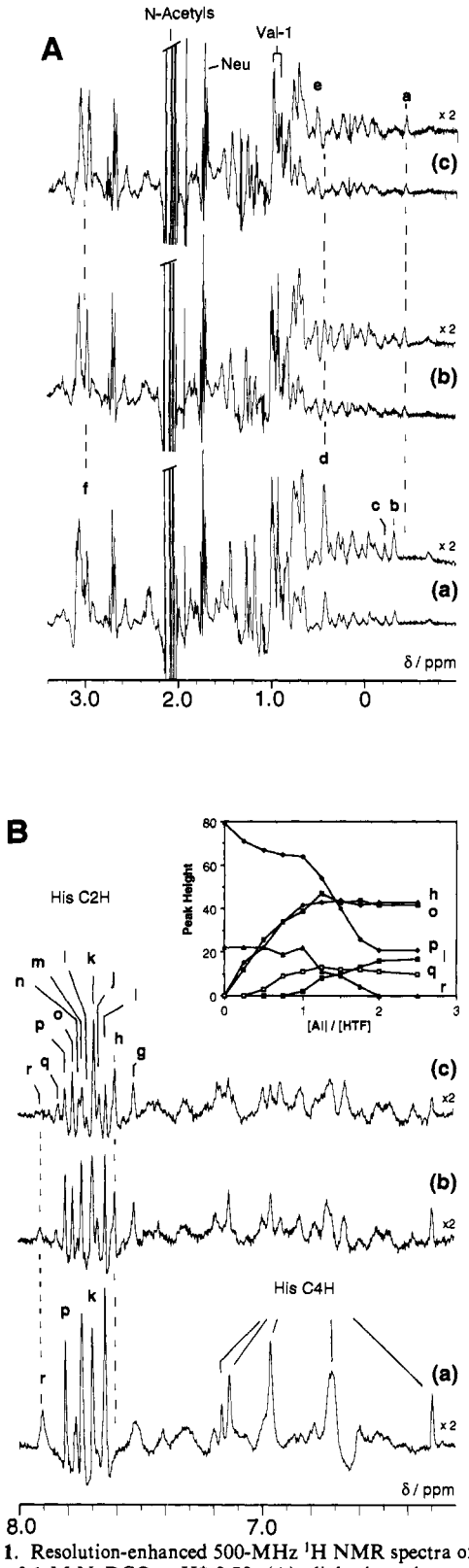

Figure 1. Resolution-enhanced 500-MHz ^{1}H NMR spectra of 0.56 mM HTF in 0.1 M $NaDCO_3$, pH* 8.78, (A) aliphatic region and (B) aromatic region: (a) before and (b) after addition of 1.25 molar equiv of Al^{3+} and (c) 2.5 molar equiv of Al^{3+}. Assignments: Val-1, valine-1 CH_3; *N*-acetyls, MeCO of NAcGlc and NAcNeu of glycan chains (C-lobe); Neu, NAcNeu; a–f, peaks perturbed by Al^{3+} binding; g–r, His C2H peaks. The intensities of His C2H peaks h, l, o, p, q, and r are plotted in the inset in part B; these data are from a second (and more complete) titration using 0.35 mM HTF. With broad overlapping peaks, low S/N ratios (low protein concentrations), and the use of resolution enhancement, peak heights (or areas) are subject to error from the choice of base lines. The data indicate slow exchange of HTF with Al-HTF on the NMR time scale.

orientation of these methyls with respect to certain aromatic side chains of other residues, perhaps due to closure of the metal-binding cleft.[12]

These methods can now be used to test both the thermodynamic and kinetic effectiveness of therapeutic chelating agents designed to remove Al^{3+} from HTF and the body, such as desferrioxamines, hydroxypyridinones, and catecholates.[13-16] For example, incubation of an Al_2-HTF sample with 3 molar equiv of desferrioxamine (Ciba) for 4 h at 310 K completely reversed the effects of Al^{3+} on the spectrum, and the spectrum of apo-HTF was regenerated. It should also be possible to study the sequential uptake and release of other metal ions, e.g., Ga^{3+}, In^{3+}, and perhaps Fe^{3+}, by transferrin, and with longer accumulation times it may even be possible to do experiments at HTF concentrations close to those found in vivo (ca. 37 μM).

Acknowledgment. We thank the Science and Engineering Research Council, Medical Research Council, University of London Intercollegiate Research Service, and Wolfson Foundation for their support for this work and Professor R. C. Woodworth, Dr. P. F. Lindley, and Dr. G. Williams for helpful discussion.

