COMPARATIVE CIRCULAR DICHROISM STUDIES OF IRON-FREE AND IRON-SATURATED FORMS OF HUMAN SEROTRANSFERRIN AND LACTOTRANSFERRIN

Joel MAZURIER, Jean-Pierre AUBERT*, Marie-Henriette LOUCHEUX-LEFEVRE* and Geneviève SPIK

*Laboratoire de Chimie Biologique, Université des Sciences et Techniques de Lille I et Laboratoire Associé au C.N.R.S. n° 217, B. P. n° 36, 59650 - Villeneuve d'Ascq, France and *Institut de Recherches sur le Cancer (Institut Jules Driessens) et Unité 124 de l'I.N.S.E.R.M., B. P. n° 3567, 59020 - Lille Cédex, France*

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1. Introduction

Since 1971, circular dichroism studies of chicken ovotransferrin [1] and of human serotransferrin [2] have been carried out. A comparative circular dichroism study of human serotransferrin and chicken ovotransferrin and their copper complexes [3] suggests that some dissimilarities may exist between the metal-binding sites of the transferrins. As the iron complex formed by lactotransferrin is known to be more stable than that of serotransferrin especially at low pH [4] we have undertaken a comparative circular dichroism study of human serotransferrin and lactotransferrin and their iron complexes over the wavelength range of 180–800 nm. Additional comparative circular dichroism study was done in the presence of increasing molarity of guanidine chloride in order to understand the participation of iron binding to protein conformation.

The obtained data show that (i) there is no difference in the α-helical content of apo and iron lactotransferrin complex, (ii) the value of α-helical content (28%) obtained for apolactotransferrin is greater than this one of aposerotransferrin (17%) determined either from our results or by Nagy and Lehrer [2], (iii) the extrinsic dichroic bands of lactotransferrin are twice more intense than these ones of serotransferrin, (iv) specific iron binding in sero and lactotransferrin induces three new dichroic bands at 305, 330 and 465 nm, (v) addition of increasing amount of guanidine chloride is followed in sero- and lactotransferrin by a synchronous decrease of $[\theta]305$ and $[\theta]465$ which can be explained by the loss of the interaction of tryptophan residues with iron binding sites, (vi) in the presence of guanidine chloride, iron removal from lactotransferrin immediately induces a decrease of $[\theta]220$ although for serotransferrin $[\theta]220$ remains constant till one iron atom is bound to the protein.

2. Materials and methods

Human lactotransferrin was isolated from milk [5] and human serotransferrin was prepared as described in [6]. Their apoderivatives were obtained according to [7]. The iron-transferrin complexes were formed by the addition of $FeCl_3$ in 0.2 M citrate and bicarbonate buffer pH 8.6 [8] or by the addition of Fe^{3+}-nitrilotriacetic acid [9]. Iron content was determined with ferrozine reagent [10] and protein concentration was measured spectrophotometrically by using $E^{1\%}_{280}$ of 11.2, 14, 11 and 14.3 for apo- and iron-serotransferrin, apo- and iron-lactotransferrin, respectively [7] or by the method of Lowry [11]. Guanidine chloride was prepared from carbonate according to [12] and twice cristalllized in ethanol–water (6:4, v/v). Transferrins were dissolved in 0.214 M NaF at a concentration of 0.1%. Absorption spectra were obtained with Cary model 118-C spectrophotometer with cells of 5 and 20 mm pathlength. The CD spectra were recorded with a Jobin-Yvon Dichrograph R. J. Mark III in 0.1 and 5 mm path-length cells for ultraviolet and visible region at an absorbance less than 1.5 optical density unit. For all calculations a mean residue weight of 112 was

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used. The ellipticity was expressed as mean residual molar ellipticity $[\theta]$ in degrees·cm^{-2}·dmol^{-1}.

