Preparation and Characterization of the Vanadium(III) Derivative of Transferrin

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Introduction

It is well known that a typical property of the serum protein transferrin is its capability of binding '*in vitro*' tripositive metal ions [1, 2]. Metals which can experience different oxidation numbers are bound as tripositive cations, and the formed derivatives display a very high stability [3]; for instance, the manganese(II) ion in solution in presence of apotransferrin, binds the protein experiencing fast oxidation to manganese(III) [3]. Analogously, cobalt(II) is easily oxidized to cobalt(III) by stoichiometric amounts of hydrogen peroxide in the metal sites of transferrin [3], cobalt(III)transferrin being the stable derivative. We have recently found that transferrin can also bind the thallium(III) ion [4]; such species in solution are strong oxidizing agents, the reduction potential for the semi-reaction $Tl^{3+} + 2e^- \rightleftharpoons Tl^+$ being 1.25 V.

On the basis of these findings, we thought that transferrin binding sites could be able to stabilize other tripositive cations, which otherwise would be highly reactive with respect to oxidation or reduction. This is for instance the case of the vanadium(III) cation, whose water solutions are quite unstable in the presence of oxygen, the tripositive cation being oxidized to oxovanadium(IV) ($E\ VO^{2+}/V^{3+} = 0.36$ V); the same kind of reactivity is experienced in solution by vanadium(III) complexes, the oxovanadium(IV) compounds being generally more stable than the vanadium(III) analogues [5].

Oxovanadium(IV) is known to bind transferrin, as it has been used as an EPR probe [6–8] to monitor the different properties of the two metal binding sites; the derivative can be easily prepared through stoichiometric addition of oxovanadium(IV) ions to apotransferrin solutions in the presence of synergistic anions; it is, however, air unstable and has to be handled and preserved in a nitrogen atmosphere [6]. We report here the preparation of an air stable vanadium(III)–transferrin derivative with bicarbonate as synergistic anion.

Experimental

Iron-free transferrin, purchased from the Sigma Chemical Company and purified according to described methods [9], was catalyzed against 0.1 *M* Tris·HCl or 0.1 *M* MES buffer solutions. The protein concentration was determined by measuring the absorbance at 228 nm, using a molar extinction coefficient of 92500 for the metal free protein [8]. The oxovanadium(IV) transferrin derivative was prepared as previously reported [6]. The electronic spectra in the visible and UV region were recorded on a Cary 17D spectrophotometer. CD measurements were performed on a JASCO J500C spectropolarimeter.

Results and Discussion

Addition of vanadium trichloride to water solutions of apotransferrin in 0.1 *M* Tris·HCl, 0.05 *M* $NaHCO_3$ pH 7.8 gives rise to the formation of a light green solution: its electronc spectrum shows an absorption at 15,700 cm^{-1} (ϵ = 130 M^{-1} cm^{-1} per protein unit), which is consistent with the presence of vanadium(III) species (Fig. 1); there is also evidence of shoulders at about 22,500 and 27,000 cm^{-1}, the latter showing an intensity of about 2,000 M^{-1} cm^{-1}. The intensity of the bands proportionally increases with the addition of VCl_3 up to a stoichiometric 2/1 metal/protein ratio. No increase in the overall intensity of the spectrum is observed upon further addition of vanadium(III) ions, suggesting that a specific divanadium(III) transferrin derivative is formed. The above results are confirmed by UV difference spectroscopy measurements, a commonly used technique for checking the formation of specific derivatives between metal ions and siderophilins [10–12]. No increase in the intensity of the absorption band at 41,300 cm^{-1}, caused by the absorption of tyrosinate residues bound to the metal ion in the active site, is observed in vanadium(III) transferrin solutions with respect to apotransferrin solutions at the same experimental conditions upon further addition of vanadium(III) ions, once the 2/1 metal/protein ratio is reached.