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Drug-Induced Reactions of Bovine Serum Albumin: ^{1}H NMR Studies of Gold Binding and Cysteine Release

Orla M. Ni Dhubhghaill, Peter J. Sadler,* and Alan Tucker

Department of Chemistry, Birkbeck College
University of London
Gordon House and Christopher Ingold Laboratory
29 Gordon Square, London WC1H 0PP, U.K.

Received June 3, 1991

The protein albumin plays a major role in the transport of drugs and metal ions in the blood.[1] For example, in patients treated with gold antiarthritic drugs, over 80% of the circulating gold in blood serum is carried on albumin.[2,3] However, the chemistry of serum albumin is poorly understood. Progress in this area is likely to arise from a combination of X-ray crystallography[4] and high-resolution NMR spectroscopy.[5] Albumin (MW 66.5 kDa) is a large molecule for ^{1}H NMR spectroscopy, but with high-field spectrometers and resolution-enhancement methods, resonances from the more mobile regions of the protein are well-resolved and can be used to probe drug-induced structural changes. We report here[6] evidence for specific structural changes in bovine serum albumin (BSA) on binding to an antiarthritic gold drug and the accompanying release of Cys from the protein. The fate of released Cys is determined by a ligand on the gold drug.

* To whom correspondence should be addressed.

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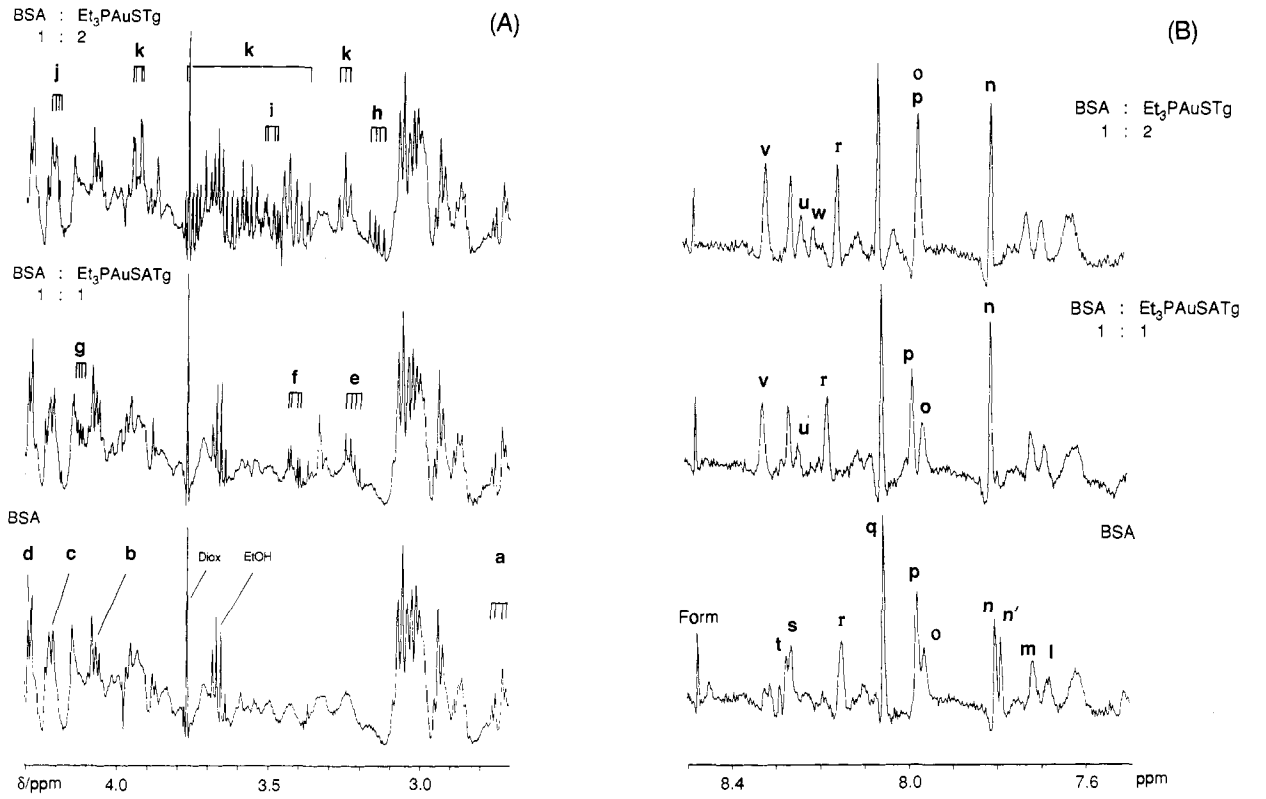

