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Brief Article

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Effect of Nonenzymatic Glycosylation on the Magnetic Resonance Imaging (MRI) Contrast Agent Binding to Human Serum Albumin

Céline Henoumont,[†] Sophie Laurent,[†] Robert N. Muller,[†,‡,*] and Luce Vander Elst[†]

[†]Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, 19 Avenue Maistriau, B-7000 Mons, Belgium

[‡]Center for Microscopy and Molecular Imaging (CMMI), Académie Wallonie-Bruxelles, Gosselies, Belgium

ABSTRACT: Enhanced nonenzymatic glycosylation (NEG) of human serum albumin (HSA) is observed in diabetic patients. This modifies some of the physiological functions of HSA, as the binding of ligands. Some gadolinium complexes, commonly used as MRI contrast agents, have a high affinity for HSA, which enhances their efficacy. The aim of this study is to evaluate the possible influence of the NEG of HSA on its affinity for some gadolinium chelates.

INTRODUCTION

Human serum albumin (HSA) is the most abundant plasma protein with a concentration of 4% or 0.6 mM. The nonenzymatic glycosylation (NEG) of HSA by free glucose molecules is a well-known phenomenon occurring for all healthy people but which is particularly important for diabetic patients. Reports in the literature indicate that the level of NEG is of 10% of the total HSA concentration for healthy people but can reach up to 30% for diabetic patients. The identified sites for NEG are the lysine residues of HSA and particularly Lys 525 and Lys 199.[1−4]

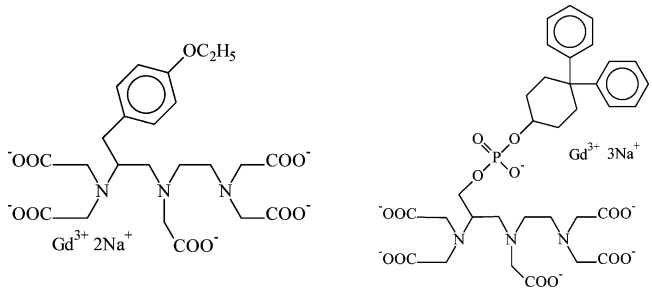
One of the most important properties of HSA is its ability to bind various kinds of endogenous and exogenous ligands, such as bilirubin, fatty acids, ibuprofen, warfarin, and so on. Its NEG induces conformational changes and, as a consequence, influences its binding properties. Several studies have indeed shown that the binding of some endogenous and exogenous molecules is reduced when HSA is glycosylated.[2,5−9]

Some gadolinium complexes used as MRI contrast agents are characterized by a relatively high affinity for HSA. This noncovalent interaction increases their efficacy (i.e., relaxivity [The relaxivity of a gadolinium complex corresponds to its efficacy and is defined as the increase of the water proton longitudinal relaxation rate induced by 1 mmol per L of the gadolinium complex aqueous solution.]) and their vascular lifetime.[10] To date, no studies have been published to evaluate the effect of the NEG of HSA on the binding of these gadolinium complexes.

Here, the effect of the NEG of HSA on the binding of three gadolinium complexes (Scheme 1) will be evaluated. **1** (Gd-EOB-DTPA) has a moderate affinity for HSA (K_a = 1500 M^{-1} with N = 1),[11−13] **2** (MS-325) binds more strongly (K_a = 6800 M^{-1} with N = 1),[11,14−16] and **3** (MP2269) has the highest affinity for HSA (K_a = 16000 M^{-1} with N = 1.6).[11,17−19]

Proton relaxometry is the most widely used technique for the evaluation of noncovalent interactions between a gadolinium complex and its target.[10−25] Indeed, its principle rests on the difference between the relaxivity of the free paramagnetic chelate and that of the supramolecular assembly resulting from its binding to HSA in fast equilibrium with its free component (eqs 1−3).

Scheme 1. Structure of the Three Studied Gadolinium Complexes



1 (Gd-EOB-DTPA) **2** (MS-325)

3 (MP2269)

