OBC
www.rsc.org/obc

Probing the interaction of the biotin–avidin complex with the relaxivity of biotinylated Gd-DTPA †

Sander Langereis, Homme-Auke T. Kooistra, Marcel H. P. van Genderen and E. W. Meijer *
Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands. E-mail: E.W.Meijer@tue.nl; Fax: +31 40 245 1036

Received 25th February 2004, Accepted 23rd March 2004
First published as an Advance Article on the web 6th April 2004

A biotinylated Gd-DTPA complex is designed to study the biotin–avidin complexation using the longitudinal relaxivity of this new MRI label, which illustrates the use of MRI contrast agents to probe the formation of supramolecular assemblies in water.

Magnetic resonance imaging (MRI) contrast agents, such as the gadolinium complex of diethylenetriaminepentaacetic acid (Gd-DTPA), are commonly utilized to increase both the contrast and the anatomical detail of an MR image, due to their ability to enhance the longitudinal relaxation rate ($R_1 = 1/T_1$) of the protons of water molecules.[1–4] The efficiency of an MRI contrast agent is often expressed in terms of relaxivity (r), *i.e.* the change in relaxation rate divided by the concentration of MRI contrast agent in mM. Low molecular weight Gd-DTPA complexes have a relatively low relaxivity and a non-specific biodistribution.[4] It is well established that MRI contrast agents can bind to macromolecules, either in a covalent[2–4] or a non-covalent[4–9] fashion, which induces a pronounced enhancement of the relaxivity, due to a decrease in the molecular tumbling rate of the gadolinium complex.[4] As a result, the relaxivity can be utilized to probe molecular weight and/or the association process of Gd-DTPA structures.

In a pioneering study of Lauffer *et al.*, the non-covalent, reversible binding of low molecular weight MRI contrast agents to human serum albumin, a plasma protein, was described.[6,10] The hydrophobic interaction between their gadolinium chelate and the plasma protein led to a substantial increase in the longitudinal relaxivity upon binding to the protein. However, the binding between their gadolinium-based MRI contrast agent and the plasma protein was rather weak and non-specific.[6] Under such conditions the association process is complicated to study (multiple steps with different association constants). Lately, several exciting approaches in the development of MRI contrast agents with a higher specifity and an increased binding affinity have been reported, including enzyme-specific MRI contrast agents,[11] DNA-specific MRI contrast agents[12] and protein-specific MRI contrast agents.[13]

To study the relation between the relaxivity and strong non-cooperative binding of guests to a multivalent host in a quantitative manner, we developed an MRI contrast agent based on biotinylated Gd-DTPA, which can be associated with avidin (Fig. 1). It is well established that avidin, a tetrameric protein, is capable of binding four equivalents of biotin in a strong, non-cooperative fashion ($K_a \sim 1.7 \times 10^{15}$ M^{-1}).[14]

The biotinylated Gd-DTPA complex **4** was synthesized *via* a three-step procedure (Scheme 1). The intermediate products **2** and **3** were characterized with ^{1}H-NMR spectroscopy, IR spectroscopy, and ES-QTOF-MS. The formation of the gadolinium complex **4** was confirmed with IR spectroscopy and ES-QTOF-MS, while the gadolinium content was determined with inductively coupled plasma (ICP).

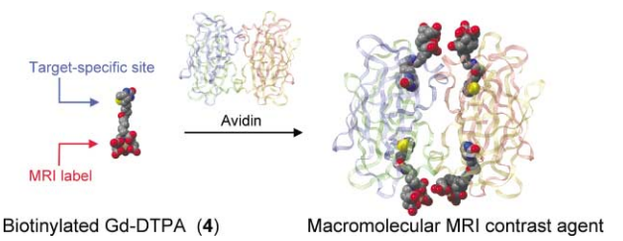
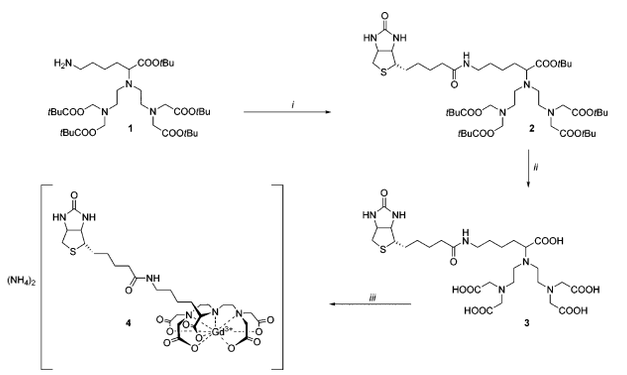