Figure 1. Resolution-enhanced 500-MHz ^1H NMR spectra of 2 mM BSA in 10 mM phosphate buffer in D_2O, before and after addition of 1 and 2 molar equiv of $Et_3PAuSATg$ and $Et_3PAuSTg$, respectively. (A) CH/CH_2 region. Assignments: a, βCH_A Asp-1; b, βCH Thr-2; c, αCH Asp-1; d, αCH Thr-2; Diox, dioxane (added reference); e and f βCH_2 and g αCH Cys-Cys; h and i βCH_2 and j αCH Cys in Cys-STg; k, STg and STg in Cys-STg. (B) His C2H region. Assignments: l–t His C2H (it seems likely that *n* and n′ are assignable to His3 in BSA-Cys34-SH and BSA-Cys34-Cys, respectively); Form, formate (impurity); u–w new peaks induced by gold binding. The pH* of the BSA solution was 7.01; the small shifts of peaks o, p, and r, after addition of gold, can be attributed to a slight decrease in pH* (<0.07). Spectra were recorded on a Bruker AM500 spectrometer using 0.5 mL in 5-mm tubes, 310 K. Pulsing conditions: 40° pulses, 2-s relaxation delay, 8K data points, (zero-filled to 16K), 128 transients, gated secondary irradiation of HOD. Sequential application of exponential (line-broadening 1 Hz) and unshifted sine-bell functions was used to enhance the resolution. Because of this, the comparison of peak intensities, e.g., with the quartet a for Asp-1, should be treated with caution.

We have studied reactions of the orally-active drug auranofin,[3,7] (1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I), $Et_3PAuSATg$, and its deacetylated metabolite[8] $Et_3PAuSTg$ (HSTg = thioglucose) with fatty acid free BSA.[9] Soon after addition of 1 molar equiv of auranofin to BSA at pH* 7,[10] three doublets of doublets characteristic of the amino acid cystine (Cys-Cys) can be identified (2D COSY, standard shifts) in the 500-MHz ^1H NMR spectrum, Figure 1A (peaks e, f, and g). In the −0.5 to 2 ppm region (figure deposited as supplementary material), a new high-field-shifted peak (likely to be a methyl resonance) is seen at −0.35 ppm, and two sets of multiplets assignable to Au–PEt_3 groups appear at 1.19 (CH_3) and 1.95 (CH_2) ppm and 1.13 and 1.83 ppm. Only the latter set increases in intensity on addition of a second equivalent of drug, whereas the peaks for Cys-Cys and the new high-field-shifted methyl are unaffected. Similarly, in the His C2H region, the collapse of two peaks assignable to His3 into a singlet and the appearance of new peaks v and u (Figure 1B) are complete after addition of 1 equiv of drug. No peaks assignable to sugar ring protons are seen.

When the auranofin metabolite $Et_3PAuSTg$ is added to BSA, some of the spectral changes are the same and are also complete after addition of 1 equiv: the new high-field methyl peak, the set of Au–PEt_3 resonances at 1.19 and 1.95 ppm, the collapse of the two peaks for His3 into a singlet, and the appearance of peaks u and v. In this case new peaks are observed for the mixed disulfide Cys-STg (Figure 1A, h, i, and j), and peaks (k) for sugar protons are clearly visible.

These observations are explicable by an initial ligand substitution reaction in which Cys34 (pK_a ca. 5)[11] of the 60% of mercaptalbumin present in the BSA displaces the STg or SATg ligand (pK_a ca. 7)[11] to form BSA-Cys34-Au(PEt_3). Thiolates with the lowest pK_a's bind to Au(I) the most strongly.[12] The displaced thiolate then undergoes thiol–disulfide interchange reactions with the remaining 40% of BSA, the mixed disulfide–BSA. Although this has been described as BSA-Cys34-Cys and BSA-Cys34-SG (GSH is glutathione),[11] only Cys derivatives appear to be seen in our spectra. BSA-Cys34-Au(PEt_3) has been detected previously by ^{31}P NMR[11,13,14] and characterized by EXAFS.[14] Curiously, when Ecker et al. reacted radiolabeled auranofin with fatty BSA under similar conditions, they found only 0.6 mol of Au/mol of BSA and 0.6 mol of SATg was released and separated by HPLC.[15]

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This might suggest that fatty acid binding to BSA protects disulfides at Cys34 from reaction with displaced ligand. In our experiments, the apparent reaction of Cys34-blocked fatty acid free BSA with displaced thiolate leads to no further binding of $AuPEt_3$ groups. Thus, drug-induced release of Cys from BSA does not simply regenerate Cys34. It is intriguing that a perturbation at Cys34 appears to be sensed by His3, one of the ligands in the N-terminal Cu(II) site. Such a communication could easily be "wired" by two intervening helices.[16] The new His peaks (u and v) could be those of His9 and His18, which are both within these helices. Albumin is known to possess binding sites for a variety of hydrophobic molecules,[1] and this may account for the broadening (beyond detection) of the resonances for the sugar ring protons of SATg in spectra of solutions containing auranofin and BSA.

