RESONANCE RAMAN SPECTROSCOPY OF IRON (III) - OVOTRANSFERRIN AND IRON (III) - HUMAN SERUM TRANSFERRIN

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SUMMARY We report the resonance Raman spectra in the frequency range 300-1800 cm^{-1} of Fe (III)-ovotransferrin and Fe (III)-human serum transferrin in aqueous solution at about 10^{-4}M protein concentration. This is the first observation of resonance Raman scattering ascribable to amino acid ligand vibrational modes of a nonheme iron protein. The resonance Raman spectra of the transferrins are similar except that the resonance band near 1270 cm^{-1} is shifted to a higher frequency for Fe(III)-human serum transferrin than that for Fe(III)-ovotransferrin. The resonance Raman bands observed near 1170, 1270, 1500 and 1600 cm^{-1} may reflect resonance enhancement of p-hydroxy-phenyl frequencies of tyrosine residues and/or imidazolium frequencies of histidine residues.

INTRODUCTION Resonance Raman (RR) spectroscopy (1) promises to be a sensitive technique for structural studies of metalloproteins as shown by recent reports of RR measurements on haemoglobin (2, 3, 4), cytochrome c (4, 5, 6), cytochrome c oxidase (7) and vitamin B_{12} and its derivatives (8, 9, 10). Long et al. (11, 12) observed RR bands for rubredoxin, a nonheme iron protein, at 368 and 314 cm^{-1} which they assigned to the antisymmetric and symmetric stretching modes, respectively,of the Fe-S_4 tetrahedron. No other Raman bands between 100 and 4000 cm^{-1} were observed for rubredoxin by those authors. Van Kreel et al. (13) reported frequency shifts at 300 and 360 cm^{-1} in the Raman spectrum of human serum transferrin-Fe(III)-HCO_3^- complex. However, these bands were not coupled with the visible absorption band at 460 nm so that they cannot be RR bands. Those authors observed no other Raman bands between 100-1400 cm^{-1} for the transferrin-Fe(III)-HCO_3^- complex.

Transferrins do not contain a prosthetic group (14) so that the chelated iron can be easily removed from the protein without causing any significant change in the structural integrity of the protein. Thus, comparison of the

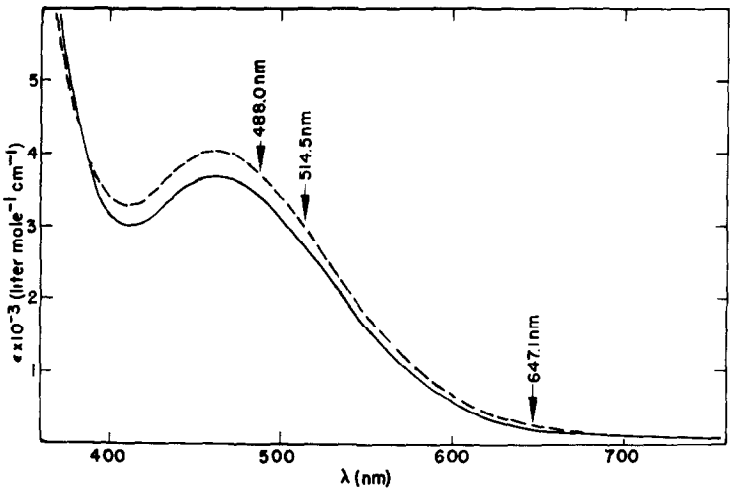

Figure 1 Absorption spectra of Fe(III)-ovotransferrin (---) and Fe(III)-Serum transferrin (___) in the 370-750 nm region. Spectra were measured with a Cary 15 recording spectrophotometer on aqueous solutions, pH 7, 1.31×10^{-4}M Fe(III)-ovotransferrin and 1.23×10^{-4}M Fe(III)-serum transferrin.

