J|A|C|S
COMMUNICATIONS

Published on Web 07/18/2002

A Paramagnetic MRI-CEST Agent Responsive to Lactate Concentration

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Received April 3, 2002

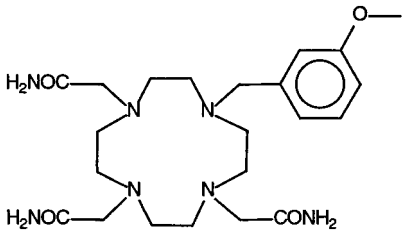
Recently, a novel class of contrast agents (CA) for magnetic resonance imaging (MRI) applications, the so-called chemical exchange saturation transfer (CEST) agents, has been disclosed. They are chemicals endowed with protons in slow exchange, on the NMR time scale, with bulk water (i.e., $k_{ex} < \Delta\nu$, where $\Delta\nu$ is the chemical shift separation in Hz between the two exchanging proton pools). Saturation transfer (ST) to the water resonance occurs upon irradiating the exchanging proton pool. The resulting decrease of the water signal intensity determines the contrast in the MR image. Balaban and co-workers investigated the CEST properties of several diamagnetic molecules (amino acids, sugars, etc.) and reported an in vivo MR image using urea as the pool of exchanging protons.[1,2] Furthermore, the same group demonstrated that the CEST agents can be successfully used as pH or temperature probes, making the observed effect independent from the absolute concentration of the agent.[3] A marked sensitivity improvement has been recently reported by van Zijl and co-workers, who exploited the ST properties of polymeric systems bearing a large number of exchangeable protons.[4]

As the ST effect is directly related to k_{ex}, it is expected that paramagnetic compounds displaying large $\Delta\nu$ values for the exchanging proton resonance[5] may improve the efficacy of the CEST agents. Actually, Sherry and co-workers obtained a good ST effect by irradiating the metal-bound water protons of a macrocyclic Eu(III) complex resonating at 50 ppm downfield from bulk water.[6] More recently, it has been reported that paramagnetic Ln(III) complexes of tetraamide derivatives of DOTA show ST properties which are markedly pH-dependent.[7,8] The results reported in this communication show that the CEST properties of a paramagnetic complex can be made sensitive to the concentration of a given metabolite. L-Lactate is the substrate of choice to prove it.

L-Lactate is a key-metabolite which is over-produced when the anaerobic glycolysis pathway becomes relevant (strokes, brain tumors, cysts, radiation therapy, metabolic disorders, brain activation, etc.).[9] Presently, the evaluation of the L-lactate levels in vivo can be pursued by looking at the NMR signal of the methyl group through magnetic resonance spectroscopy techniques. However, it might be very useful to develop an indirect method of detection based on the MRI visualization of such a key metabolite. This aim may be pursued through the development of a paramagnetic CEST agent properly designed to recognize L-lactate.

It is known that Ln(III) complexes of heptadentate ligands show a peculiar ability to interact with anionic substrates through the formation of ternary adducts in which the substrate coordinates the Ln(III) ion by replacing the metal-bound water molecules.[10] On this basis, an heptadentate ligand endowed with six exchangeable amide protons (MBDO3AM, Chart 1) has been synthesized.

Chart 1



Since Yb(III) ion has been previously shown to yield complexes displaying the highest ST efficiency, owing to its good compromise between relaxation and shift properties,[7] the $[Yb(MBDO3AM)]^{3+}$ complex has been considered for this CEST study. The ^1H NMR spectrum of a solution containing this complex and L-lactate (4:1 ratio) is characterized by the presence of two sets of signals belonging to the free and to the lactate-bound form of the chelate, respectively. This finding suggests that the exchange between the two species is slow on the NMR time scale.[11]

Increasing the amount of added L-lactate, the signals of the free complex decrease their intensity up to the point where only the lactate-bound form is observed in the spectrum.

The presaturation of the bulk water protons allowed the assignment of the amide protons of the two forms of the complex. The amide protons of the free chelate resonate at −28.5 ppm as a very broad signal ($\Delta\delta_{1/2}$ of ca. 1500 Hz at pH 6.4 and 298 K), whereas the same protons for the bound species resonate between −14 and −20 ppm as two narrower doublets ($\Delta\delta_{1/2}$ of ca. 300 Hz). Thus, the resonances of the amide protons in the ternary adduct with L-lactate resonate at more than 10 ppm downfield with respect to the free complex. The high affinity shown by this complex toward lactate, coupled with the slow free-bound exchange, suggests that the amide protons of the two forms of the complex may be selectively irradiated, thus providing a route to have a saturation transfer effect sensitive to the presence of the metabolite (provided that the [Lac]/[YbL] ratio is lower than 1).

Figure 1 reports the CEST spectra for the two forms of the complex at 312 K and pH 7.4. The spectra indicate that there is a partial overlapping between the CEST peaks of the free and bound complex mainly due to the severe broadening of the amide proton signals observed at higher pH and temperature. Thus, the irradiation at the frequency corresponding to the resonance of the amide protons of the lactate-bound form of the complex (−15.5 ppm) allows the detection of a saturation transfer even in the absence of lactate. The broader CEST peak of the bulk water protons in the absence of lactate may be ascribed to their T_2 shortening caused by the presence of two water molecules coordinated to the Yb(III) ion in the free chelate.