$$R_1^{P} = R_{1f}^{P} + R_{1b}^{P} \quad (1)$$

with

$$R_{1f}^{P} = r_1^{f} \cdot 1000 \cdot [L] \quad (2)$$

and

$$R_{1b}^{P} = r_1^{b} \cdot 1000 \cdot [LP] \quad (3)$$

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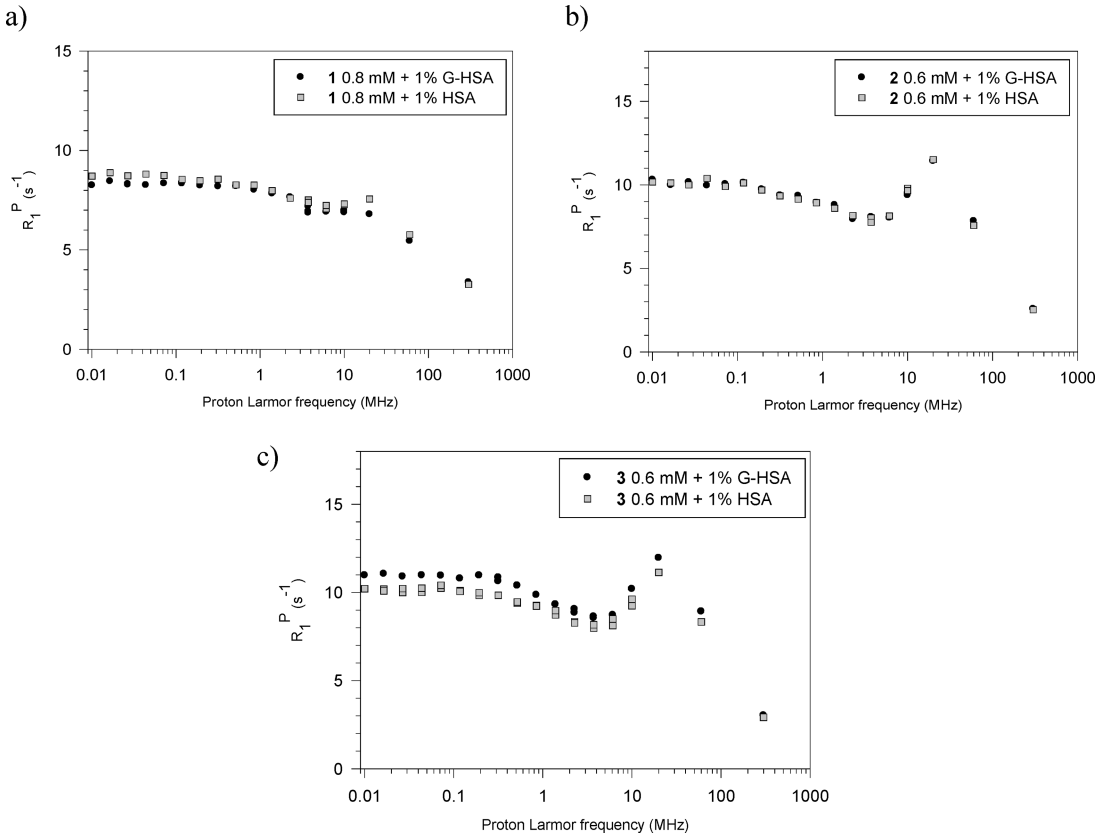

Figure 1. NMRD profiles obtained at 310 K on solutions with 1% of nonglycosylated or glycosylated HSA and (a) 0.8 mM of **1**, (b) 0.65 mM of **2**, (c) 0.65 mM of **3**.

where R_{1f}^P and R_{1b}^P are the water proton paramagnetic relaxation rates induced by the unbound and the bound gadolinium complex, respectively, r_1^f and r_1^b are the corresponding water proton relaxivities, and [L] and [LP] are the concentrations of the unbound and the bound gadolinium complex, respectively. The factor 1000 allows taking into account the fact that the water proton relaxivities are expressed for 1 mmol per L of gadolinium complex.

Classically, the procedure to evaluate the binding parameters of a gadolinium complex to HSA consists of measuring the water proton relaxation rate on solutions containing increasing concentrations of gadolinium complex and a fixed concentration of HSA. The titration curve is then fitted with eq 4 in order to estimate the association constant and the number of binding sites, assuming that all sites are identical and independent.

$$R_{1obs}^P = 1000 \times \left\{ r_1^f L^0 + (r_1^b - r_1^f) \left\{ \left(Np^0 + L^0 + K_a^{-1} - \sqrt{(Np^0 + L^0 + K_a^{-1})^2 - 4NL^0p^0} \right) \Big/ 2 \right\} \right\} \quad (4)$$

In this equation, L^0 and p^0 are the total concentrations of gadolinium complex and HSA, respectively, r_1^f and r_1^b are the relaxivities of the free and bound complex, respectively, K_a is the association constant, and N is the number of binding sites, assumed to be identical and independent.

RESULTS

Proton nuclear magnetic relaxation dispersion (NMRD) profiles were recorded on solutions containing 1% of nonglycosylated or glycosylated HSA and 0.65 mM of **2** or **3**, or 0.8 mM of **1** (Figure 1).