Fig. 1 Supramolecular approach to macromolecular MRI contrast agents composed of biotinylated Gd-DTPA **4** and avidin.



Scheme 1 Synthesis of biotinylated Gd-DTPA **4**. *i*) biotin-4-nitrophenyl ester, DMF; *ii*) trifluoroacetic acid, dichloromethane, 20 °C; *iii*) $GdCl_3 \cdot 6H_2O$, NH_4OH, water, 20 °C.

The longitudinal relaxivity (r_1) of **4** was determined by measuring the concentration dependency of its relaxation time at 1.5 T and 20 °C. The data gave a good linear fit ($R^2 > 0.999$) to the equation $(1/T_1)_{observed} = (1/T_1)_{diamagnetic} + r_1[Gd]$ and an r_1 of 6.1 ± 0.2 mM^{-1} s^{-1} was calculated. The r_1 of **4** is slightly higher than the r_1 of the parent Gd-DTPA complex ($r_1 = 4.2$ mM^{-1} s^{-1}, 1.5 T and 20 °C). This can be explained in terms of molecular weight (0.8 kDa for **4** *versus* 0.5 kDa for Gd-DTPA).

To investigate the effect of binding on the longitudinal relaxivity, MRI titration experiments with **4** and avidin were performed. It was found that the addition of a concentrated stock solution of avidin to **4**, a so-called E-titration[15] in which the multivalent protein is added to the substrate, results in a linear increase in the longitudinal ionic relaxivity (ionic r_1, *i.e.* relaxivity per gadolinium) (Fig. 2). This is indicative of strong binding between **4** and the biotin-binding pocket of avidin. A plateau value for the ionic r_1 was reached at higher ratios between avidin and **4**.

Both the longitudinal relaxivity of **4** bound to avidin ($r_{1,bound}$) and the binding stoichiometry between **4** and avidin (N) were calculated with a mathematical model describing the binding of multiple substrates to a multivalent protein with N identical, independent binding sites (see ESI†). Fitting the E-titration data to this model, taking into account that $K_a = 1.7 \times 10^{15}$ M^{-1}

† Electronic supplementary information (ESI) available: further results and experimental details. See http://www.rsc.org/suppdata/ob/b4/b402917h/

DOI: 10.1039/b402917h

and $r_{1,\ free} = 6.1\ mM^{-1}\ s^{-1}$, resulted in $r_{1,bound} = 17.5 \pm 0.3\ mM^{-1}\ s^{-1}$ and $N = 4.4 \pm 0.2$. The $r_{1,bound}$ is approximately three times higher than $r_{1,free}$. This observed gain in the ionic r_1 of **4** upon binding to avidin is indicative for a decrease in the molecular tumbling rate. The calculated value for the binding stoichiometry between avidin and **4** is only slightly higher (~10%) than the theoretical value.

Non-specific binding (physical adsorption) of **4** to avidin was ruled out by performing a control experiment, wherein the biotin binding pockets of avidin are presaturated with (+)-biotin. In that case, no enhancement of the ionic r_1 was observed (Fig. 2), implying that ligand exchange between **4** and biotin is extremely slow on the MRI timescale and, therefore this exchange process can be ignored under the applied experimental conditions.[14]

