# Direct Detection of Albumin in Human Blood Plasma by $^1$H NMR Spectroscopy. Complexation of Nickel$^{2+}$

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Blood plasma is a heterogeneous mixture of lipoprotein particles and high $M_r$ proteins such as albumin, IgG, $\alpha_1$-acid glycoprotein, and transferrin, together with low $M_r$ substances such as amino acids, anions, and metal ions.[1] Even at the highest available frequency, $^1$H NMR spectra of plasma (or serum) consist of a broad envelope of overlapping resonances.[2,3] Only the sharper peaks due to mobile small molecules, *N*-acetyls of glycan side chains of acute-phase glycoproteins, and lipids in lipoproteins have been previously assigned.[4,5] In particular, no resonances have been specifically assigned to amino acid residues of plasma proteins; they are broad and readily filtered out by spin–echo methods using short refocusing times.[4-6] Complexation of $Ni^{2+}$ in blood plasma is of interest because nickel is an essential element with a poorly understood biochemistry[7,8] and a potentially potent allergen.[9] $Ni^{2+}$ is known to bind strongly to the N-terminus of albumin,[9,10] Even though albumin is a large protein (66.5 kDa, 585 amino acids)[10] for $^1$H NMR work, we have recently assigned[11,12] resonances for the N-terminal amino acids of both albumin and Ni–albumin. We now show[13] that specific complexation sites for $Ni^{2+}$ on albumin can be detected in single-pulse and 2D NMR spectra of human blood plasma and that competitive binding to the free amino acid L-His can be studied. Novel features observed for the $Ni^{2+}$ binding site of isolated albumin, not found previously with its 1–24 peptide, are also detected for albumin in plasma.

A resolution-enhanced 500-MHz (Bruker AM500) single-pulse spectrum[14] of the aromatic region of human blood plasma[15] is shown in Figure 1. A large number of the peaks have chemical shifts, intensities, and line widths similar to those observed in spectra of defatted purified human albumin (Figure D1, supplementary material) over a range of pH* values. High-field-shifted resonances are also observable in plasma spectra (0.5 to -0.5 ppm) corresponding to those of isolated albumin, and several peaks in the aromatic region undergo pH-dependent shifts (data not shown) similar to peaks of isolated albumin assigned to His $\epsilon$CH (C2H) imidazole ring protons.[16] Additional small sharp peaks in the plasma spectrum are assigned to formate and the free amino acids His, Tyr, and Phe (confirmed by the spectrum of the low $M_r$ ultrafiltrate[17] from the same plasma sample, shown in the supplementary material, Figure D1).

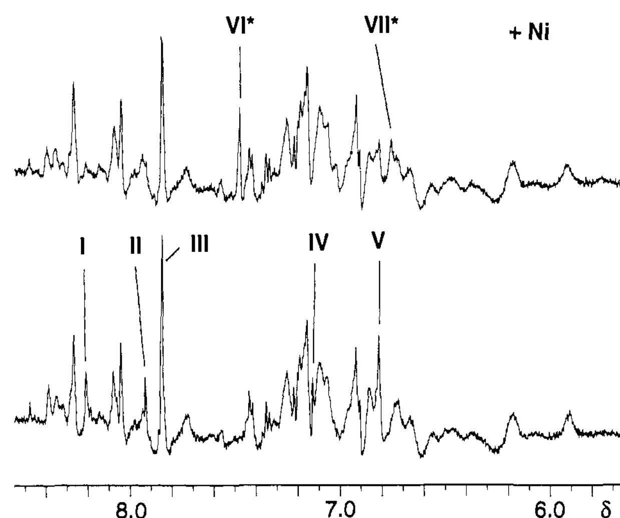


**Figure 1.** Resolution-enhanced 500-MHz $^1$H NMR spectra of the aromatic region of human blood plasma, before (lower) and after (upper) addition of $Ni^{2+}$ (ca. 0.5 mol equiv with respect to albumin). Assignments are as follows. Albumin: I (His $\epsilon$CH, tentative; see text), III (His3 $\epsilon$CH, overlapped with another $\epsilon$CH peak), V (His3 $\delta$CH). Ni–albumin: VI* (His3 $\epsilon$CH), VII* (His3 $\delta$CH). L-His: II ($\epsilon$CH), IV ($\delta$CH).