The similarity of the NMRD profiles recorded on these solutions containing a large excess of Gd complexes show that the NEG of HSA has a negligible effect on the relaxation rates of the three gadolinium chelates. Nevertheless, because these profiles have been obtained at a high concentration of ligand compared to HSA (4-fold excess or more), the measured water proton paramagnetic relaxation rate is mainly influenced by the free gadolinium complex (as shown by eqs 1−3, the paramagnetic relaxation rate is indeed a weighted average between that coming from the unbound and bound gadolinium complexes, respectively). Possible changes in the binding affinity caused by the NEG of HSA could thus be missed in these experiments.

To complete these data, titration experiments on solutions with 1% glycosylated or nonglycosylated HSA and containing each of the gadolinium chelates at concentrations ranging from 0.026 or 0.027 mM to 0.65 or 0.8 mM were performed at 20 MHz and 310 K. Whereas slightly lower relaxation rates were observed for **1** and **2** over the whole studied concentration range in the presence of glycosylated HSA, no change was observed for **3**. The obtained experimental data were fitted with eq 4, where the relaxivities of the free gadolinium complexes r_1^f were fixed at the values previously obtained in aqueous solution and the relaxivities of the bound gadolinium complexes r_1^b were allowed to vary by 10% around the values previously obtained

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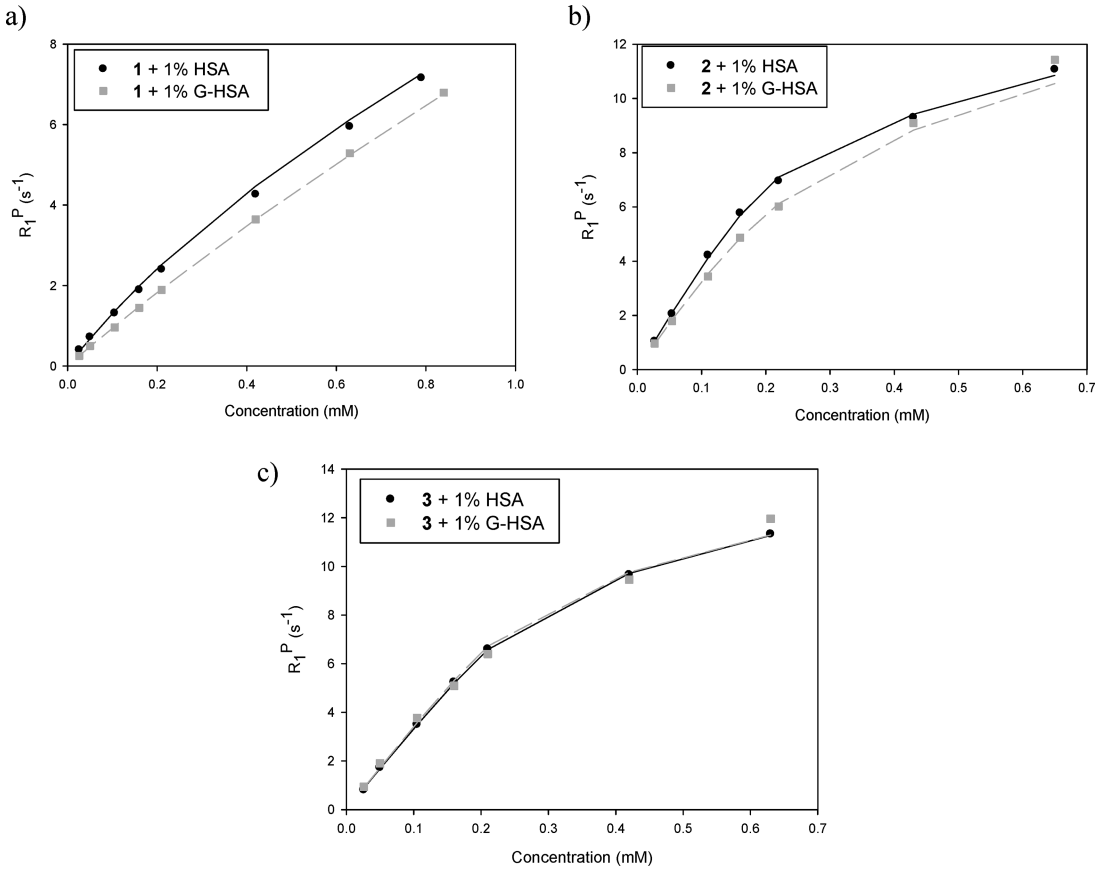

Figure 2. Evolution of the water proton paramagnetic relaxation rate in the presence of a fixed concentration (1%) of glycosylated or nonglycosylated HSA and of variable concentrations of (a) **1**, (b) **2**, and (c) **3** (B_0 = 0.47 T, T = 310 K).

with nonglycosylated HSA[11] (Figure 2). The results of the fitting are presented in Table 1.