The mechanisms of the reactions leading to the formation of either Cys-Cys or Cys-STg are curious, as is the transfer of the hydrophobic SATg ligand from mercaptalbumin to blocked albumin. This work provides a basis for investigations of the involvement of albumin in the promotion of metal–ligand substitution reactions via both metal and ligand recognition processes, thiol–disulfide interchanges, and thiol–disulfide transport and delivery.

Acknowledgment. We thank the Wellcome Trust, Cancer Research Campaign, MRC, and Wolfson Foundation for their support for this work. We are grateful to the MRC Biomedical NMR Centre, Mill Hill, for the provision of NMR facilities.

Supplementary Material Available: The complementary spectra to Figure 1 covering the region −0.5 to 2.2 ppm showing triethylphosphine resonances and high-field-shifted albumin peaks (1 page). Ordering information is given on any current masthead page.

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Molecular Recognition and Catalysis. Acceleration of Phosphodiester Cleavage by a Simple Hydrogen-Bonding Receptor

Vrej Jubian, Robert P. Dixon, and Andrew D. Hamilton*

Department of Chemistry, University of Pittsburgh Pittsburgh, Pennsylvania 15260

Received September 5, 1991

The design of artificial catalysts for phosphodiester cleavage (Scheme I) is an important goal that has implications for the controlled hydrolysis of DNA and RNA.[1] Of the many strategies investigated,[2-5] none approaches the efficiency of phosphodiesterase enzymes such as staphylococcal nuclease (SN), which hydrolyzes DNA 10^{16}-fold faster than the background reaction.[6] The active site of this remarkable enzyme contains two key arginines at positions 35 and 87.[7] Site directed mutagenesis studies[6,8] have established that only Arg35 binds to the monoanionic substrate whereas both residues stabilize the dianionic, trigonal-bipyramidal intermediate (Scheme I) with Arg87 playing the additional role of general acid and protonating the leaving group.

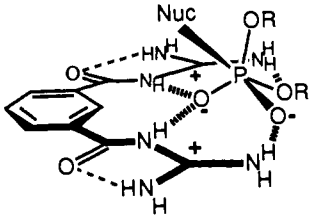


Figure 1. Possible mode of interaction between **1** and trigonal-bipyramidal intermediate in Scheme I.[13]

Scheme I

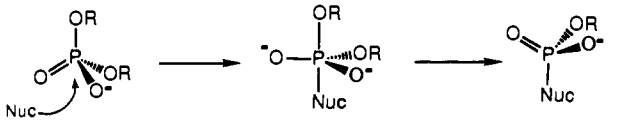
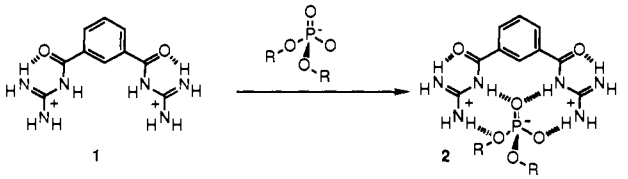


Table I.[a] Rate Constants for Phosphodiester Cleavage Reactions

receptor (concn, mM)	$k_{obsd} \times 10^5$ s^{-1}	k_{obsd}/k_{uncat}
Intramolecular Reaction of **3**[b]		
none	0.038	1
1 (30)	26.5	700
1 (20)	24.5	645
1 (10)	17	450
1 (5)	11	290
5 (10)	0.094	2.5
Intermolecular Thiolysis of **4**[c] with Ethyl Mercaptoacetate		
none	0.042	1
1 (5)	1.5	36

[a] In CH_3CN, [diester] = 1×10^{-4} M, [lutidine] = 1.25×10^{-2} M, at 25 °C. All k_{obsd} values are the average of at least three runs which differed by less than 5%. [b] Barium salt.[16] [c] Pyridinium salt.

In this paper we report the acceleration of both inter- and intramolecular phosphodiester cleavage by a synthetic receptor based on the active site of SN. Bis(acylguanidinium) **1** is formed in a single synthetic step from dimethyl isophthalate and guanidinium hydrochloride and forms strong complexes of type **2** with phosphodiesters in CH_3CN ($K_a \sim 5 \times 10^4$ M^{-1}).[9] Earlier work



from this group on barbiturate recognition[10] and on the accelerated aminolysis of phosphorodiamidates[11] indicated that the isophthaloyl spacer in **1** was well-suited to position the two guanidiniums to

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