Raman spectrum of the iron chelate to that of the metal-free protein provides a convenient test for the presence of RR bands in the spectrum for Fe(III)-transferrin. We have obtained the Raman spectra of the iron chelates of ovotransferrin and human serum transferrin using 488.0, 514.5 and 647.1 nm as exciting wavelengths, all of which fall within the broad Fe(III)-transferrin absorption band centered at 460 nm. Four intense bands are observed near 1170, 1270, 1500 and 1600 cm^{-1} which are apparently due to resonance enhancement of vibrational modes of amino acid residues which serve as ligands for the metal-protein chelate.

EXPERIMENTAL* Chicken ovotransferrin (Lot No. 106B-8510) and human serum transferrin (Lot No. 121C-2730) were purchased from Sigma Chemical Company. Human serum transferrin, essentially free of iron contamination ($E^{1cm}_{1\%}$<0.01 at 460 nm), was used without further purification. Chicken ovotransferrin was purified on a cellulose exchange column (15). The iron chelates were prepared following the procedure of Warner and Weber (16). A Coherent Radiation 52B argon ion laser was used for 488.0 and 514.5 nm excitation, and a Spectra Physics 165 krypton ion laser was used for 647.1 nm excitation. Raman scattering at 90° to the incident beam was analyzed by a Spex 1401 double monochromator and detected by a RCA C31034 gallium-arsenide photomultiplier. Details of the data acquisition system and the procedure for correcting photomultiplier and optical efficiency have

* Reference to a company product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

