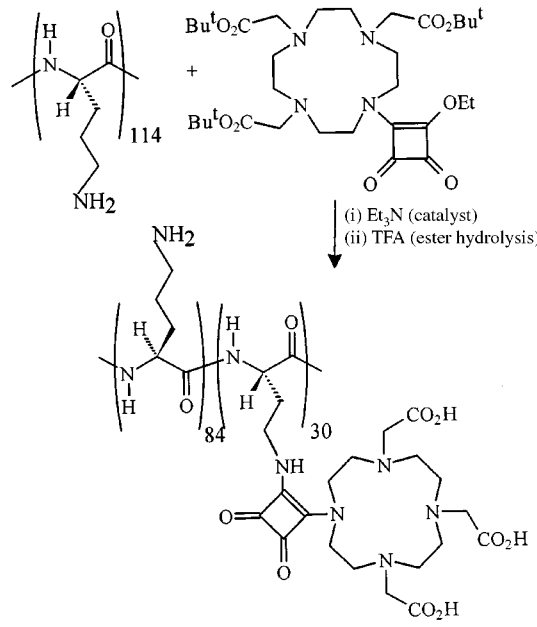
The ability of the paramagnetic macromolecular complex $(GdDO3ASQ)_{30}$-Orn_{114} to enhance the water proton relaxation rate has been found to be a function of pH; this behaviour is related to the structural changes occurring in the polypeptide upon protonation of the side-chain NH_2 groups.

Magnetic resonance imaging (MRI) is a powerful diagnostic technique based on the acquisition of images which are topological representations of NMR parameters, including the T_1 and T_2 of water protons. Contrast agents (CAs) for MRI are mainly represented by Gd(III) chelates whose high paramagnetism causes a marked increase of the water proton relaxation rates in the bodily regions where they distribute.[1–3] The effectiveness of a Gd(III) complex as a CA is measured by the relaxivity, r_{1p}, which represents the net increment of the water proton relaxation rate in a 1 mmol l^{-1} solution of the paramagnetic solute. The development of a second generation of Gd(III) based CAs stems from systems endowed either with higher relaxivities or with an improved ability to act as reporters of their biochemical environment. Among the various possibilities, the availability of a pH-sensitive Gd(III) chelate appears particularly interesting since it is known that increased glycolytic activity may cause a significant pH decrease in the extracellular region of certain tumours.[4] At the magnetic fields usually employed in MRI (0.5–1.5 T) the relaxivity of a Gd(III) chelate is mainly determined by the molecular reorientational time τ_r.[5] Thus a means of assaying pH may be pursued by designing systems whose molecular mobility is a function of pH. It is well established that the structures of poly(amino acid)s like polylysine and polyornithine are strongly dependent upon the pH of the solution. In fact, at acidic pHs, the repulsion among positively charged amino groups induces the occurrence of a highly flexible structure whereas at basic pHs the formation of intra-chain hydrogen bonds yields the formation of an α-helix resulting in an overall rigidity of the macromolecular system.

On this basis a novel macromolecular system has been recently synthesised, containing thirty Gd(III) chelates bound to a poly(amino acid) chain made of 114 ornithine residues. Since it has been recently reported that squaric esters readily react with amines,[6] we exploited this finding by using the squaric acid moiety as a linker between the tetraazamacrocyclic structure of DO3A and the amino groups of the polyornithine chain (Scheme 1).[7] In addition to acting as linker between the poly(amino acidic) chain and the DO3A ligand, the squaric acid moiety may participate in the coordination of the Gd(III) ion through the oxygen atom of the carbonyl group. Such a reaction scheme has some advantage with respect to that previously reported for $(GdDTPA)_n$-Lys_m.[8] In fact, the synthesis of the latter system implies the use of the bifunctional DTPA bis-anhydride which leads to undesirable intra- and inter-molecular cross-linking reactions with a consequent reduction of the thermodynamic stability of the metal complexes.[9] The use of a monoreactive chelate derivative allows a controlled and specific activation of the amino groups in the poly(amino acid) macromolecule.