The CD spectrum of the vanadium(III)–transferrin derivative, which is shown in Fig. 1, is more resolved than the electronic spectrum and can therefore be helpful in the characterization of the electronic properties of this derivative. It shows two negatively polarized bands at 15,700 cm^{-1} and 26,300 cm^{-1}, and a positively polarized band at 22,500 cm^{-1}. Octahedral vanadium(III) complexes are expected to give rise to three electronic transi-

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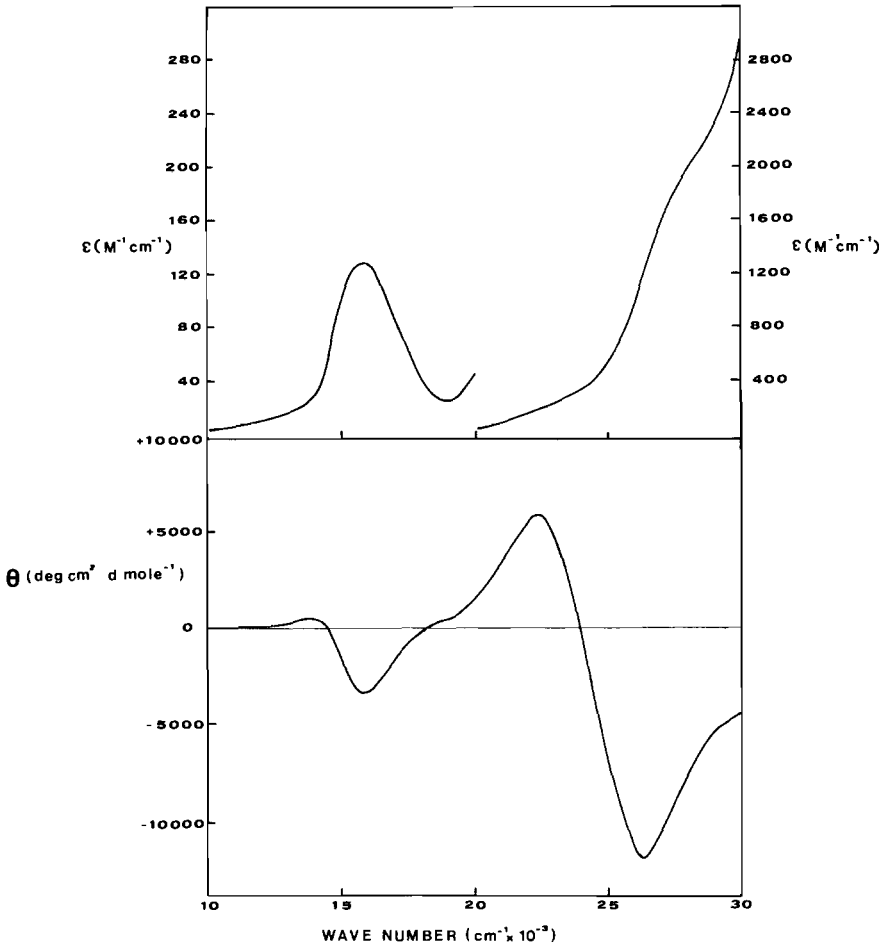

Fig. 1. Electronic (upper) and C.D. (lower) spectra of the divanadium(III) derivatives of transferrin in Tris·HCl 0.1 *M*, $NaHCO_3$ 0.05 *M*, pH 7.8. Molar absorbances and ellipticities are referred to protein unit.

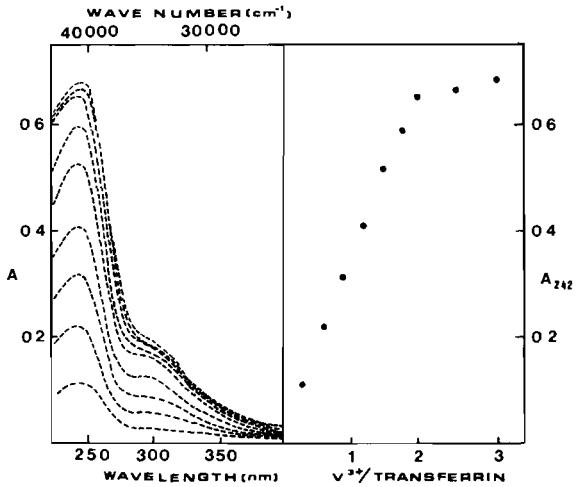

Fig. 2. Difference UV spectra of 3 ml apotransferrin solutions 2.05×10^{-5} *M*, Tris·HCl 0.1 *M*, $NaHCO_3$ 0.1 *M*, pH 8.3 upon stepwise addition of VCl_3 solutions (left), and variation of the absorbance at 242 nm as function of the metal/apoprotein ratio (right).