Next we titrated $Ni^{2+}$ into plasma.[18] Most notable was the progressive appearance of a new peak at 7.48 ppm (VI*) and the disappearance of His $\epsilon$CH peaks I and II, together with a reduction in intensity of peak III, Figure 1. In the His $\delta$CH (C4H) region, peaks IV and V disappear, and a new peak appears at 6.73 ppm (VII*). Peaks II and IV can be assigned to the $\epsilon$CH and $\delta$CH, respectively, of free His (confirmed by a standard addition), while peaks III and V correspond to resonances assigned to $\epsilon$CH and $\delta$CH of His3 of isolated human albumin.[11,16] Peaks VI* and VII* have shifts similar to those of the His $\epsilon$CH and $\delta$CH peaks of Ni–albumin, Table I. Changes in the aliphatic regions of spectra of plasma on addition of $Ni^{2+}$ also show a striking resemblance to those observed for isolated albumin.[12] Cross-peaks in 2D TOCSY spectra of plasma assignable to Asp1 $\alpha/\beta$

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(1) (a) Composition of Blood. *Geigy Scientific Tables*; Lentner, C., Ed.; CIBA-GEIGY Ltd.: Basel, Switzerland, 1984; Vol. 3. Albumin accounts for ca. 60% of the total protein in blood serum with a concentration of ca. 42–52 g $L^{-1}$ (0.60–0.78 mM). An average concentration of 0.69 mM was assumed in this work. (b) Bell, J. D.; Brown, J. C. C.; Sadler, P. J. *Chem. Br.* **1989**, *24*, 1021–1024.

(2) Bock, J. L. *Clin. Chem.* **1982**, *28*, 1873–1877.

(3) Rabenstein, D. L.; Millis, K. K.; Strauss, E. J. *Anal. Chem.* **1988**, *60*, 1380–1391.

(4) Bell, J. D.; Brown, J. C. C.; Sadler, P. J. *NMR Biomed.* **1989**, *2*, 246–256.

(5) Nicholson, J. K.; Wilson, I. D. *Prog. Nucl. Magn. Reson. Specrosc.* **1989**, *21*, 465–501.

(6) Nicholson, J. K.; Buckingham, M. J.; Sadler, P. J. *Biochem. J.* **1983**, *211*, 605–615. No resonances remain in the aromatic region with refocusing times >ca. 30 ms.

(7) Articles in *Met. Ions. Biol. Syst.* **1988**, *23*.

(8) Normal concentration of Ni in plasma ca. 8 nM, up to 0.6 $\mu$M in exposed workers: Nieboer, E.; Tom, R. T.; Sanford, W. E. *Met. Ions Biol. Syst.* **1988**, *23*, 91–121.

(9) Menné, T.; Nieboer, E. *Endeavour* **1989**, *13*, 117–122. $Ni^{2+}$-specific antibodies recognize an antigenic determinant formed by complexation of $Ni^{2+}$ to albumin: Dolovich, J.; Evans, S. L.; Nieboer, E. *Br. J. Indust. Med.* **1984**, *41*, 51–55.

(10) Peters, T., Jr. *Adv. Protein Chem.* **1985**, *37*, 161–245.

(11) Sadler, P. J.; Tucker, A. *Eur. J. Biochem.* **1992**, *205*, 631–643.

(12) Sadler, P. J.; Tucker, A.; Viles, J. H. *Proceedings of the Sixth Symposium of the Protein Society*; San Diego, CA, July 25–29, 1992; Abstract S145, p 71; manuscript in preparation.

(13) Patel, S. U.; Sadler, P. J.; Tucker, A.; Viles, J. H. *J. Cell Biochem.* **1993**, *17C*, 303.

(14) Resolution enhancement, see, e.g., Lindon, J. C.; Ferrige, A. G. *Prog. Nucl. Magn. Reson. Spectrosc.* 1981, *14*, 27–66. A combination of (unshifted) sine-bell and exponential functions (line-broadening, 2 Hz) achieves a reasonable compromise of signal-to-noise and resolution enhancement. Typical pulsing conditions: 65° pulses, spectra width 6 kHz, 16K data points, acquisition time 1.36 s, relaxation delay 2 s, 512 transients, 310 K. Dioxan was added an an internal reference (3.765 ppm relative to sodium 3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropanoate).

(15) Heparinized blood was obtained from a healthy human volunteer. The plasma was separated by centrifugation at 277 K, freeze-dried, and reconstituted in half the volume of 0.1 M deuteriated phosphate buffer pH* 7.0 (meter reading). Buffering is essential because the His $\epsilon$CH resonances are extremely sensitive to small pH changes around neutral pH. In blood, the main buffer system is $CO_2/HCO_3^-$, but this is difficult to handle *in vitro*. We have obtained similar spectra from fresh (non-freeze-dried) plasma.

(16) Bos, O. J. M.; Labro, J. F. A.; Fischer, M. J. E.; Wilting, J.; Janssen, L. H. M. *J. Biol. Chem.* **1989**, 264, 953–959.