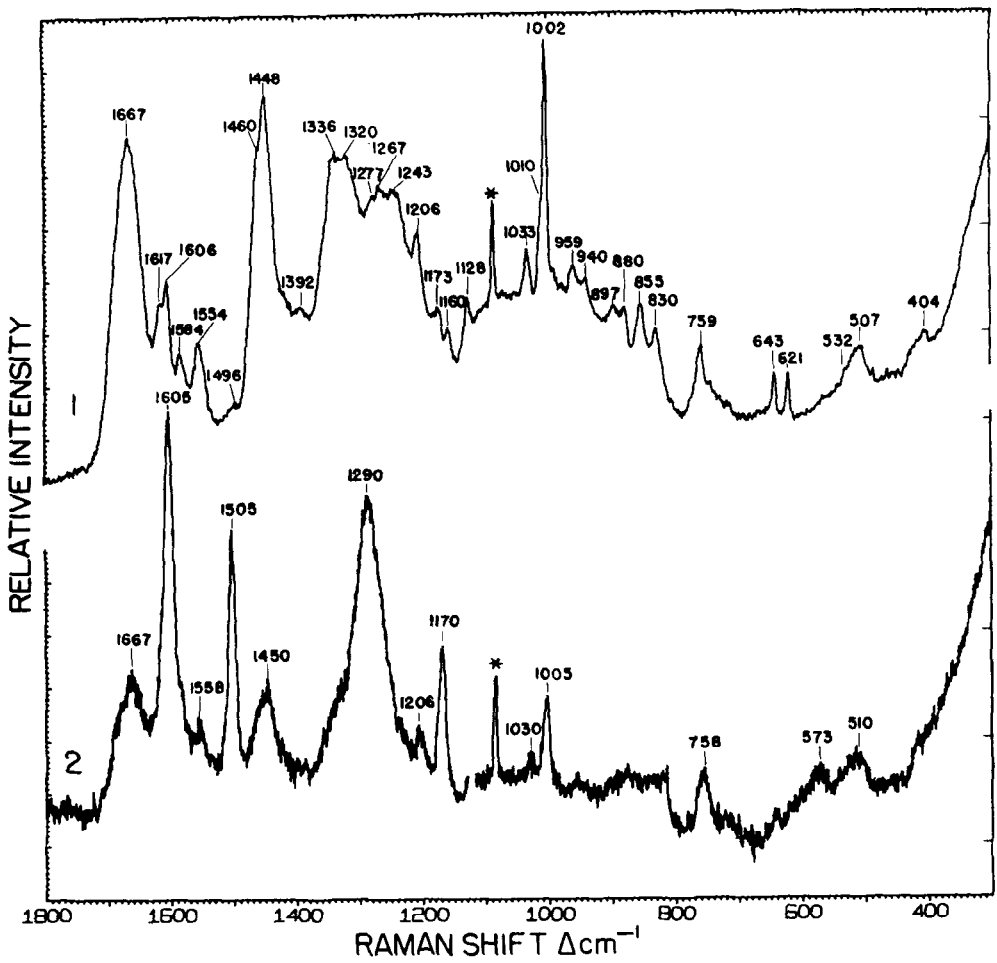

Figure 2 Raman spectra of solid ovotransferrin (curve 1) and solid Fe(III)-ovotransferrin (curve 2) in the 300-1800 cm^{-1} region. Spectra were measured at 25°C using 514.5 nm excitation. Starred (*) band in both curves is a calcium carbonate Raman line excited in the polarization scrambler plate at the entrance slit of the spectrometer. Instrumental conditions: curve 1, 360 mW power, 3 cm^{-1} slit width, 5 cm^{-1}/min scan rate, 3.3 sec time constant; curve 2, 100 mW power, 5 cm^{-1} slit width, 5 cm^{-1}/min scan rate, 3.3 sec time constant.

been reported (17). Solution Raman spectra were measured near 0°C in capillary cells of 0.25 mm inside diameter (18) and corrected for contributions due to water by subtraction of a pure water spectrum. Solid Raman spectra were measured at 25°C using a 180° back-scattering geometry (19).

RESULTS AND DISCUSSION The visible absorption spectra of Fe(III)-ovotransferrin and Fe(III)-serum transferrin shown in Figure 1 are similar. Both broad absorption bands peak at 460 nm and extend out to about 700 nm so that the 488.0,

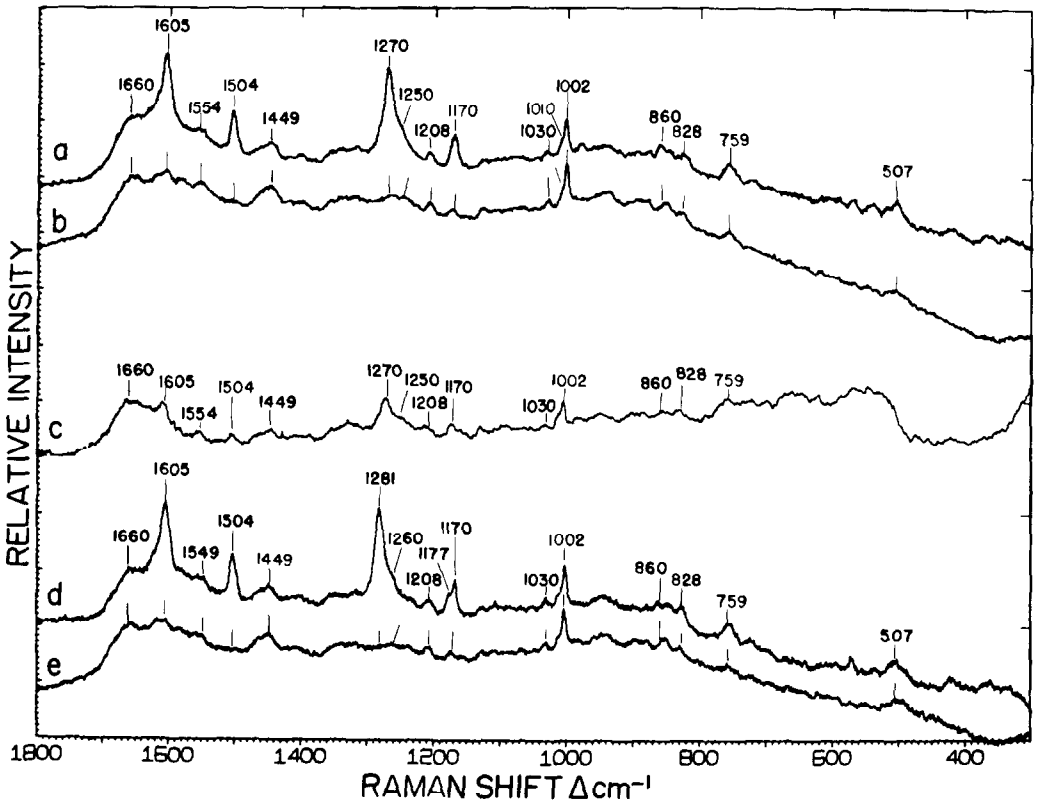

Figure 3 Solution Raman spectra of Fe(III)-ovotransferrin (curves a and c), ovotransferrin (curve b), Fe(III)-serum transferrin (curve d), and serum transferrin (curve e) near 0°C, pH 7. Experimental conditions:

Curve	Protein Conc. X10⁴(M)	Excitation (nm)	Power (mW)	Slit Width (cm^{-1})	Scan Rate (cm^{-1}/Min)	Time Constant (Sec)
a	1.01	488.0	150	4	5	10
b	1.27	488.0	250	4	5	10
c	1.01	647.1	200	4	5	3.3
d	1.23	488.0	200	4	5	10
e	1.03	488.0	200	4	5	10

514.5 and 647.1 nm laser excitations used fall within the electronic absorption band. Raman spectra of solid, metal-free ovotransferrin and its Fe(III) chelate are shown in Figure 2. The vibrational frequencies of ovotransferrin (curve 1) are listed in Table I, together with tentative assignments in terms of amino acid residues based on Raman data on other proteins (20, 21, 22) and on amino

Table I. Raman Spectrum of Solid Ovotransferrin (300-1800 cm^{-1})*

Frequency (Δcm^{-1})		Tentative Assignment	Frequency (Δcm^{-1})		Tentative Assignment
404	(1)	Skeletal bending	1173	(1)	Tyr
507	(2B)	ν(S-S)	1206	(2)	Phe-Tyr
532	(1s)	Skeletal bending	1243	(2)	Amide III
621	(2)	Phe	1267	(2)	
643	(1)	Tyr	1277	(1)	
759	(2)	Trp	1320	(4)	δCH
830	(1)	Tyr	1336	(4)	
855	(2)	Tyr	1392	(OB)	$\nu(coo^-)$ (Symmetrical)
880	(1)	Trp	1448	(10)	δCH_2
897	(0)	ν(C-C)	1460	(8s)	
940	(1)		1496	(OB)	Hist
959	(1)		1554	(2)	Trp
1002	(10S)	Phe	1584	(1)	Phe
1010	(3s)	Trp	1606	(3)	Phe
1033	(2)	Phe	1617	(2s)	Tyr-Trp
1128	(2)	ν(C-N)	1667	(10)	Amide I
1160	(1)				

*Numbers in ( ) are relative peak intensities based on a value of 10 for the strongest line in the spectrum (Figure 1). The symbol B denotes broad, S sharp, s shoulder, ν bond stretch, δ bond deformation, Tyr Tyrosine, Phe phenylalanine, Trp tryptophan and Hist histidine.

acids (23, 24). The spectrum for Fe(III)-ovotransferrin (curve 2) obtained using a laser power less than one-third of that used for the metal-free protein, shows intense bands at 1170, 1290, 1505 and 1605 cm^{-1} which appear to be superimposed upon a normal, but poorly defined ovotransferrin spectrum. The possibility that those four intense bands are resonance Raman bands is verified by the solution Raman spectra presented in Figure 3. The Fe(III)-ovotransferrin spectrum obtained with 488.0 nm excitation (curve a and Table II) shows intense peaks at 1002, 1170, 1270, 1504 and 1605 cm^{-1} and other less intense peaks and shoulders of which the broad band at 1449 cm^{-1} and shoulder at 1660 cm^{-1} are of particular interest. In the corresponding spectrum obtained with 647.1 nm excitation (curve c), the intensities of the peaks at 1170, 1270, 1504 and 1605 cm^{-1} are greatly reduced while those of the peak at 1002 cm^{-1}, the broad band at 1449 cm^{-1} and the shoulder at 1660 cm^{-1} remain virtually unchanged. The

