Molecular Interaction and Energy Transfer between Human Serum Albumin and Polyoxometalates

Guangjin Zhang,[†] Bineta Keita,[†] Jean-Claude Brochon,[‡] Pedro de Oliveira,[†] Louis Nadjo,*[,†] Constantin T. Craescu,[§] and Simona Miron[§]

Laboratoire de Chimie Physique, Equipe d'Electrochimie et Photoelectrochimie, UMR 8000 CNRS, Universite Paris-Sud, Bâtiment 350, 91405 Orsay Cedex, France, Laboratoire de Biotechnologies et de Pharmacologie Génétique Appliquee, UMR 8113 CNRS, Ecole Normale Supérieure de Cachan, 61 Avenue de Président Wilson, 94235 Cachan Cedex, France, and INSERM U759/Institut Curie-Recherche, Centre Universitaire Paris-Sud, Bâtiment 112, 91405 Orsay Cedex, France

Received: June 16, 2006; In Final Form: December 6, 2006

As a step toward the elucidation of the mechanistic pathways governing the known bioactivity of polyoxometalates (POMs), two representative molecules of this class of chemicals, the wheel-shaped $[NaP_5W_{30}O_{110}]^{14-}$ (P5W30) and the Keggin-type anion $[H_2W_{12}O_{40}]^{6-}$ (H2W12), are shown, by two independent techniques, to interact with the fatty-acid-free human serum albumin (HSA). The excited-state lifetime of the single tryptophan molecule of this protein is dramatically decreased by the binding. The quenching mechanism is found to constitute the first example of energy transfer between HSA and POMs. Such molecular recognition is believed to be a key step for subsequent evolution of the systems. Circular dichroism (CD) was used to assess the structural effects of POM binding on HSA and to confirm the interaction revealed by fluorescence studies. CD experiments showed that the two POMs have different effects on the secondary structure of the protein. Binding P5W30 partially unfolds the protein whereas H2W12 has no remarkable effect on the structure of the protein.

1. Introduction

Polyoxometalates (POMs) are early transition metal−oxygen anionic clusters. Their properties make them attractive for applications in a variety of domains, including catalysis, electrocatalysis, photochemistry, analytical chemistry, and medicine.[1] Their promising antibacterial, antiviral, antitumor, anticancer, and anti-HIV activities arouse more and more research interest.[2] Specifically, recent research has recognized POM-induced cell apoptosis, inhibition of virus bonding to receptor, and enhancement of β-lactam antibiotics as well as the inhibition of bacterial growth.[2] However, the mechanisms of these phenomena at a molecular level remain unclear. As a consequence, preliminary studies of interactions, in a broad sense, between POMs and several proteins of interest, are one of the necessary steps toward an understanding of POMs' biological activity.

Human serum albumin (HSA) was selected as a particularly relevant protein in the present work. HSA aids in transport, distribution, and metabolism of many endogenous and exogenous ligands, including fatty acids, amino acids, metals ions, and numerous pharmaceuticals, and contributes significantly to colloid osmotic blood pressure.[3−5] Almost every pharmaceutical compound injected in the blood finds itself in the presence of a high concentration of HSA, known to have a strong affinity for a variety of chemical species. As a consequence, HSA should play an important role in the biological fate of POMs. To our knowledge, however, there has been no study probing the nature of the interactions of POMs with HSA. Here, we have investigated HSA intrinsic fluorescence quenching by two selected polyoxometalates, $[H_2W_{12}O_{40}]^{6-}$ (H2W12) and the wheel-shaped structure $[NaP_5W_{30}O_{110}]^{14-}$ (P5W30), which are very stable at the physiologically relevant pH values. Herein, we report our initial findings and identify both electrostatic interactions and excited-state energy-transfer processes. The currently presented work is restricted to the report of the unprecedented finding of interaction energy transfer between HSA and POMs. POM−HSA interaction was confirmed by circular dichroism (CD), which also gave insight into the structural influence of POM binding to HSA.

2. Experimental Section

Materials. Fatty-acid-free human serum albumin was purchased from Sigma. The samples were dissolved in Tris buffer solution (0.05 M Tris, pH = 7.5, 0.15 M NaCl, unless otherwise stated when modifying the ionic strength). All of the chemicals were of high-purity grade and used as purchased without further purification. The concentration of the protein was determined spectrophotometrically using an extinction coefficient (ϵ_{280}) of 36 600 M^{-1} cm^{-1}. Ultrapure water with a resistivity of 18.2 MΩ cm was produced by passage through a RiOs 8 unit followed by a Millipore-Q Academic purification set. Polyoxometalates were synthesized by the method in literature.[6,7] The concentrations of the POMs were determined directly by the weight of POMs and volume of the solution.

Measurement and Methods. All fluorescence spectra were recorded on a SPEX spectrofluorimeter (Jobin-Yvon-Horiba) equipped with a 250 W xenon lamp and a thermostat bath, which was calibrated using a standard lamp. Quartz cuvettes with 1.0 cm optical path were used. The excitation wavelength was

* Author to whom correspondence should be addressed. Phone: 33 1 69 15 77 51. Fax: 33 1 69 15 43 28. E-mail: nadjo@lcp.u-psud.fr.
[†] Universite Paris-Sud.
[‡] Ecole Normale Supérieure de Cachan.
[§] Centre Universitaire Paris-Sud.