(17) Amicon Centricon filter, <5 kDa. Average concentrations in plasma: His 89 $\mu$M; Phe 65 $\mu$M; Tyr 72 $\mu$M (ref 1a).

(18) Four aliquots of 4.7 $\mu$L, followed by one of 18.7 $\mu$L, of a 50 mM solution of $NiCl_2$ in $D_2O$ were added to plasma (0.7 mL, 2× concentrated, 1.38 mM albumin assumed), giving Ni:albumin ratios of 0.24, 0.48, 0.72, 0.95, and 1.84. The pH* was maintained at 7.0 by addition of NaOD solution as appropriate. The spectra were obtained within ca. 30 min of additions, and no attempt was made to study kinetics.

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**Table I.** $^1$H NMR Chemical Shifts for Ni–Albumin, Ni–Albumin Formed in Blood Plasma, and the $Ni^{2+}$ Complex of the 1–24 Peptide of Human Albumin

| proton | | Ni–albumin[a] | Ni–plasma[b] | Ni–[1–24]peptide[c] |
|---|---|---|---|---|
| Asp1 | αCH | 3.60 | 3.58 | 3.58 |
| | βCH$_2$ | 2.45 | 2.45 | 2.43 |
| | β'CH$_2$ | 2.60 | 2.59 | 2.60 |
| Ala2 | αCH | 3.63 | 3.65 | 3.68 |
| | βCH$_3$ | 1.27 | 1.25 | 1.30 |
| His3 | εCH | 7.47 | 7.48 | 7.56 |
| | δCH | 6.75 | 6.73 | 6.94[d] |
| Lys4(?) | δCH$_2$ | 1.50 | 1.52 | |
| | εCH$_2$ | 2.84 | 2.87 | |

[a] Reference 12, pH* 7.8. [b] This work, pH* 7.04. [c] Reference 19, pH* 8.0. [d] This shift is notably 0.2 ppm to low field of that for Ni–albumin, perhaps due to the lack of interaction with Lys.

and $\alpha/\beta'$ protons decrease in intensity, and new cross-peaks assignable to the same protons in the $Ni^{2+}$ complex appear, Figure 2. The cross-peak for Ala2 $\alpha/\beta$ protons is obscured by lipid peaks in the spectrum of plasma but can be assigned in the $Ni^{2+}$ complex, Figure 2 and Table I. Little change was observed in the high-field region of the spectrum (0.5 to -0.5 ppm) on addition of $Ni^{2+}$. No further increases in the intensities of peaks VI* and VII* occurred after addition of ca. 075 mol equiv $Ni^{2+}$ Figure D2, supplementary material), but only a general broadening of peaks was observed, attributable to the formation of paramagnetic $Ni^{2+}$ complexes.

These changes are consistent with the strong binding (slow exchange) of $Ni^{2+}$ to the N-terminus of albumin in blood plasma. Indeed, the shifts are similar to those for the $Ni^{2+}$ complex of the 1–24 peptide of human albumin.[19] The latter is a diamagnetic square-pyramidal complex with $Ni^{2+}$ bound to the N-terminal Aspl-Ala2-His3- residues via Asp $\alpha$-$NH_2$, two deprotonated-peptide nitrogens (Ala2 NH and His 3 NH), and the imidazole ring δN of His3, with weak axial coordination to the $CO_2^-$ group of Aspl. UV-visible and CD studies show that this site is similar in albumin and smaller model peptides.[20–22]

There are some curious differences between the binding of $Ni^{2+}$ to the 1–24 peptide of albumin and to isolated albumin which are also detected for blood plasma: a triplet at 2.94 ppm progressively decreases in intensity on addition of $Ni^{2+}$, and a new triplet appears at 2.87 ppm (supplementary material, Figures D2 and D3). Two-dimensional TOCSY spectra of isolated human albumin[12] allow assignment of the latter peaks to Lys $\epsilon CH_2$ protons ($\delta/\epsilon$ cross-peak at 1.52/2.87 ppm, Figure 2 and Table I; cross-peaks for β and γ protons are also resolved). No Lys resonances are perturbed on complexation of $Ni^{2+}$ to the 1–24 peptide.[19] We have previously suggested[12] that the NMR shifts of these Lys β, γ, δ, and ε protons for Ni–albumin are consistent with this side chain being positioned above the imidazole ring of coordinated His3, much as in the X-ray structure of [Cu(Gly-His-Lys)].[23] This structural feature may be of significance to the antigenicity of Ni–albumin. The Lys is most probably Lys4, although it could also be a Lys from a more distant part of the sequence.[24]

There is an apparent involvement of a second His residue (peak I, Figure 1) in $Ni^{2+}$ binding. This could arise from a weak axial interaction of $Ni^{2+}$ with a His residue distant in the sequence at the N-terminal site[24] or perhaps reflect population of a second site. Previous UV–visible and CD measurements on isolated albumin have suggested that an octahedral site can be partially populated with paramagnetic $Ni^{2+}$ at pH 7.[21,25] However, a definite assignment of peak I to albumin cannot be made.