At acidic pH the unreacted amino groups in $(GdDO3ASQ)_{30}$-Orn_{114} are protonated and highly hydrated, thus they tend to stay as far apart as possible. The paramagnetic moieties too maintain a relatively high degree of mobility along the lateral chain of each substituted ornithine and the molecular reorientation of the complex only in part 'feels' the large size of the whole macromolecule. The progressive deprotonation of the $-NH_3^+$ groups occurring with the increase of pH induces a 'rigidification effect' due to the formation of intramolecular hydrogen bonds between adjacent peptidic linkages. The progressive changes in the overall structure of $(GdDO3ASQ)_{30}$-Orn_{114} are reflected in the relaxivity parameter as shown in Fig. 1. At pH < 4 r_{1p} has a constant values of *ca.* 23 mM^{-1} s^{-1} which is about five times higher than the r_{1p} value normally found for related monomeric Gd(III) complexes. The increase of the pH of the solution causes a linear increase of r_{1p} which reaches the value of 32 mM^{-1} s^{-1} at pH 8. Further increase of pH above this value does not cause any change in the observed relaxivity. To check that the observed behaviour is a con-



Scheme 1

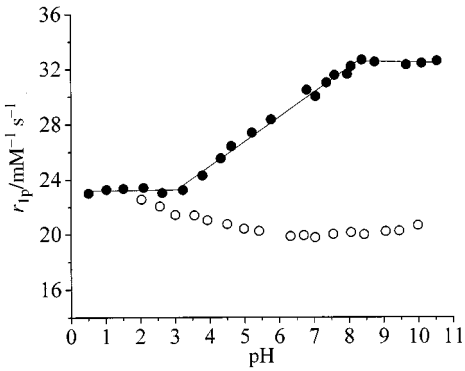


Fig. 1 pH dependence of r_{1p} values for $(GdDO3ASQ)_{30}$-Orn_{114} (●) and $(GdDO3ASQ)_{50}$-Orn_{50} (○). The latter system has been chosen because its molecular weight is similar to that of $(GdDO3ASQ)_{30}$-Orn_{114}.

sequence of a change in τ_r, the $1/T_1$ dependence on the Larmor frequency (ω_0) (NMRD profile) has been measured at three pH values (Fig. 2). The shape of the NMRD profile of Gd(III) chelates, as measured on a field-cycling relaxometer over the frequency range from 0.01 to 50 MHz, is determined by a number of parameters including the number of water molecules in the inner coordination sphere, the molecular reorientational time (τ_r), the exchange lifetime of the coordinated water (τ_m), the electronic relaxation time (τ_s), and the Gd-bound water proton distance (r).[5] The NMRD profiles of paramagnetic macromolecular systems show a characteristic hump in the 20–35 MHz region whose height is determined by the relative ratio of τ_s to τ_r.[10] In fact, in the low frequency range r_{1p} is determined primarily by τ_s whose value, being field dependent, increases steadily with ω_0 causing an increase of the observed r_{1p}. As τ_s becomes longer than τ_r, the observed r_{1p} is determined by the latter parameter and the $1/T_1$ dispersion takes place. As shown in Fig. 2, a significant increase in the height of the relaxivity peak in the 20–35 MHz region occurs as the pH of the solution passes from 4.5 to 8.5. This behaviour is consistent with an increase of τ_r with pH. Furthermore, the increase in pH also induces an increase in relaxivity at low magnetic field strength. Such behaviour, which is reminiscent of that observed upon formation of macromolecular adducts between a paramagnetic Gd(III) chelate and HSA,[2] has been accounted for in terms of an additional contribution to the overall relaxivity arising from water molecules surrounding the complex and those belonging to the hydration layers of the macromolecule. We take this finding as further evidence of structure formation in the polypeptide chain upon deprotonation of the -NH_3^+ side chain groups.