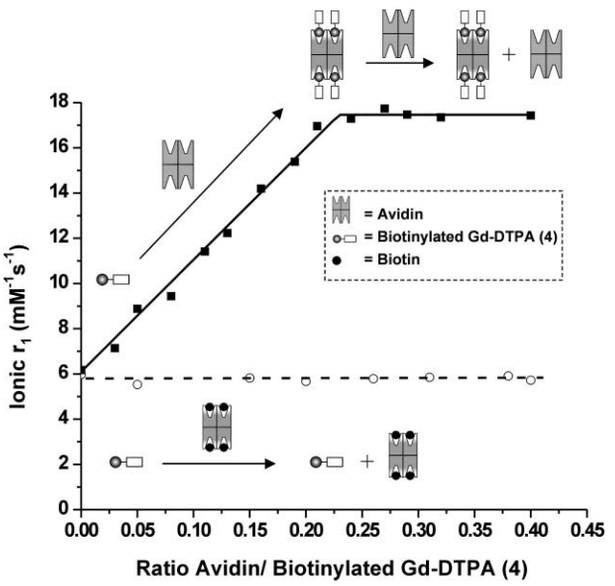


Fig. 2 E-titration[15] of a 67 μM solution of biotinylated Gd-DTPA **4** with avidin (■) at 1.5 T and 20 °C. Fitted data with $r_{1,free} = 6.1\ mM^{-1}\ s^{-1}$, yielding $r_{1,bound} = 17.5 \pm 0.3\ mM^{-1}\ s^{-1}$ and $N = 4.4 \pm 0.2$ (solid line). Control experiments showing the titration of a solution of 67 μM of biotinylated Gd-DTPA **4** with (+)-biotin blocked avidin (O) at 1.5 T and 20 °C. A horizontal line is drawn to guide the eye (dashed line). All MR titration experiments were performed in a 0.1 M PBS buffer at pH 7.4.

Additionally, the amount of binding sites of the protein was validated independently using the HABA-avidin assay.[16] In this experiment, the organic dye HABA (4′-hydroxyazobenzene-2-carboxylic acid) is expelled from the biotin binding pocket of avidin by the biotin, resulting in a decrease in the UV/Vis absorption band of the HABA dye at 500 nm. The HABA assay with biotin and avidin revealed that on average 3.9 molecules of biotin bind to avidin. Similar experiments with **4** and avidin resulted in a similar binding stoichiometry of 3.8 (see ESI†). This value is in reasonable agreement with the calculated binding stoichiometry between **4** and avidin based on the E-titration.

The titration experiment was also performed in the reversed way, a so-called M-titration[15] in which the substrate is added to a solution of the multivalent protein (Fig. 3). Upon increasing the amount of **4** initially a minor increase in the ionic relaxivity is observed. Such a small increase may be attributed either to the increase in molecular weight upon complexation or to interactions between individual gadolinium moieties bound to the same avidin molecule. The latter effect was observed for higher generations of the Gd-DTPA based poly(propylene imine) dendrimers.[17] The use of an excess of **4** results in a decrease of the average ionic relaxivity as a consequence of the increasing amount of unbound **4** in solution. The data for the

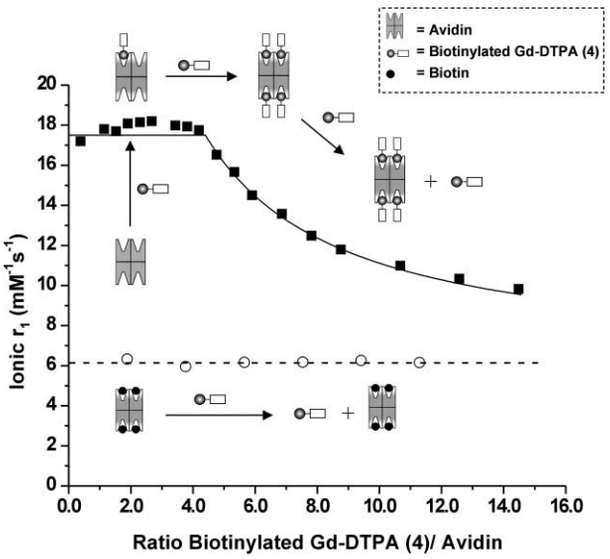


Fig. 3 M-titration[15] of a 70 μM solution of avidin with biotinylated Gd-DTPA **4** (■) at 1.5 T and 20 °C. Using the data from the E-titration, the solid line represents the expected average ionic relaxivity. Control experiments showing the titration of 70 μM avidin with (+)-biotin blocked avidin and biotinylated Gd-DTPA **4** (O) at 1.5 T and 20 °C. A horizontal line is drawn to guide the eye (dashed line). All MR titration experiments were performed in a 0.1 M PBS buffer at pH 7.4.