10.1021/jp063758z CCC: $37.00
Published on Web 01/26/2007

selected at 295 nm, which excited only tryptophan residues and avoided the excitation of tyrosine residues. The UV spectra were recorded at room temperature on a Perkin-Elmer Lamda19 spectrophotometer. All measurements were performed at room temperature (20 °C) in aqueous solution unless otherwise stated. Typically, 2 mL of a solution containing an appropriate concentration of HSA or tryptophan or *N*-acetyl-L-tryptophanamide (NATA) was titrated by successive additions of polyoxometalate solutions. Titrations were performed manually by using trace syringes. At each titration, the fluorescence spectrum was collected. Raw data were systematically corrected for inner filter effects. The inner filter effect correction was carried out as follows: In our systems, POMs have substantial absorption in the excitation wavelength (295 nm) of HSA. With the gradual addition of polyoxometalates, the excitation light is absorbed by both of the entities, which we designate the inner filter effect. Thus it is very important to subtract such an effect from the raw quenching data. At the position of emission, which is in the half of the cell length, the absorption of the excitation light by the polyoxometalates can be calculated by the following set of equations

$$\mathrm{OD} = \log \frac{I_0}{I} = \frac{1}{2}\epsilon_{295}\mathrm{CL}$$

$$F = F_1 \frac{I_0}{I}$$

where ϵ_{295} is the molar absorption coefficient constant of the POMs at 295 nm, F is the real fluorescence intensity after deduction of the inner filter effect, and F_1 is the original fluorescence intensity measured from the spectra.

The displacement experiments of dansylamide (DNSA) by POMs were performed by a fluorescence technique, using a method reported in the literature.[8] In short, the fluorescence of a solution containing DNSA (2.0×10^{-2} mM) and HSA (1.0×10^{-2} mM) was measured before and after the addition of the relevant POM. The wavelength of excitation was 350 nm, and the emission was detected at 475 nm.

CD experiments were performed on a Jasco 715 CD spectrometer equipped with a Peltier temperature control unit. Far-UV spectra were recorded between 200 and 260 nm at 20 °C using 1 cm or 1 mm quartz cells for 0.2 μM and 0.4 μM or 10 μM concentration of the protein, respectively. Samples were dissolved in 10 mM Tris buffer (pH = 7.5). Temperature denaturation curves were recorded between 5 and 95 °C with a rate of temperature increase of 1 °C/min.

Time-resolved fluorescence lifetimes were obtained from the fluorescence decays using a time-correlated single photon counting technique. The instrumentation setup was essentially similar to those previously described.[9−11] The excitation light pulse source was a Ti sapphire femtosecond laser (Tsunami, Spectra Physics) associated with a third harmonic generator tuned to 295 nm. Fluorescence emission was detected through a polarizer set at the magic angle and a monochromator (ARC SpectraPro-150) set at 350 nm ($\Delta\lambda = 15$ nm). Analysis of the decay curves was performed by the quantified maximum entropy method.[11]

Fatty-acid-free human serum albumin was studied, in the absence and the presence of the two POMs, $[H_2W_{12}O_{40}]^{6-}$ (H2W12) and $[NaP_5W_{30}O_{110}]^{14-}$ (P5W30). Stern−Volmer analysis was applied to the fluorescence quenching data of fatty-acid-free HSA in Tris buffer according to eq 1

$$\frac{F_0}{F} \quad \text{or} \quad \frac{\tau_0}{\tau} = 1 + k[\mathrm{Q}] \tag{1}$$