3. Results and discussion

3.1. *Transferrins in non-denaturing solution*

The shapes of the intrinsic dichroic spectra are different for sero- and lactotransferrin (fig.1) but the far ultraviolet CD spectra from 180–250 nm of apolactotransferrin and its iron complex are identical and show two well distinct negative extrema at 208 and 220 nm with identical ellipticity. These results differ from those obtained from serotransferrin as the CD spectra of aposerotransferrin and its iron

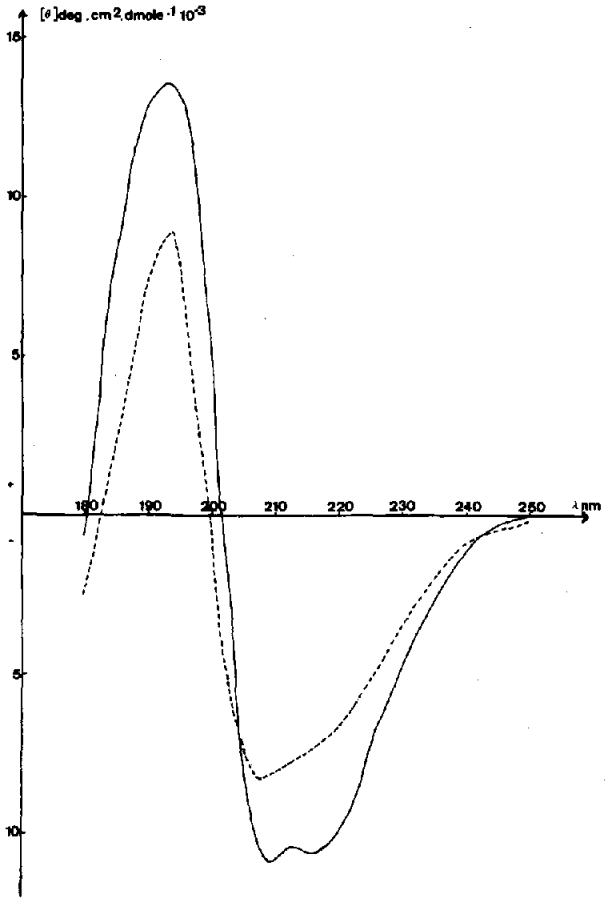

Fig.1. Circular dichroism spectra of iron lactotransferrin (—) and of iron serotransferrin (- - - -) between 180 and 250 nm.

Table 1
Percentage of α-helix and β-sheet in iron-saturated and iron-free human serotransferrin and lactotransferrin determined by circular dichroism[a]

	α-Helix	β-Sheet[b]
Serotransferrin		
iron-saturated	23	62
iron-free	17	68
Lactotransferrin		
iron-saturated	26	57
iron-free	28	64

[a] Since the content of *N*-acetylglucosamine and *N*-acetylneuraminic acid residues in both transferrins is low [15], their optical activity was neglected.

[b] Computer evaluation of the β-sheet content may not lead to correct values consequently the above data may be discussed.

complex show a negative extremum around 208 nm and a shoulder near 220 nm. Identical results were obtained by [3,13]. Application of the equation of Chen, Yang and Martinez [14] allows one to determine the percentage of α-helix and β-sheet in both iron-containing and iron-free transferrins (table 1).

The CD spectra for aposero- and apolactotransferrin from 245 to 350 nm are given in fig.2. For both apotransferrins the CD spectra are composed of bands centered around 250, 283 and 291 nm superimposed on a broad negative band at about 276 nm. The $[\theta]$ at 283 and 291 available for tyrosine and tryptophan residues and the broad band at 276 nm probably due to disulfide bridges and to phenylalanine residues are 3 and 2.5 times, respectively, more intense in apolactotransferrin than in aposerotransferrin. Since no major differences exist in amino acid composition of the two transferrins [15] the greatest magnitude of the apolactotransferrin CD spectra is probably due to a stronger organization. The negative band near 252 nm for apolactotransferrin is shifted to 256 nm for aposerotransferrin.