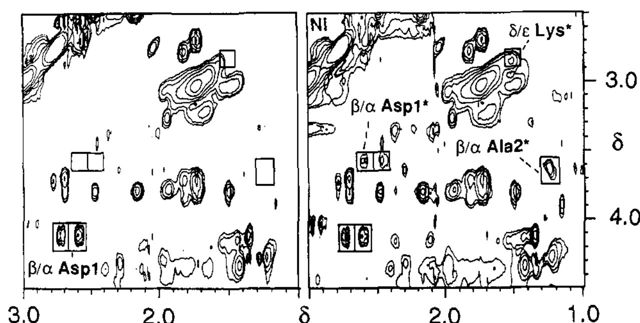


**Figure 2.** 500-MHz 2D TOCSY $^1$H NMR spectra (spin-lock time 35 ms) of human blood plasma, before (left) and after (right) the addition of $Ni^{2+}$ (ca. 0.5 mol. equiv with respect to albumin). New peaks (labeled with an asterisk) appear which are assignable to the N-terminal amino acid residues of Ni–albumin (Asp1-Ala2-) and a Lys residue. Very similar changes are observed when isolated defatted human albumin binds $Ni^{2+}$, Table I.

Notable also are the parallel decreases in intensities of peaks for both albumin (III, V, and possibly I) and free His (II, IV, supplementary material, Figure D2) in spectra of plasma as Ni–albumin is formed (VI* and VII*). Clearly L-His is a strong competitor for $Ni^{2+}$ binding in plasma and may play a critical role in regulating $Ni^{2+}$-induced structural changes in albumin, as has been noted previously,[21,25] although it is not yet possible to distinguish by NMR between the formation of $Ni^{2+}$–His complexes, which are paramagnetic (broad peaks), and ternary Ni–His–albumin complexes.

The ability to detect resonances for albumin in spectra of intact blood plasma has a wide range of potential applications, from studies of the kinetics of metal binding (e.g., $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, and $Cd^{2+}$) and ligand competition (including L-His and therapeutic chelating agents) to interactions with organic drugs and xenobiotics and investigations of abnormal albumins in various clinical states. The system will also be valuable for investigating how the chemistry of an isolated protein differs from that in its native biological medium, for example, the nature of the pH-induced N–B structural transition.[10]

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**Supplementary Material Available:** Figures showing the aromatic regions of 500-MHz $^1$H NMR spectra of albumin, plasma, and plasma ultrafiltrate (Figure D1), changes in peak heights of resonances of plasma affected by $Ni^{2+}$ addition Figure D2), and the effect of $Ni^{2+}$ on the 2.2–3.4-ppm region of the 1D $^1$H NMR spectrum of plasma Figure D3) (4 pages). Ordering information is given on any current masthead page.

(19) Laussac; J.-P.; Sarkar, B. *Biochemistry* **1984**, *23*, 2832–2838.
(20) Tsangaris, J. M.; Chang, J. W.; Martin, R. B. *Arch. Biochem. Biophys.* **1969**, *103*, 53–58.
(21) Glennon, J. D.; Sarkar, B. *Biochem. J.* **1982**, *203*, 15–23. Log(association constants): Ni–albumin 9.57, HSA-Ni-His 16.23; Ni(His) 8.57; Ni(His)$_2$ 15.57.
(22) E.g., [Cu(GGH-*N*-methyl amide)]: Camerman, N.; Camerman, A.; Sarkar, B. *Can. J. Chem.* **1967**, *54*, 1309–1316.
(23) Perkins, C. M.; Rose, N. J.; Weinstein, B.; Stenkamp, R. E.; Jensen, L. H.; Pickart, L. *Inorg. Chim. Acta.* **1984**, *82*, 93–99.
(24) Carter, D. C.; He, X.-M. *Nature* **1992**, *358*, 209–215. The N-terminus of crystalline albumin is highly structured beyond Lys4, unlike the 1–24 peptide in solution (ref 19).
(25) Laurie, S. H.; Pratt, D. E. *J. Inorg. Biochem.* **1986**, *28*, 431–439.