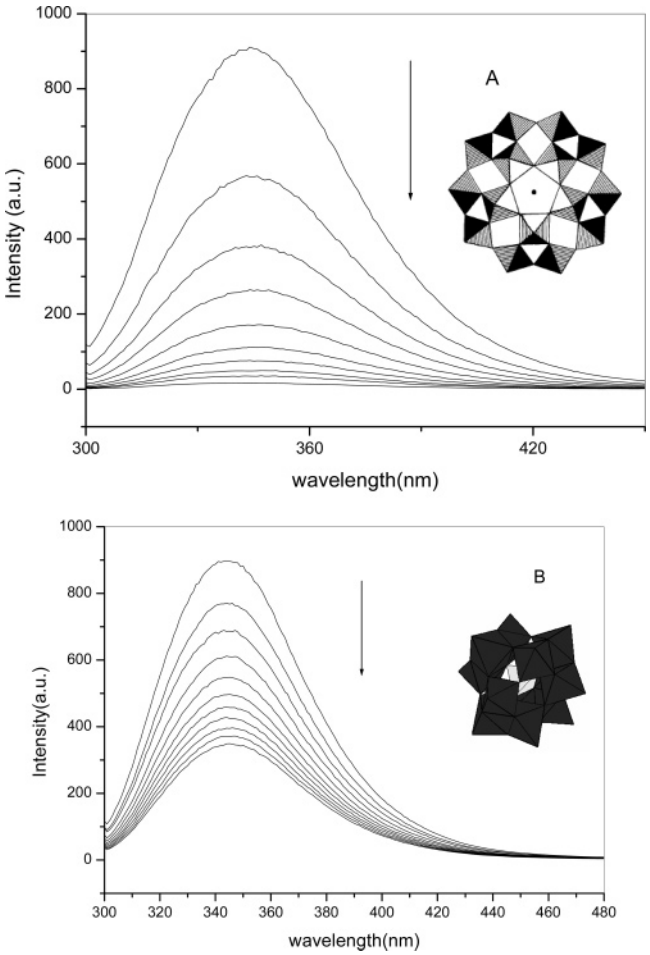

Figure 1. Emission spectra of HSA in the absence and the presence of various concentrations of POMs ([HSA] = 1.0×10^{-2} mM). (A) Fluorescence quenching by P5W30. (B) Fluorescence quenching by H2W12. (From top to bottom, the concentration of POMs increased from 0 to 2.0×10^{-2} mM with a step of 2.0×10^{-3} mM.) The inset is the structure of the corresponding POM.

where τ_0 and τ and F_0 and F, respectively, are the relative lifetimes and the relative steady-state fluorescence intensities in the absence and the presence of the quencher and [Q] is the concentration of the quencher. The significance of k depends on the nature of the quenching process: It may represent k_d, the rate of dynamic quenching, or the static quenching constant, K_q.

3. Results and Discussion

HSA contains a single tryptophan residue, Trp214.[3] Fluorescence quenching efficiency and aspects of the quenching mechanism of the HSA excited state by POMs were studied by both steady-state and time-resolved fluorescence measurements. At first, we found that the fluorescence of both isolated tryptophan and NATA are not quenched by the selected POMs, thus indicating an absence of direct interaction between these POMs and the indole group (Figure SI1 in the Supporting Information). This observation suggests that the binding site of the POMs on HSA is not directly the Trp214 residue. The variation of the HSA fluorescence spectrum upon the addition of two POMs is shown in Figures 1A and 1B, respectively, along with a schematic structure of the relevant POM. A Stern−Volmer analysis was applied to the fluorescence quenching data. In the first step, analysis of relative emission intensities was for both POMs as shown in Figure 2. In the range of POM

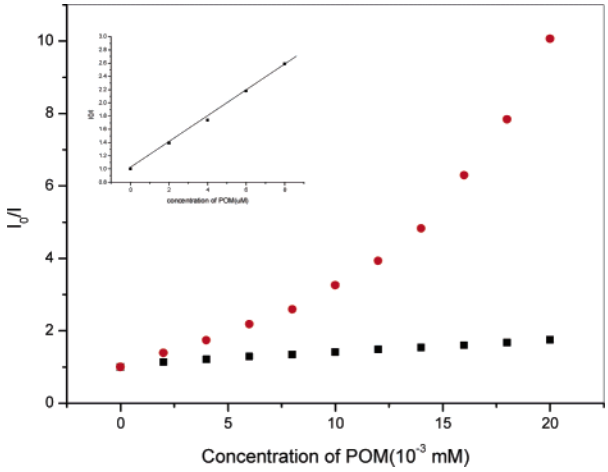

Figure 2. Stern−Volmer plot of the quenching of HSA's fluorescence by P5W30 (red circles) and H2W12 (black squares). The data were corrected for the inner filter absorption effects of the POMs. The inset is the linear part of the Stern−Volmer plot for lower concentrations of P5W30.

concentrations used, the Stern−Volmer plot for HSA−P5W30 shows an upward curvature at high quencher concentrations, whereas the plot for H2W12 remains linear. The behavior of the HSA−P5W30 system suggests a static quenching process.[12] For H2W12, the quenching constant calculated from the Stern−Volmer plot is $K_q = 3.5 \times 10^4$ mol^{-1} L at 293 K. For P5W30, the quenching constant can be calculated to be $K_q = 2.0 \times 10^5$ mol^{-1} L at 293 K. This value is larger than that of H2W12, as suggested by Stern−Volmer plots. These estimated values of quenching constant are within the limits of the reported data, albeit for organic quenchers.[13,14]

