SPECTROSCOPIC EVIDENCE FOR PERTURBATION OF TRYPTOPHAN IN AL(III) AND GA(III) BINDING TO OVOTRANSFERRIN AND HUMAN SERUM TRANSFERRIN

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1. Introduction

Ultraviolet spectroscopy has been widely used to study metal binding to transferrins [1–8]. The observed difference spectra are usually interpreted in terms of changes in ionization of tyrosyl residue, although earlier work suggested perturbation [5] or direct involvement [3,9] of tryptophan in Fe(III) and Cu(II) binding to transferrins. But Fe– and Cu–transferrins have broad charge-transfer absorption bands in the near ultraviolet [5,10,11] which extend into the wavelength region in which the tyrosine and tryptophan chromophores absorb. This makes interpretation difficult. Difference spectra obtained at pH 11.0 have been used to correct for this background absorption of Fe–transferrins [2,4]. But, ionization of tyrosines may not be complete at pH 11.0 [1] and time-dependent exposure of 'buried' chromophores at alkaline pH [12] and alkaline hydrolysis of disulfide bonds [13] may further complicate determination of background absorption by this method.

Colorless transferrin metal complexes appear to be more suitable for difference spectral studies. Zn(II) [4,7] and trivalent lanthanides [6] give difference spectra which are similar to that for tyrosine ionization, with no apparent interfering absorption due to the metal ion. Ga(III) binding to ovotransferrin has been reported [8,14] to give a difference spectrum characteristic of tyrosine ionization. A difference was noted between the spectrum of the first Ga bound and that of the second [8]. We have measured, (i) the difference spectra produced by Zn, Ga and Al(III) binding to ovotransferrin and human serum transferrin, and (ii) the change in difference spectra produced by the addition of Zn to Al– and Ga–transferrins. We found that, (i) binding of Zn to transferrins gives difference spectra which are consistent with tyrosine ionization but binding of Ga and Al produced difference spectra characteristic of perturbation of tryptophan and tyrosine, and (ii) addition of Zn transforms Al– and Ga–transferrin difference spectra into that for the Zn complex. Thus, Zn competes with Al and Ga for the same binding sites, but not necessarily for the same ligands. These results suggest that tyrosine is not a ligand in Al and Ga binding to transferrins and that tryptophan is in or near the metal binding sites.

2. Experimental

Chicken ovotransferrin was purchased from Sigma Chemical Co. and purified on a CM-cellulose column [15]. Human serum transferrin, purchased from Behring Diagnostics (Batch No. 13471A), was used without further purification. The Cary 15 spectrophotometer was checked by the procedure of Mihalyi [16]. There were no stray light effects up to an absorbance of 3.6 (K_2CrO_4 solutions) and no fluorescence effects up to an absorbance of 2.0 (ovotransferrin solutions). Difference spectra were obtained at protein concentrations which gave an absorbance of less than 1.2 (0.5 nm band width) at the peak of the lowest wavelength difference band. Protein concentrations were determined at 280 and 278 nm for apo-ovotransferrin and apo-human serum transferrin, respectively, using an absorptivity of 85 800 liter mol^{-1} cm^{-1} for

both proteins. Concentrations (about 4×10^{-4} M metal ion) of stock metal solutions ($ZnSO_4$, $Al_2(SO_4)_3$ and $Ga_2(SO_4)_3$ in 10^{-3} N HCl) were determined by atomic absorption analyses. Difference spectra were obtained in 1 cm cells using the 0.1 absorbance scale, at a scan rate of 6 nm/min, after addition of microliter volumes of metal ion to the sample cell and the same volume of 10^{-3} N HCl to the reference cell. Data were corrected for dilution effects. Measurements made with tandem cells [17] gave similar results, so that for Zn–, Al– and Ga–transferrin, there is no absorption by complexes between metal and buffer. Complete titration curves ($\Delta\epsilon$ versus metal added, data not shown) were used to establish that the difference spectra presented were obtained at full saturation of the metal binding sites with Zn, Al and Ga.

3. Results and discussion

Difference spectra for tyrosine ionization and for perturbation of tyrosine and tryptophan are shown in fig.1. Difference spectra produced by Zn, Al and Ga binding to ovotransferrin and human serum transferrin are shown in fig.2. The locations and absorptivities of the more prominent difference bands are summarized in table 1. The shapes, locations of the difference bands, and the absorptivity ratio ($\Delta\epsilon_3/\Delta\epsilon_1$) of the shortest to the longest wavelength positive bands for the Zn–transferrins are similar to those for tyrosine ionization. However, small irregularities in the difference spectra between 275–287 nm, particularly for Zn–human serum transferrin, suggest that perturbation of tyrosine and tryptophan also occurs. The spectra for Al– and Ga–transferrins are distinctly different from Zn– . Positive bands appear at 287–291 nm, 281–282 nm and 237–241 nm and negative troughs appear at 305 nm and 252–257 nm. These appear to be characteristic of perturbation of tryptophan and of tyrosine, with the former readily