Table 1. Results of the Fittings of the Relaxometric Data According to eq 4

		K_a (10^3 M^{-1})	N	r_1^b (s^{-1} mM^{-1})	r_1^f (s^{-1} mM^{-1})
1	HSA	2.6 ± 0.3	1.00 ± 0.09	36.0 ± 3.0	5.5
	HSA_{gly}	0.9 ± 1.8	1.08 ± 0.06	37.4 ± 4.8	5.5
2	HSA	58.7 ± 78.4	1.4 ± 0.2	42.3 ± 7.6	5.5
	HSA_{gly}	19.7 ± 4.4	1.4 ± 0.3	42.5 ± 4.0	5.5
3	HSA	84.7 ± 59.3	1.8 ± 0.2	35 ± 4.1	6.2
	HSA_{gly}	87.7 ± 83.6	1.7 ± 0.2	36 ± 1.0	6.2

These results show that the NEG of HSA induces a small decrease in the affinity of **1** and **2** and has no effect on the affinity of **3**.

This effect is, however, limited because a closer look at the curve of **2** shows that, to obtain the same relaxation rate of, for example, 5.78 s^{-1} (fourth point of the curve), a concentration of 0.16 mM is needed with nonglycosylated HSA, whereas a concentration of 0.20 mM should be required with glycosylated HSA.

DISCUSSION

Previous studies have shown that the NEG of HSA causes conformational changes.[2−4] This has some consequences on the functions of HSA such as the binding and the transport of numerous ligands. It has been shown for example that the NEG of HSA influences the binding of bilirubin,[2] thyroxine,[9] phenylbutazone,[6,7] dansylproline,[7] or also ibuprofen,[7] but has no influence on the binding of L-tryptophan,[5] warfarin,[6,7] or dansylamide.[7] All these endogenous or exogenous compounds are known to bind on different binding sites of HSA. Bilirubin, phenylbutazone, warfarin, and dansylamide bind on the Suddlow site I of HSA, whereas dansylproline, ibuprofen, or L-tryptophan bind on the Suddlow site II[7,26−28] and thyroxine binds on both sites.[29] It thus seems that the influence of the NEG of HSA on its binding capacity does not depend on the localization of the binding region, as it was already shown by the study of Koyama et al.[8] This is confirmed by our study because **1** binds on the Suddlow site I of HSA, **2** binds on the Suddlow site II, and **3** has its primary binding site on the Suddlow site II and a weaker binding site on the Suddlow site I.[13,15] The difference in the influence of NEG on the binding affinity of HSA for the three studied contrast agents could thus be explained by the different structures of the gadolinium complexes and hence their affinity for HSA. Contrarily to **1** and **2**, **3**, for which no influence of NEG on its binding affinity for HSA has been observed, is indeed nonaromatic but is substituted by a lipophilic bicyclic system. It has been mentioned in the literature[7] that the glycosylation of HSA induces some alterations in the microenvironment of the binding sites. This study seems to show that these modifications do not influence systematically the binding of all ligands of HSA and that the structure of the ligand is an important factor.

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CONCLUSION

The slight decrease in the affinity of **1** and **2** for HSA, observed when the protein is glycosylated, is quite negligible. Thus, the efficacy of the three studied contrast agents in the presence of glycosylated HSA is not really affected. Efficacy and pharmacokinetic profiles should not be markedly different in diabetic patients.

EXPERIMENTAL SECTION

Chemicals. **1** (Primovist) and **2** (Vasovist) were provided by Bayer HealthCare (Berlin, Germany), and **3** was provided by Mallinckrodt (Saint-Louis, MO). Nonglycosylated (product A-1653, 96−99%) and glycosylated (G-HSA, 1−5 hexoses per molecule of HSA) human serum albumin were obtained from Sigma (Bornem, Belgium) and were used without further purification. The HSA concentration in all the experiments was fixed to 1% or 0.15 mM. All the experiments were conducted in water.

Relaxometry Method. Longitudinal proton relaxation rates were measured at 0.47 T and 310 K on a Minispec mq-20 (Bruker, Karlsruhe, Germany) by a standard inversion−recovery sequence.

NMRD profiles were recorded on a Stelar relaxometer (Mede, Italy) working between 0.24 mT and 0.24 T. The additional relaxation rates at 0.47 T, 1.41 T, and 7.05 T were measured on mq-20 and mq-60 minispec systems and on an AMX300 spectrometer (all from Bruker, Karlsruhe, Germany).

AUTHOR INFORMATION

Corresponding Author

*Phone: +32-65-373520. Fax: +32-65-373533. E-mail: robert.muller@umons.ac.be.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

NEG, nonenzymatic glycosylation; NMRD profiles, nuclear magnetic relaxation dispersion profiles

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