For both POMs, the quenching efficiency decreases when temperature increases as seen in Figures 3A and 3B, an observation confirming static quenching in these HSA−POM systems. As a matter of fact, the opposite trend is expected for dynamic quenching.[12] Both systems exhibit the same ionic strength dependence trend: Upon increase of ionic strength by NaCl addition, the quenching efficiency decreased. Raising the ionic strength up to 1.0 M weakens considerably the HSA−POM interactions. The observation indicates an electrostatic interaction between POMs and HSA macromolecules. A semiquantitative treatment of the ionic strength effect was performed as follows. From the quenching constant at different ionic strengths, useful information on the interactions can be extracted, based on the following experimentally derived equation[13,14]

$$\log k = \log k_0 + 0.5\Delta z^2 \mu^{1/2}$$

where k is the binding constant at ionic strength μ for a bimolecular reaction involving ionic species in solution (k is the same as in eq 1), k_0 is the bimolecular rate constant at $\mu = 0$, and Δz^2 is the difference between the square of the charge of the intermediate complex formed from the reactants and the sum of the squares of the charges of the reactant molecules. It is pointed out that the calculation is straightforward for HSA−H2W12 but is performed only with the linear portion of the Stern−Volmer plot for HSA−P5W30. Both systems show a good linear relationship between log k and $\mu^{1/2}$ (Figure SI2 of the Supporting Information). Interestingly, Δz^2 was calculated to be −1.3 from the HSA−H2W12 system and −2.1 from the HSA−P5W30 system. The small but negative values of Δz^2 indicate that there are very few positive charges on the protein and that raising the ionic strength decreases the quenching

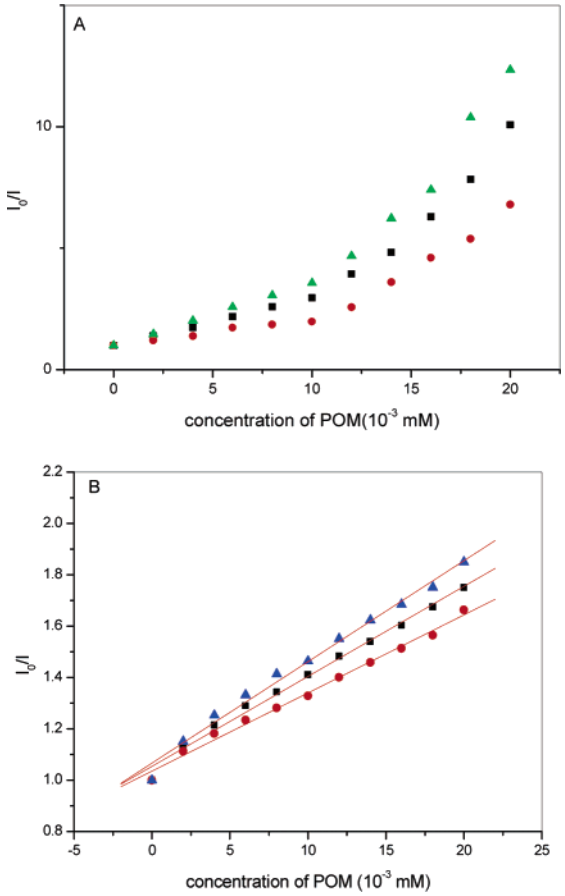

Figure 3. Stern−Volmer plot of the HSA−POM system at different temperatures: (A) HSA−P5W30 system at 10 °C (triangles), 20 °C (squares), and 37 °C (circles); (B) HSA−H2W12 system at 5 °C (triangles), 20 °C (squares), and 37 °C (circles).

TABLE 1: Fluorescence Average Lifetime (ns) of the HSA with Different Concentrations of Polyoxometalates

γ = [POM]/[HSA]	P5W30 (20 °C)	H2W12 (20 °C)	P5W30 (37 °C)
0	3.82	3.82	3.57
0.4	3.21		3.30
		3.67	
	2.32		2.62
		3.49	
0.5	1.76		
0.8	1.08		1.32
1.0	0.77	3.17	0.75

action, a result reinforcing the conclusion that ionic interactions are important in the present HSA−POM systems.

Altogether, the results fit in an overall static quenching mechanism in the two HSA−POM systems.

For further insight into the fluorescence quenching processes, the lifetimes of the excited state of tryptophan within the protein were measured in the absence and the presence of POMs. Fluorescence decays are correctly fitted by a lifetime distribution consisting of four peaks as previously described.[11] The peak centroid values and corresponding weights as well as the average lifetime are gathered in the Supporting Information (Tables SI1 and SI2). These components correspond to different environments of the single tryptophan residue. Table 1 gives the values of the fluorescence average lifetimes for both systems. In the absence of POM, the average lifetime falls within the recent estimations of lifetime for the single tryptophan of HSA.[15] Surprisingly, for both systems, the average lifetime decreased with increasing POM concentration. Figure 4 shows the

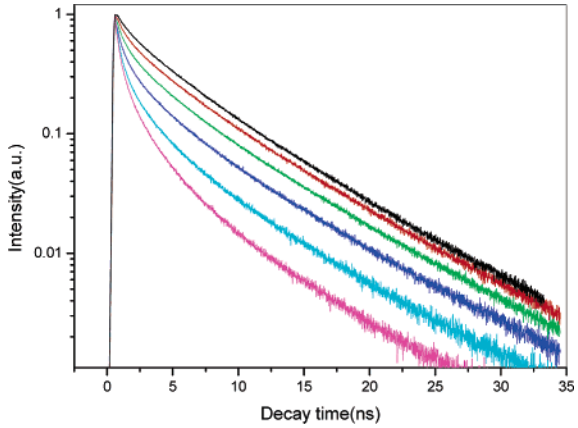

Figure 4. Fluorescence decay of HSA at different concentrations of P5W30 ([HSA] = 1.0×10^{-2} mM). The protein was excited at 295 nm and monitored at 355 nm. From right to left, the concentration of P5W30 increased from 0 to 2.0×10^{-2} mM with a step of 4.0×10^{-3} mM.