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10.1021/ja0264044 CCC: $22.00

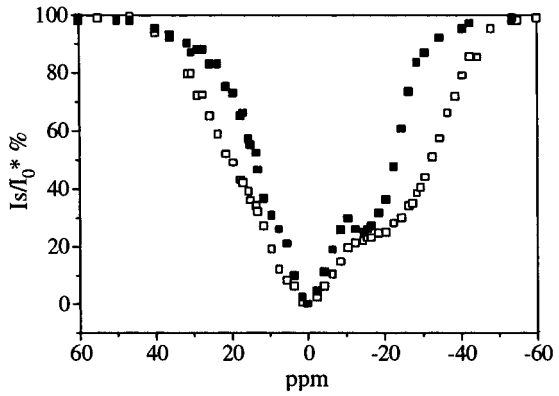

Figure 1. CEST spectra of a 30 mM solution of $[Yb(MBDO3AM)]^{3+}$ complex: free (□) and fully bound to L-lactate (■) (pH 7.4, 312 K, 7.05 T). The intensities of the water signal have been normalized by taking as reference the intensity measured upon irradiating at 60 ppm.

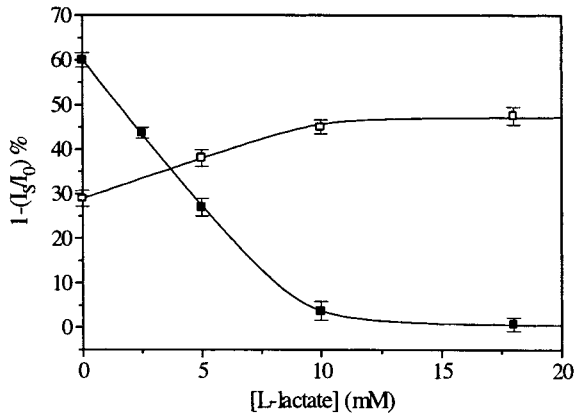

Figure 2. ST effect (pH 7.4, 312 K, 7.05 T) for a 9.3 mM solution of $[Yb(MBDO3AM)]^{3+}$ as a function of L-lactate concentration. The irradiation field was set to the frequency corresponding to the amide protons of free (■) and L-lactate-bound (□) complex.

To limit the disadvantage of the partial overlapping of the two CEST peaks without reducing ST effect, the irradiation of the amide protons of the free chelate has been upfield-shifted to −29.1 ppm, whereas the irradiation field for the bound form has been located at −15.5 ppm.

In Figure 2, the ST values measured for a 9.3 mM solution of $[Yb(MBDO3AM)]^{3+}$ as a function of L-lactate concentration, are reported.

The irradiation was performed using a CW pulse of 6 s corresponding to an irradiation power of 25 μT. To take into account the direct saturation of the bulk water caused by the irradiation pulse, the ST effects have been measured as I_S/I_0, where I_S is the intensity of the bulk water upon irradiating the amide protons (at a frequency ν^{on} from bulk water) and I_0 is the intensity measured by placing the irradiation offset at $\nu^{off} = -\nu^{on}$.

Upon irradiating the frequency corresponding to the resonance of the amide protons of the free complex, the measured ST values show a remarkable dependence (from 60 to 0%) on the L-lactate concentration in the 0−10 mM range. As expected, the responsiveness of the method is not so good (ST values from 30 to ca. 50%) when the amide protons of the lactate-bound complex are irradiated.

Quantitatively, the ST values measured by irradiating the amide protons of the free complex may be conveniently analyzed by using the following set of equations:[7]

$$\left(1 - \frac{I_S}{I_0}\right)^{free} = \left[\frac{k_{NH}^{free} n^{free} [C]_{free}}{111.2 R_{1irr}^{free} + k_{NH}^{free} n^{free} [C]_{free}} \left(1 - \exp\left[-\left(R_{1irr}^{free} + \frac{k_{NH}^{free} n^{free} [C]_{free}}{111.2}\right) t\right]\right)\right] \quad (1)$$

where 111.2 is the molar concentration of the bulk water protons, k_{NH}^{free} is the pseudo-first-order kinetic constant rate for the exchange of the amide protons for the free chelate, n^{free} is their numbers (6 in $[Yb(MBDO3AM)]^{3+}$), R_{1irr}^{free} represents the longitudinal relaxation rate of the bulk water protons in the presence of the irradiation field and $[C]_{free}$ is the molar concentration of the free complex. The amount of the free complex is correlated to $[Lac]_T$ and $[C]_T$ through the value of the thermodynamic association constant K_A:

$$[C]_{free} = \frac{-(K_A[Lac]_T - K_A[C]_T + 1) + \sqrt{(K_A[Lac]_T - K_A[C]_T + 1)^2 + 4K_A[C]_T}}{2K_A} \quad (2)$$

By using this model, a nice fitting of the data reported in Figure 2 was obtained ($K_A = 8 \times 10^3$; k_{NH}^{free} at 298 K = 1180 s^{-1}).

It is worth noting that, differently from the pH measurements,[7] an accurate determination of the lactate concentration by this method needs the knowledge of the local concentration of the paramagnetic probe, $[C]_T$, and furthermore, the $[Lac]_T/[C]_T$ ratio should be lower than 1.

In summary, the results herein reported further widen the scope of paramagnetic CEST agents. In fact, they show that a novel class of probes responsive to metabolites' concentration can be developed through a proper design of the paramagnetic CEST complex that has to fit the coordination requirements of the substrate of interest.

Acknowledgment. Financial support from MIUR (PRIN), CNR (Biotechnology PF and L. 95/95), and Bracco Imaging S.p.A (Milano Research Center) is gratefully acknowledged.

Supporting Information Available: ^{1}H NMR spectrum at 7.05 T, 298 K and pH 6.4 of a solution containing $[Yb(MBDO3AM)]^{3+}$ (28 mM) and L-lactate (7 mM) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0264044