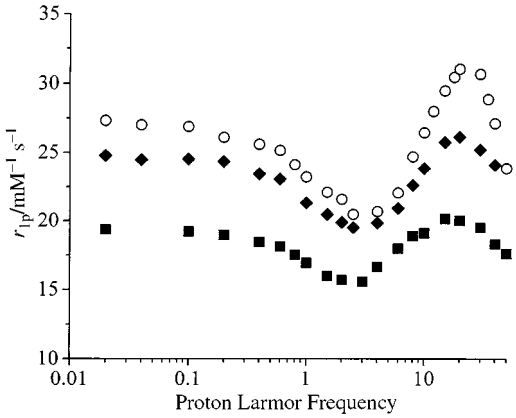


Fig. 2 $1/T_1$ NMRD profiles (298 K) of $(GdDO3ASQ)_{30}$-Orn_{114} (1 mM) at pH 4.5 (■), 7 (◆) and 8.5 (○) respectively.

The role of the free amino groups on the macromolecule in determining the dependence of r_{1p} upon the pH of the solution is clearly assessed by compairing the pH dependence of r_{1p} for $(GdDO3ASQ)_{30}$-Orn_{114} and r_{1p} of a related system containing no free NH_2 groups (Fig. 1). Such a system has been obtained by following an analogous procedure to that used for $(GdDO3ASQ)_{30}$-Orn_{114} but with a higher DO3A ester : poly-(amino acid) ratio.[7] Clearly, the lower r_{1p} values shown by the saturated conjugate indicate that, in this system, the chelates moieties undergo larger motions than in $(GdDO3ASQ)_{30}$-Orn_{114}. It is likely that the steric requirements of the bulky chelates do not allow the poly(amino acid) chain to adopt, at basic pHs, a compact α-helix structure as occurs for the partially substituted derivative. Rather, the flexibility of the main chain is transfered to the side-chains bearing the paramagnetic chelates which, in turn, are responsible for the partial 'quenching' of the relaxivity potentially expected for systems of this size.

In summary $(GdDO3ASQ)_{30}$-Orn_{114} is an interesting macromolecular MRI agent whose high relaxivity is made strongly pH dependent through structural changes which limit the internal mobility of the chelate moieties. The pH range at which r_{1p} changes fits better the physiological values than that recently observed for another macrocyclic Gd(III) complex whose pH-dependent r_{1p} is the result of variations in its hydration sphere.[11] Furthermore it appears to provide an alternative MRI route to recently reported procedures for the determination of the extracellular pH of tumours based on ^1H, ^{19}F or ^{31}P probe molecules, known to have pH-dependent MR properties which titrate in the physiological range.[12,13]

Notes and references

‡ Synthesis of $(GdDO3ASQ)_{30}$-Orn_{114}. The triester (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, tri-*tert*-butyl ester) is reacted with diethyl squarate in ethanol, overnight at room temperate (RT) to yield the DO3A5Q ligand. The synthesis of the polyornithine conjugate is pursued by reacting the latter compound and the poly(amino acid) (at the molar ratio of *ca.* 30 : 1) at RT for 72 h in ethanol (in the presence of triethylamine as catalyst). Finally hydrolysis of *tert*-butyl ester groups is carried out with TFA. The complexation was carried out by adding a stoichiometric amount of $GdCl_3$ to the aqueous solution of the polyornithine conjugate at neutral pH and at RT. The eventual excess of free Gd(III) ions is easily removed by centrifugation of the solution brought to basic pH. All new compounds exhibited satisfactory spectral and elemental analysis. The CD spectra between 200 and 250 nm of $polyOrn_{114}$ and of $(GdDO3ASQ)_{30}$-Orn_{114} appear similar, indicating that the conformation of the polypeptide backbone is unaltered upon partial conjugation of lateral amino groups to squaric acid moieties.

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