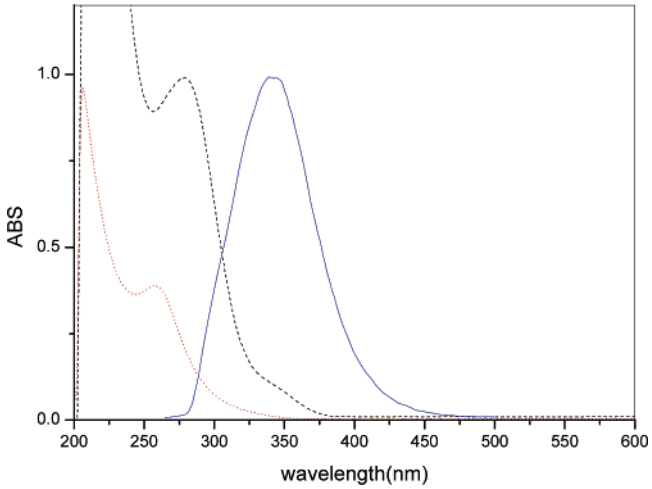

Figure 5. Spectral overlap between absorption of POMs (1.0×10^{-2} mM) and normalized emission of HSA. The solid line is the emission of HSA, the dashed line is the absorption of P5W30 and the dotted line is the absorption of H2W12.

fluorescence decay profiles of the protein at different concentrations of P5W30, from which it can directly be seen that the lifetime decreased significantly with an increase of P5W30 concentration. The general trend, in Table 1, is a lifetime decrease when temperature increases. In the present work, fluorescence originates from the single tryptophan residue in a protein structure that is rather sensitive to temperature. The addition of POM has a smoother effect on average lifetime at 37 °C than at 20 °C. In addition, we should mention that average lifetime may fluctuate by 5% for different samples. Detailed examination of lifetime component values (Tables SI1 and SI2 of the Supporting Information) reveals that only long lifetimes are modified by POM binding, thus indicating that this binding affects the environment of tryptophan. Since ground-state static quenching is not expected to shorten the lifetime, this lifetime decrease for both systems should be attributed to another mechanism. A temperature increase did not significantly change the average lifetime decrease trend upon addition of POM; that confirms the absence of a dynamic quenching mechanism.[11] Taking into account an overlap between the emission spectrum of HSA and the absorption spectra of the POMs (Figure 5), an excitation energy-transfer mechanism might be assumed. Such a mechanism is not uncommon. Recently, energy transfer was described for the ensemble of HSA and a flavonoid antioxidant quercetin.[15] The large difference in absorption overlap for P5W30 and H2W12 with HSA emission can explain their difference in energy transfer and therefore HSA lifetime changes. This overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor is the essential prerequisite for the possibility of an energy-transfer mechanism. In our examples, it can be stressed that the absorption spectrum of the acceptor is located on the higher energy side of the donor emission spectrum, which is, in turn, not common. However, energy transfer was demonstrated, recently, between cromolyn sodium, an organic acceptor, and HSA (donor) with the same order of spectra overlap as observed here.[16] To the best of our knowledge, this is the first example of energy transfer between a protein and POMs. The upward curvature of the quenching plot for P5W30 can be attributed to energy-transfer-enhanced static quenching.[11]

The apparent distance, r, between partners within the HSA−POM assembly and the critical distance R_0 related to the spectroscopic properties of the donor and the acceptor were calculated along classical procedures.[17−21] The distance r was evaluated from the energy-transfer efficiency

$$E = 1 - \frac{\tau}{\tau_0} = \frac{1}{1 + (r/R_0)^6}$$

where E is the efficiency of transfer between the donor and the acceptor and R_0 is related to the properties of the donor and was calculated by following equation

$$R_0^{\,6} = 8.79 \times 10^{-25} \kappa^2 n^{-4} \phi J$$

where κ is the orientation factor related to the geometry of the donor and acceptor and $\kappa^2 = {}^2/_3$ for random orientation as in fluid solution, n is the average refractive index of the medium in the wavelength range where spectral overlap is significant ($n = 1.4$ for the current condition), ϕ is the fluorescence quantum yield of the donor ($\phi = 0.15$ for HSA), and J is the effect of the spectral overlap between emission of the donor and absorption of the acceptor and can be calculated by the following equation

$$J = \frac{\int_0^\infty F(\lambda)\epsilon(\lambda)\lambda^4 \, d\lambda}{\int_0^\infty F(\lambda) \, d\lambda}$$

where $F(\lambda)$ is the corrected fluorescence intensity of the Trp residue in the wavelength range from λ to $\lambda + \Delta\lambda$ and $\epsilon(\lambda)$ is the extinction coefficient of the POM at λ.