M-titration were described with the equation for the average ionic relaxivity (see ESI†) and the parameters obtained from the E-titration. This graph shows a good correspondence with the data.

The number of binding sites found with the new MRI label is higher than the value found on the traditional HABA-test. Since it is outside of our experimental error, further studies are foreseen to elucidate the differences found.

In conclusion, MRI titration experiments demonstrated that the strong and specific interaction between biotin and avidin can be probed with the relaxivity of biotinylated Gd-DTPA **4**. The quantitative model presented in this paper shows the strength of this approach and provides an elegant tool to study in detail the formation of supramolecular assemblies in water.

Acknowledgements

This work was financially supported by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO).

References

1 P. C. Lauterbur, M. H. Mendoca-Dias and A. M. Rudin, *Frontiers of Biological Energetics*, AcademicPress, New York, 1978.
2 R. B. Lauffer, *Chem. Rev.*, 1987, **87**, 901–927.
3 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293–2352.
4 A. E. Merbach and E. Toth, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, John Wiley & Sons, New York, 2001.
5 S. Aime, L. Frullano and S. Geninatti Crich, *Angew. Chem., Int. Ed.*, 2002, **41**, 1017–1019.
6 P. Caravan, N. J. Cloutier, M. T. Greenfield, S. A. McDermid, S. U. Dunham, J. W. M. Bulte, J. C. Amedio, Jr., R. J. Looby, R. M. Supkowski, W. D. Horrocks, Jr., T. J. McMurry and R. B. Lauffer, *J. Am. Chem. Soc.*, 2002, **124**, 3152–3162.
7 V. Jacques and J. F. Desreux, *Top. Curr. Chem.*, 2002, **221**, 123–164.
8 S. A. Anderson, R. K. Rader, W. F. Westlin, C. Null, D. Jackson, G. M. Lanza, S. A. Wickline and J. J. Kotyk, *Magn. Reson. Med.*, 2000, **44**, 433–439.
9 H. Kobayashi, S. Kawamoto, R. A. Star, T. A. Waldmann, M. W. Brechbiel and P. L. Choyke, *Bioconjugate Chem.*, 2003, **14**, 1044–1047.

10 R. B. Lauffer, D. J. Parmelee, S. U. Dunham, H. S. Ouellet, R. P. Dolan, S. Witte, T. J. McMurry and R. C. Walovitch, *Radiology*, 1998, **207**, 529–538.
11 P. L. Anelli, I. Bertini, M. Fragai, L. Lattuada, C. Luchinat and G. Parigi, *Eur. J. Inorg. Chem.*, 2000, 625–630.
12 P. Caravan, J. M. Greenwood, J. T. Welch and S. J. Franklin, *Chem. Commun.*, 2003, 2574–2575.
13 L. M. De Leon-Rodriguez, A. Ortiz, A. L. Weiner, S. Zhang, Z. Kovacs, T. Kodadek and A. D. Sherry, *J. Am. Chem. Soc.*, 2002, **124**, 3514–3515.
14 N. M. Green, *Methods Enzymol.*, 1990, **184**, 51–67.
15 R. A. Dwek, *Nuclear Magnetic Resonance in Biochemistry. Applications to Enzyme Systems*, Clarendon Press, Oxford, 1973.
16 N. M. Green, *Biochem. J.*, 1965, **94**, 23c–24c.
17 S. Langereis, Q. G. Lussanet, M. H. P. van Genderen, W. H. Backes and E. W. Meijer, *Macromolecules*, 2004, in press.