Table II. Raman Spectrum of Fe(III)-Ovotransferrin in Water (300 - 1800 cm^{-1})

	Frequency (Δcm^{-1})		ρ‡	Tentative Assignment
	507	(3)		ν(S-S)
	759	(2)		Trp
	828	(1)		Tyr
	860	(2)		Tyr
	1002	(4)		Phe
	1010	(2s)		Trp
	1030	(1)		Phe
*	1170	(4)	.33	Tyr
	1208	(1)		Phe-Tyr
*	1250	(3s)		Hist-Tyr
*	1270	(10)	.22	Hist-Tyr
	1449	(2)		δCH_2
*	1504	(5)	.17	Hist
	1554	(1)		Trp
*	1605	(8)	.32	Tyr
	1660	(2s)		Amide I

‡ Depolarization defined such that a depolarized line has a value of 0.75.
* Resonance Raman bands.

spectrum for ovotransferrin (curve b) obtained with 488.0 nm excitation shows bands of comparable intensities with those of the iron chelate at 1002, 1449 and 1660 cm^{-1}, negligible intensities at 1270 and 1504 cm^{-1} and some indication of residual intensities at 1170 and 1605 cm^{-1}. The above observations may be summarized as follows: (1) Visual subtraction of the ovotransferrin spectrum (curve b) from that of its iron chelate (curve a) leaves only the intense bands at 1170, 1270, 1504 and 1605 cm^{-1}; (2) The large decrease in intensities of these four bands upon excitation with 647.1 nm line (curve c) is consistent with the expected behavior of RR lines, i.e., their intensities decrease as the wavelength of the exciting line becomes further removed from the wavelength of maximum absorption; and (3) The invariant intensity bands (which served as convenient markers in this work) in all three spectra (curves a, b, and c) at 1002, 1449, and 1660 cm^{-1} are normal Raman bands of high intensity, assigned to phenylalanine ring, CH_2 deformation and amide I vibrations, respectively (see Figure 1 and Table I).

The Raman spectra of human serum transferrin (Figure 3, curve e) and its iron chelate (curve d), are similar to those for ovotransferrin and its iron chelate (curves b and a) except that the 1270 cm^{-1} RR band of Fe(III)-ovotransferrin appears shifted to 1281 cm^{-1} in Fe(III)-serum transferrin and a shoulder appears at 1177 cm^{-1} in the Fe(III)-serum transferrin spectrum. We believe that these shifts indicate a difference between the binding sites of the two transferrins which is in accord with similar conclusions made from circular dichroism measurements (25) on the copper chelates of the two transferrins. The effect of 647.1 nm excitation (not shown) on Fe(III)-serum transferrin was similar to that observed for Fe(III)-ovotransferrin (curve c).

The considerable experimental evidence which implicate oxygen and nitrogen containing ligands at the iron binding sites of transferrins has been reviewed by Feeney and Komatsu (26). Since Fe-O and Fe-N vibrational bands are expected to appear at the low end of the frequency range covered in this work (27), the observed RR bands are probably associated with vibrational modes of the ligands themselves. Tyrosine, tryptophan and histidine residues are the most likely ligands. The following observations are based on the Raman data on these amino acids and locations of the p-hydroxy-phenyl, indole and imidazolium frequencies (23, 24): (1) The band at 1170 cm^{-1} is probably due to a p-hydroxy-phenyl vibration of tyrosine residues; (2) The band at 1504 cm^{-1} is most likely attributable to an imidazolium frequency of histidine although there is a possible contribution from tyrosine residues; (3) The band near 1270 cm^{-1} is probably due to contributions from both histidine and tyrosine residues. Note that the resonance band near 1270 cm^{-1} for both transferrins show a definite shoulder on the low frequency side; and (4) The band at 1605 cm^{-1} is probably due to tyrosine residues although there is a possibility of small contributions from the other two amino acids. Although definite assignments of each of the resonance bands to a particular vibrational mode of a particular amino acid residue will depend upon a study of suitable model systems, the preliminary results presented above show that resonance Raman is a promising technique for elucidating the binding sites of metal-protein complexes.

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