Taking the E value at $\gamma = ([\text{POM}]/[\text{HSA}]) = 2$, at which the lifetime decrease reached a limit, a r value can be calculated.[17] From the data of P5W30, R_0 was calculated to be 2.56 nm, leading to an estimate for r, the apparent distance between Trp214 and the POM, of 2.04 nm. Analogously, $R_0 = 1.82$ nm and $r = 2.21$ nm were obtained for a HSA−H2W12 system. The values for both systems fall in a range favorable ($0.5R_0 < r < 1.5R_0$) for a significant probability of energy transfer between HSA and this POM. Both the absolute value of the average distance r between the donor fluorophore and the acceptor on the 2−8 nm range and the fulfilment of the required condition, $0.5R_0 < r < 1.5R_0$, converge to indicate that energy transfer from HSA to POM occurs with high probability.[22]

The possible binding position of polyoxometalates on the protein can be discussed from energy-transfer parameters. HSA conformational dynamics studies[18,20,21,23] reported that the Trp214 moiety is freely rotating within a cone of a semiangle

of 30°. The corresponding distance variation coefficient can be estimated to be between 0.76 and 1.25 nm. In our measurements, this gives a donor−acceptor actual distance within the range from 1.55 to 2.55 nm for the HSA−P5W30 system and from 1.68 to 2.76 nm for the HSA−H2W12 system. Then a tentative identification of the specific HSA binding site(s) of POMs can be proposed. HSA is known to have its 585 amino acids distributed in three homologous domains, I (1−195), II (196−383), and III (384−585),[4,5,17] as sketched in Figure SI3 in the Supporting Information. The crystallographic structure has allowed us to estimate distances from Trp 214 to amino acids involved in ligand binding.[18] At least, there are two recognized binding sites for drugs and other compounds.[3] For amino acids of site I located in the same subdomain IIA as Trp214, this distance ranges between 0.3 and 1.6 nm. The entrance of this pocket is surrounded by positively charged residues, such as Arg257, Arg222, Lys199, His242, Arg218, and Lys 195.[3,24] For site II amino acids, the range extends from 0.9 to 2.4 nm. The entrance of this pocket is less positively charged than that of site I, as only Arg410 is located at the mouth of the pocket.[21] Taking into account the distances, the overall charge distribution on the surface of HSA and the average diameters of the POMs (2 nm for P5W30 and 1 nm for H2W12),[1,25] both sites are suitable for binding POMs. However, to identify and map the exact binding position of the POMs, further detailed work is necessary and is underway.

Among the new complementary directions, preliminary competitive displacement and CD experiments are described briefly.

As the data in the preceding discussion do not allow us to give the precise binding sites of POMs on the protein, competitive displacement experiments by fluorescence were undertaken. For example, it is shown that the fluorescence of dansylamide, which binds specifically on site I, is not affected by the binding of POMs, thus indicating that the POMs may bind on another site. More detailed and complementary work is underway in this direction.

Also, preliminary results of CD experiments provide an independent approach to study HSA−POM interactions. To assess the structural effects of H2W12 binding to HSA we used CD spectroscopy to evaluate the secondary structure content and the structural stability of the protein. As shown in Figure 6A, binding of H2W12 has no significant effect on the secondary structure content of the protein, which is characterized by a high helical proportion. The same results are obtained at higher concentrations (10 μM) of HSA and H2W12. In the absence of any ligand, HSA shows a cooperative thermal unfolding in the range from, 55 to 85 °C with a mid-temperature at approximately 67 °C (Figure 7A). This relatively high thermal stability is not altered by a 2-fold excess of H2W12 at a low concentration of protein (2.0×10^{-4} mM). However, at higher concentrations (1.0×10^{-2} mM) of protein and H2W12, we observed a dramatic decrease of the mid-transition temperature (to ~60 °C) of the protein by binding with H2W12 (Figure 7B). CD experiments were also performed to determine the effect of P5W30 binding on the secondary structure and stability of the protein. Figure 6B shows the far-UV CD spectra of the protein in the absence and the presence of increasing concentrations of P5W30. It can be observed that with the increase in P5W30 concentration the CD signal of HSA decreased significantly. The decrease of the CD signal indicates a loss of helical secondary structure content. The thermal denaturation experiment shows (Figure 7A) that binding of P5W30 has a remarkable effect on the thermal unfolding process of the protein. After