tions assigned as $^3T_{1g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), $^3T_{1g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_2) and $^3T_{1g}(F) \rightarrow {}^3A_{2g}(P)$ $(\nu_3)^5$, if the bands at 15,700 and 22,500 cm^{-1} are assigned as ν_1 and ν_2 in the present divanadium(III)–transferrin complex, simple calculations [13] yield the ligand field splitting parameter 10 Dq = 16.8×10^3 cm^{-1} and the Racah parameter B = 0.53×10^3 cm^{-1}, both reasonable values. From the above values the ν_3 transition is expected to be at 32.5×10^3 cm^{-1}. The latter transition would be hard to detect, since it is expected to display lower intensity than ν_1 and ν_2 and should be covered by the high absorptions present in the UV region. The relatively high intensity of the d–d transitions is indicative of a low symmetry chromophore.

On the ground of its intensity, the band at about 27,000 cm^{-1} can be assigned as a charge transfer transition, indeed, metal(III) transferrin derivatives show absorptions of intensity of the order 10^3–10^4 M^{-1} cm^{-1} [1, 3], which are assigned as tyrosinate to metal charge transfer transitions [14]. Such

to metal charge transfer transitions [14]. Such bands have been recently analyzed in terms of optical electronegativities [14], and found to be perfectly consistent with the above assignment. By using 2.8 as the value of the ligand electonegativity (as proposed for several transferrin derivatives [14]) and that for vanadium(III) of 2.1 [15] as well as the B value previously determined, the energy of the charge band is predicted to be at 39.5×10^3 cm^{-1}. Indeed the shape of the difference absorption spectrum in the UV region indicates that a further intense band probably lies below the tyrosinate bands at 33,900 and 41,300 cm^{-1}, which are typical of all the metallotransferrins [10]. If the above analysis is correct, then two charge transfer bands are present in the vanadium(III) transferrin derivative, *i.e.* at 27,000 and 40,000 cm^{-1}. Two intense bands have also been found in the manganese(III) derivative [3].

The vanadium(III) transferrin has also been characterized with respect to reactivity. The studies performed have shown that it is quite stable: the metal is not removed from the protein binding sites through exhaustive dialysis against water or buffer solutions, nor is substituted by iron (at least within several days) when the latter ion is added as iron nitrilotriacetate or iron oxalate to vanadium(III)–protein solutions. Vanadium(III) can be removed only with the typical procedures through chelating agents used for iron removal from the native transferrin. The most interesting properties are, however, those related to redox stability. The vanadium(III) ion in the derivative is stable towards oxidation by atmospheric oxygen, and no change in the spectral properties have been observed after air exposure for a month. Moreover, if the reducing agent dithionite is added to oxovanadium(IV) transferrin solutions, a rapid development of the vanadium(III) transferrin spectrum is observed, indicating that the oxovanadium(IV) ion is reduced into the transferrin binding sites and the vanadium(III) derivative is formed. For instance for a 10^{-3} *M* solution of oxovanadium(IV)–transferrin, complete reduction is observed in the presence of 5×10^{-2} M dithionite ions within three hours. Addition of strong oxidizing agents, such as hydrogen peroxide or peroxodisulfate ions to the vanadium(III) transferrin, does not cause any change in the spectral properties of this derivative, indicating that (at variance with the other vanadium(III) compounds) the protein derivative is totally stable towards oxidation.

Finally, if vanadium(III) is allowed to react with apotransferrin at pH = 6.0, only one metal ion is taken by the apoprotein. This is consistent with the pH dependent affinity of the binding sites of transferrin for metal ions [16].

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