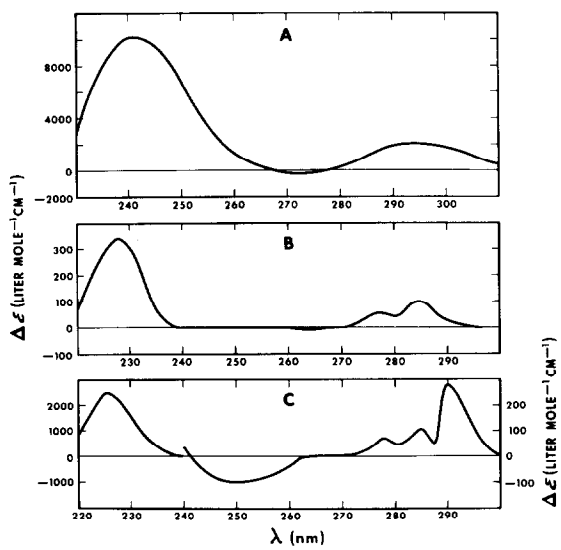

Fig.1. Ionization difference spectrum of *N*-acetyl tyrosine, pH 11.3 versus pH 7.6 (A) and perturbation difference spectra produced by 20% (v/v) ethylene glycol for tyrosine (B) and tryptophan (C).

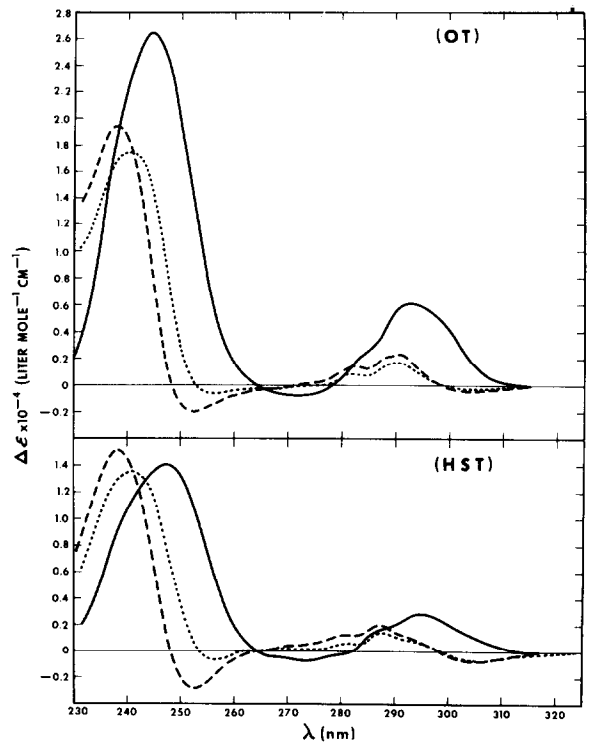

Fig.2. Difference spectra produced by Zn(II) (——), Al(III) (- - -) and Ga(III) (· · ·) binding to ovotransferrin (OT) and human serum transferrin (HST). Experimental conditions: pH 8.4, 0.025 M borate buffer containing 0.025 M KCl and 5×10^{-4} M $NaHCO_3$; transferrin concentrations between 5.6 and 6.4×10^{-6} M; and (Zn)/(OT) = 2.64, (Al)/(OT) = 3.08, (Ga)/(OT) = 2.58, (Zn)/(HST) = 2.45, (Al)/(HST) = 2.38, (Ga)/HST) = 2.34.

Table 1
Wavelength and molar absorptivities of the difference bands for tyrosine ionization, solvent perturbation of tyrosine and of tryptophan, and for metal binding to transferrins

Metal	λ_1	$\Delta\epsilon_1$	λ_2	$\Delta\epsilon_2$	$\Delta\epsilon_2/\Delta\epsilon_1$	λ_3	$\Delta\epsilon_3$	$\Delta\epsilon_3/\Delta\epsilon_1$	λ_4	$\Delta\epsilon_4$	λ_5	$\Delta\epsilon_5$
a	293	2067				242	10 175	4.9	273	− 218		
b	285	99	277	56		227.5	336	3.4				
c	290	278	282	181	0.65	225	2460	8.8	250	− 100		
	Ovotransferrin											
Al	291	2380	282	1510	0.63	238	19 380	8.1	252	−2010	305	−470
Ga	290	1750	282	960	0.55	237	16 020	9.2	257	− 610	305	−280
Zn	292	6090	283	2020		245	26 380	4.3	271	− 670		
	Human serum transferrin											
Al	287	2040	281	1300	0.64	238	15 080	7.4	252	−2840	305	−800
Ga	288	1470	281	620	0.42	241	13 530	9.2	257	− 620	305	−700
Zn	295	2940	287	1720		247	14 050	4.8	273	− 740		

[a]*N*-acetyl tyrosine ionization
[b]Tyrosine, 20% (v/v) ethylene glycol perturbation
[c]Tryptophan, 20% (v/v) ethylene glycol perturbation

recognizable near 292–295 nm in the case of human serum transferrin. The absorptivity ratio $\Delta\epsilon_3/\Delta\epsilon_1$ ranges from 7.4–9.2, similar to that observed for tryptophan perturbation (8.8), and considerably larger than that observed for tyrosine ionization (4.9) or for perturbation of tyrosine (3.4).