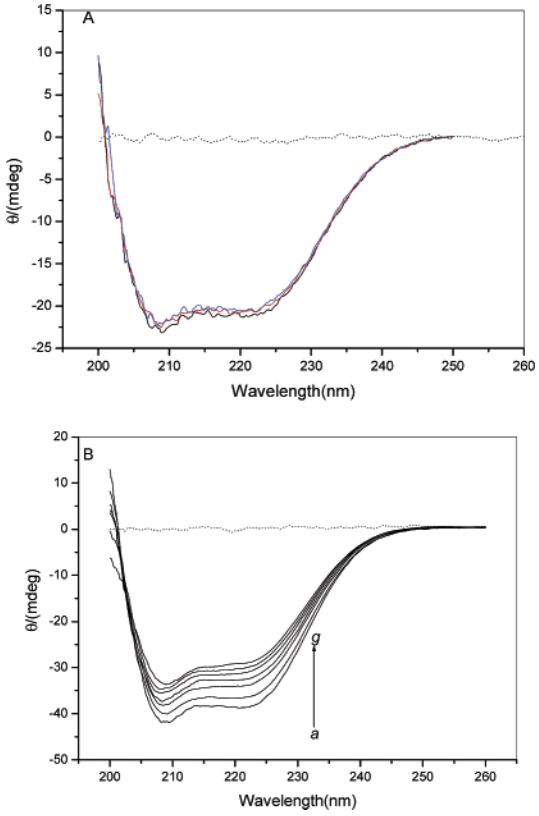

Figure 6. CD spectra of HSA in the absence and the presence of different concentrations of POMs (in 10 mM Tris/HCl buffer, pH 7.5, 20 °C). (A) The black line is the CD spectrum of 2.0×10^{-4} mM HSA, the red and blue lines are the spectra in presence of H2W12 at 2.0×10^{-4} and 4.0×10^{-4} mM, respectively, and the dotted line is the CD spectrum of 4.0×10^{-4} mM H2W12. (B) [HSA] = 4.0×10^{-4} mM. For spectra from a to g, the molar ratios of [P5W30]/[HSA] are 0, 0.2, 0.6, 1, 1.5, 2, and 3, respectively. The dotted spectrum is the CD signal of 4.0×10^{-4} mM P5W30.

the initial unfolding of the protein by binding with P5W30, further thermal denaturation induced little unfolding of the protein.

It should be noted that for P5W30 such effects of protein denaturation can occur at low concentrations of protein, whereas for H2W12 such effects were observed only at high concentration of protein.

The CD experiment indicates that the molecular interaction between POMs and HSA may produce changes in the secondary structure content and stability of the protein, and the shape effect of the POMs may be significant for such an interaction.

In this paper, three different approaches were used for studying the interaction between POMs and HSA. Particular emphasis was put on fluorescence quenching experiments as a means to reveal the very existence of such interactions and the type of mechanism through which they occur. The quenching mechanism was found to constitute the first example of energy transfer between HSA and POMs. Further and complementary steps in the understanding of this interaction were provided by competitive displacement experiments and CD experiments. Attempts at displacement, by POMs, of dansylamide, which binds specifically on site I of HSA, were unsuccessful, thus indicating that this site is probably not affected by POM binding. Finally, CD results, in addition to presenting an interest in their own right, shed light on the influence of the POMs used in this work on the secondary structure of HSA. Even though much

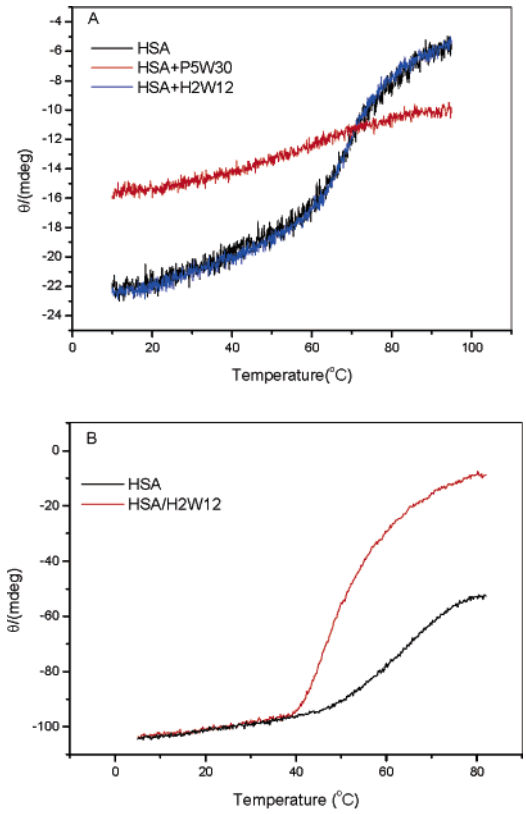

Figure 7. Thermal denaturation of the protein monitored by the CD signal at 222 nm in the absence and the presence of POMs at different concentrations of protein: (A) [HSA] = 2.0×10^{-4} mM, [POM] = 4.0×10^{-4} mM; (B) [HSA] = 1.0×10^{-2} mM, [H2W12] = 1.0×10^{-2} mM.

work remains before reaching the applicability conditions of selected POMs as drugs, the present results altogether shed some light on different aspects of the interaction between these chemicals and HSA, which is a prerequisite for the action of pharmaceutical compounds.