Specific iron binding in both transferrins induces three additional dichroic bands at 305, 325 and 456 nm and a decrease of the magnitude of the ellipticity of all the other bands (fig.2). The well defined 305 nm dichroic band is probably induced by tryptophan residues which have a disturbed electronic environment. The dichroic band at 325 nm may be

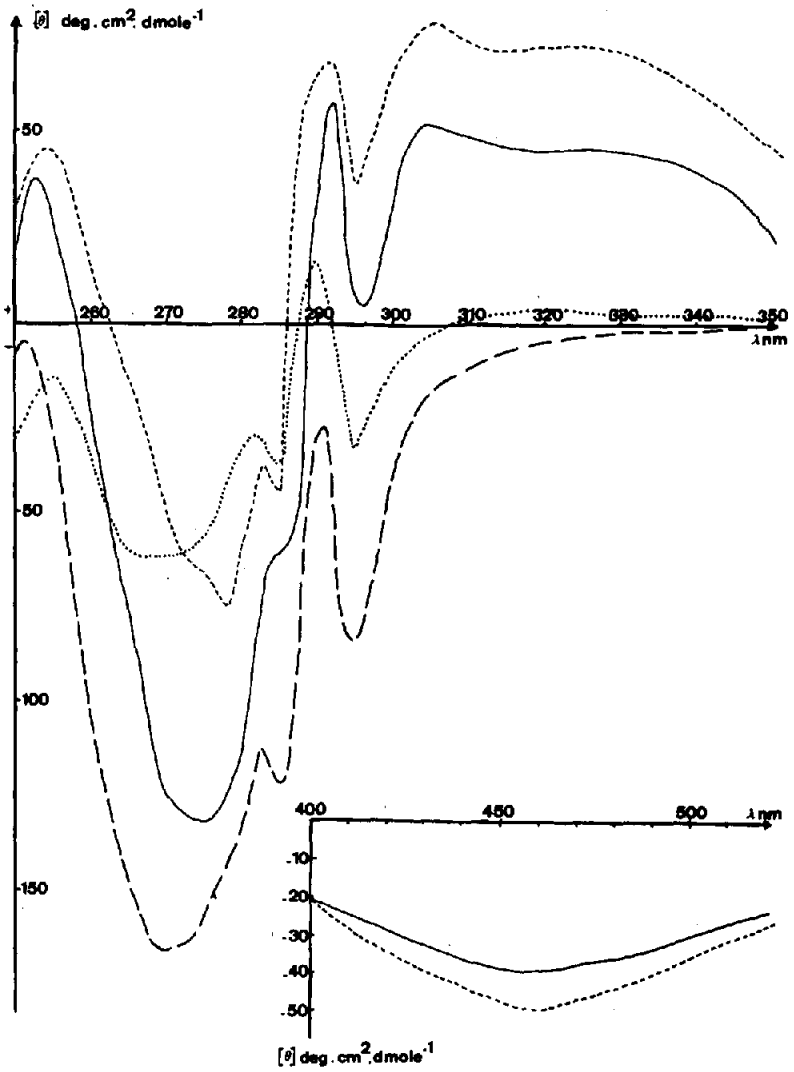

Fig.2. Circular dichroism spectra between 250–350 nm and 400–500 nm of iron lactotransferrin (——), iron-free lactotransferrin (– –), iron serotransferrin (- - -), iron-free serotransferrin (. . . .).

due to the tail of the band at 465 nm or, may be, as suggested by Tan [1] for ovotransferrin to the change in dihedral angle of at least one disulfide bridge upon iron binding.

3.2. *Transferrins in denaturing solution*

The CD spectra of iron sero- and lactotransferrin and their apo derivatives have been recorded in NaF solution in the presence of increasing molarity of guanidine chloride from 1 to 6 M. In the presence of 2.2 to 3.7 M, the decrease of the values of $[\theta]220$ for iron serotransferrin appears to be linear (fig.3A), however increasing molarity above 3.7 M does not introduce any additional modification of the value of $[\theta]220$. As the value of $[\theta]220$ of aposerotransferrin remains constant only in the presence of 6 M guanidine chloride, the secondary structure of aposerotransferrin appears to be more resistant upon denaturation.