Zn displacement of Al from Al–ovotransferrin and of Ga from Ga–human serum transferrin is shown in fig.3. Similar results (not shown) were obtained for Zn displacement of Al in Al–human serum transferrin

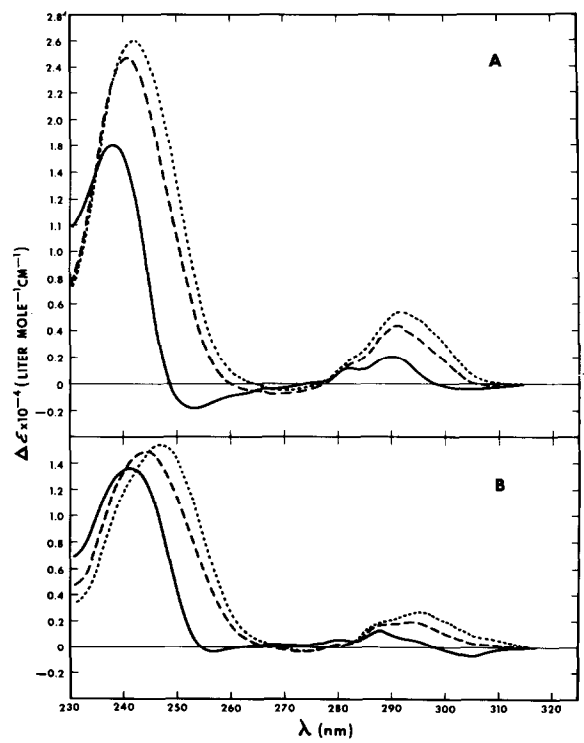

Fig.3. Zn(II) displacement of Al(III) in Al(III) ovotransferrin (A) and of Ga(III) in Ga(III) human serum transferrin (B). Experimental conditions: pH 8.4, 0.025 M borate buffer containing 0.025 M KCl and 5×10^{-4} M $NaHCO_3$, transferrin concentration between 6.0 and 6.4×10^{-6} M and

Metal(s)	Curve	(M)[a] (Transferrin)	(Zn) (Transferrin)
(A)Al	(——)	2.80	–
(A)Al, Zn	(- - - -)	2.80	1.32
(A)Al, Zn	(· · ·)	2.80	2.64
(B)Ga	(——)	2.34	–
(B)Ga, Zn	(- - - -)	2.34	1.22
(B)Ga, Zn	(· · ·)	2.34	2.45

[a](M) is concentration of Al for (A) and of Ga for (B).

and Ga in Ga–ovotransferrin. Addition of less than saturating amounts of Zn resulted in difference spectra intermediate between that for the metal being displaced and that for Zn–transferrin. Addition of more than saturating amounts of Zn gave difference spectra for Zn–transferrin (compare fig.2), indicating complete displacement of the original metal in the complex. These results indicate that Zn, Al and Ga occupy the same binding sites.

The tryptophan perturbation produced by Al and Ga binding to transferrin could be due to solvent or charge effects [18]. Perturbation difference spectra of proteins often show a negative trough near 300 nm, ascribed to the effects of nearby charged groups on tryptophyl residues [19]. The binding of Al and Ga most likely increases the net positive charge at the binding site. But the sign of the absorption change near 290 nm is not consistent with introduction of positive charge near tryptophan [20]. Furthermore, the appearance of the negative trough at 305 nm and the $\Delta\epsilon_2/\Delta\epsilon_1 > 0.5$ (table 1) suggest a solvent induced perturbation rather than a charge effect [21]. Accordingly, the difference bands are consistent with solvent perturbation (perhaps burial) of tryptophan. The $\Delta\epsilon_1$ for Al– and Ga–transferrins, 735–1190 (neglecting any perturbation of tyrosine and assuming perturbation of one tryptophan per metal atom bound to transferrin, table 1), is at the high end of the range of values observed for tryptophan [21,22]. $\Delta\epsilon_1$ for 'burying' a tryptophan is about 1600 [18], so that Al and Ga binding (possibly to carboxylates) may cause a crevice containing tryptophan to be closed. Chemical modification of tryptophan does not interfere with Fe binding to human serum transferrins [23], but this does not preclude tryptophan as a component of the binding sites.

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

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