4. Conclusions

The interaction between HSA and two POMs with different structures was studied by static and time-resolved fluorescence quenching. Electrostatic interaction between POMs and the protein was found. The wheel-shaped polyoxometalate, P5W30, shows much better binding than the small Keggin polyoxometalate, H2W12. Energy transfer was also observed clearly in the case of P5W30. From competition with dansylamide binding, it appears that the binding site of POMs on the protein is likely on site II, but further work is necessary to reach a precise conclusion. These results were complemented by CD experiments on the HSA−POM systems, thus giving more insight into the structural influence of the binding of POMs to HSA. Among numerous future challenges, studies will concern the influence of POM structures, size, charge, and composition on the interaction with HSA and other proteins of interest, and pH effects on both the HSA conformation and the protonation state of the POMs.

Acknowledgment. This paper is dedicated to Dr. Roland Contant on the occasion of his retirement. This work is supported by the CNRS (UMR 8000 and 8113, CNRS), the Université Paris-Sud 11, Ecole Normale Supérieure de Cachan, and INSERM U759/Institut Curie-Recherche. G.Z. thanks the University Paris-Sud 11 for a postdoctoral fellowship and the European Community for the beginning of a Marie Curie Incoming Fellowship (Contract No. 040487).

Supporting Information Available: Stern−Volmer plot of the quenching of NATA's fluorescence by P5W30, plot of log k_q vs $\mu^{1/2}$ for both systems, lifetimes of HSA as a function of increasing addition of P5W30 and H2W12, and the domain structure of HSA. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Pope, M. T. *Heteropoly and Isopoly Oxometalates*; Springer-Verlag: New York, 1983. (b) Pope, M. T.; Müller, A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 34. (c) Keita, B.; Nadjo, L. Electrochemistry of isopoly and heteropoly oxometalates. In *Encyclopedia of Electrochemistry*; Bard, A. J., Stratmann, M., Scholz, F., Pickett, C. J., Eds.; Wiley-VCH: Weinheim, Germany, 2006; Vol. 7, pp 607−700.

(2) (a) Judd, D. A.; Nettles, J. H.; Nevins, N. N.; Snyder, J. P.; Liotta, D. C.; J. Tang, J.; Ermolieff, J.; Schinazi, R. F.; Hill, C. L. *J. Am. Chem. Soc.* **2001**, *123*, 886. (b) Rhule, J. T.; Hill, C. L.; Judd, D. A.; Schinazi, R. F. *Chem. Rev.* **1998**, *98*, 327. (c) Yamase, T. *J. Mater. Chem.* **2005**, *15*, 4773.

(3) He, X. M.; Carter, D. C. *Nature* **1992**, *358*, 209.

(4) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153−203.

(5) Peters, T. *All About Albumin: Biochemistry, Genetics, and Medical Applications*; Academic Press: San Diego, 1996.

(6) Contant, R.; Ciabrini, J. P. *J. Chem. Res.* **1977**, 2601.

(7) Jeannin, Y.; Martin-Frère, J. *Inorg. Synth.* **1990**, *27*, 115.

(8) Sudlow, G.; Birkett, D. J.; Wade, D. N. *Mol. Pharmacol.* **1976**, *12*, 1052.

(9) Deprez, E.; Tauc, P.; Leh, H.; Mouscadet, J. F.; Auclair, C.; Brochon, J. C. *Biochemistry* **2000**, *39*, 9275.

(10) Deprez, E.; Tauc, P.; Leh, H.; Mouscadet, J. F.; Auclair, C.; Hawkins, M. E.; Brochon, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10090.

(11) Brochon, J. C. *Methods Enzymol.* **1994**, *240*, 262−311.

(12) Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, Germany, 2002.

(13) Stoughton, R. W.; Rollefson, G. K. *J. Am. Chem. Soc.* **1939**, *61*, 2634.

(14) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Plenum Press: New York, 1999.

(15) Mishra, B.; Barik, A.; Priyadarsini, K. I.; Mohan, H. *J. Chem. Sci.* **2005**, *117*, 641.

(16) Hu, Y-J.; Liu, Y.; Pi, Z-B.; Qu, S−S. *Bioorg. Med. Chem.* **2005**, *13*, 6609.

(17) Murphy, C. B.; Zhang, Y.; Troxler, T.; Ferry, V.; Martin, J. J.; Jones, W. E., Jr. *J. Phys. Chem. B* **2004**, *108*, 1537.

(18) Il'ichev, Y. V.; Perry, J. L.; Simon, J. D. *J. Phys. Chem. B* **2002**, *106*, 452.

(19) Ahmad, B.; Parveen, S.; Khan, R. H. *Biomacromolecules* **2006**, *7*, 1350.

(20) Dale, R. E.; Eisinger, J. *Biopolymers* **1974**, *13*, 1573.

(21) Stryer, L. *Annu. Rev. Bochem.* **1978**, *47*, 819.

(22) Weiss, S. *Science* **1999**, *283*, 1676.

(23) Selvin, P. R. *Methods Enzymol.* **1995**, *246*, 300.

(24) Sugio, S.; Kashima, A.; Mochizuki, S.; Noda, M.; Kobayashi, K. *Protein Eng.* **1999**, *12*, 439.

(25) Alizadeh, M. H.; Harmalker, S. P.; Jeannin, Y.; Martin-Frère, J.; Pope, M. T. *J. Am. Chem. Soc.* **1985**, *107*, 2662.