Reverse phenomenon is observed for lactotrans-

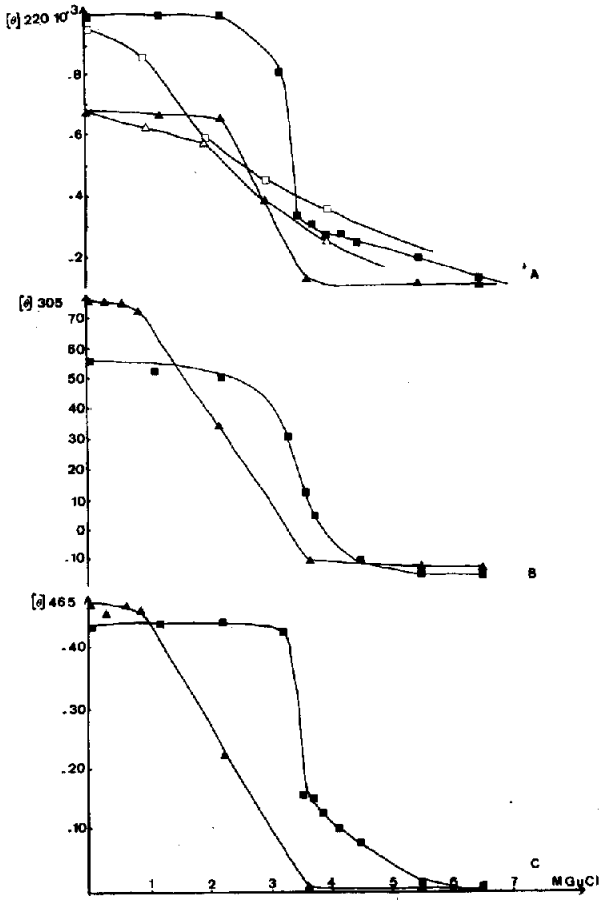

Fig.3. Ellipticity at different wavelengths versus the molar concentration of guanidine chloride. [θ] in deg cm^2 dmol^{-1}, (■) iron lactotransferrin, (□) iron-free lactotransferrin, (▲) iron serotransferrin, (△) iron-free serotransferrin.

ferrin since the ironlactotransferrin complex appears to be more stable in the presence of guanidine chloride.

Similar results were obtained [16] by ORD studies in the presence of urea.

For iron-lactotransferrin, the loss of the perturbation of tryptophan residues measured at [θ]305 (fig.3B) and the loss of iron binding ability determined at [θ]465 (fig.3C) strictly follow the evolution of [θ]220. Such a parallelism is not observed for iron-serotransferrin, since the evolution of the values [θ]305 and [θ]465 are identical and differ from the evolution of the values of [θ]220. The ellipticity of this latter band remains constant till 2.2 M guanidine chloride, when the values of [θ]305 and [θ]465 are reduced to their half values.

So, the removal of one iron atom in serotransferrin does not affect the secondary structure, but, the removal of the second atom occurs simultaneously with the unfolding of the protein.

In the presence of guanidine chloride 1 to 6 M the behaviour of [θ] at 220, 305 and 465 nm for iron-ovotransferrin appears to be similar to the one observed for iron-serotransferrin.

4. Conclusion

These data demonstrate that the secondary structure is lactotransferrin is different from that of serotransferrin since its α-helix content is greater. Moreover, the greatest ellipticity of extrinsic dichroic bands for lactotransferrin may be explained by stronger interactions between the side chains chromophores or by structural differences in the specific iron-binding sites. Specific addition of two iron atoms in lacto- and serotransferrin does not affect the secondary structure and among the newly appeared CD bands, one at 305 nm is characteristic of perturbed tryptophan residues and, may be, of small local conformation changes around these tryptophan. This result suggests that tryptophan may be involved in iron binding sites, which is in a good agreement with the data obtained from difference spectral studies [19].

It is interesting to compare the results we have obtained from a dichroic study of ovotransferrin which show that both ovo- and serotransferrin give exactly the same dichroic spectra either in non-denaturating or in denaturating conditions. Therefore it seems, on the one hand, that the two iron binding sites of iron sero- and ovotransferrin and on the other hand, or iron lactotransferrin do not have the same behaviour. In iron sero- and ovotransferrin one of the iron binding sites, which can be removed without alteration of the secondary structure may be located near the outer surface of the molecule.

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