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Preparation and properties of an Fe(III)-complex with an Amadori compound derived from L-tyrosine

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The complexation of Fe(III) with an Amadori compound derived from L-tyrosine was studied. The isolated complex was characterized by elemental analyses, Fourier transform infrared (FTIR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Analyses indicate that the ligand is coordinated through the amino and carboxylate groups of the tyrosine part of the molecule.

Keywords: Amadori, complex, Fe(III), glycation, tyrosine

Introduction

Nonenzymatic glycation of proteins consists of a series of reactions including, in the first step, condensation of the acyclic form of a sugar with the reactive amino groups of protein molecules (Ledl & Schleicher 1990). The early steps in this reaction assume that the Schiff base resulting from the initial sugar attachment undergoes a rearrangement to yield a more stable but still chemically reversible Amadori product. These products slowly degrade at the sites of sugar binding to form complex advanced glycation end products (AGE) which may cause the denaturation, polymerization and cross-linking of tissue proteins *in vivo* (Brownlee 1995).

It has been reported (Namiki & Kato 1986, Ando & Morooka 1988) that the active oxygen species generated from Amadori compounds under physiological conditions induced cleavage of nucleic acid and inactivation of viruses. In addition, it was clarified that the oxidative damage of some glycated proteins was produced directly by Cu(II)-catalysed autooxidation of the corresponding Amadori compounds (Cheng *et al*. 1991). Furthermore, increased glycation of haemoglobin in the presence of Fe(II) ions as well as generation of superoxide during the oxidation of glycated polylysine in the presence of iron–ADP have been reported (Sakurai *et al*. 1990, Ricart *et al*. 1993). It was suggested that the oxidative reaction was perhaps generated via the Amadori compound–iron complex (Sakurai *et al*. 1990).

Whereas the chemistry of biological systems involving glycation of proteins is extremely complex, the reactions involving amino acids can be delineated with well-defined Amadori compounds. In spite of the biological relevance of these monomeric compounds, only a few studies have been conducted to investigate their complexation with transition metal ions (Cheng & Kawakishi 1993, Gyurcsik *et al*. 1993). In order to obtain a better insight into the complexation behaviour of these compounds, in the present study we have chosen *N*-(1-deoxy-D-fructos-1-yl)L-tyrosine (DFT, Figure 1) as the starting Amadori compound; the new Fe(III)–DFT complex was then isolated and characterized.

The ability of Fe(III) to form complexes with amino acids and sugars or sugar-like compounds is of considerable interest in view of the role that these complexes may have in the transport of iron across cell membranes (Charley *et al*. 1963, Saltman 1965). The complexes of Fe(III) with amino acids and various kinds of sugars and polyols have been characterized by molecular weight determination, Mössbauer and infrared spectroscopy, EPR spec-

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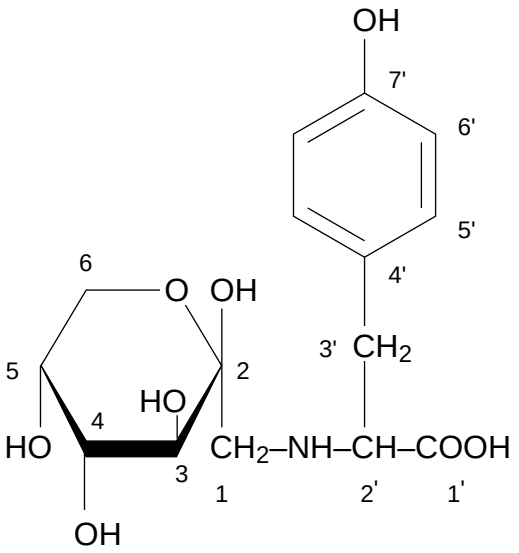

Figure 1. *N*-(1-deoxy-D-fructos-1-yl)L-tyrosine (DFT).

troscopy, extended X-ray absorption fine structure spectroscopy (EXAFS) and ^{13}C NMR spectroscopy (Puri & Asplund 1981, 1982, Tonković *et al*. 1982, 1983, Burger *et al*. 1983, Nagy *et al*. 1986, 1989 Tonković 1994). The compounds of Fe(III) with more complex molecules such as peptidoglycan monomers, or with amino acid glycosides are also described (Tonković *et al*. 1989, 1990). Solid complexes of Cu(II), Pd(II) and Pt(II) with Amadori compounds were isolated and characterized by elemental analyses and infrared spectroscopy (Chen *et al*. 1989).

Materials and methods

Ligand DFT was prepared according to published procedure (Röper *et al*. 1983). The product was purified on a Varian 9010 HPLC system with a Eurospher 100 reversed-phase C-18 (5 μm), semipreparative column (250 × 8 mm) using 15% methanol in 0.1% trifluoroacetic acid.

All other reagents used were of analytical grade. For the Job's method of continuous variations (Bauman 1962) the Fe(III) stock solution (0.1 mol dm^{-3} in 0.1 mol dm^{-3} HCl) was prepared from Fe(III) chloride hexahydrate (Merck, Germany). The solution was standardized by the titrimetric method using Titriplex III (Merck) and sulfosalicyclic acid as indicator. The DFT solution was prepared by direct weighing. KCl–HCl buffer (pH 3) was prepared by mixing 0.5 ml of 0.2 mol dm^{-3} HCl with 25 ml of 0.2 mol dm^{-3} KCl solution and addition of water to 100 ml. Aliquots comprising 0–5.0 ml of 5×10^{-3} mol dm^{-3} DFT and 5.0–0 ml of 5×10^{-3} mol dm^{-3} $FeCl_3$ (to give a total volume of 5.0 ml) were mixed in 10 ml volumetric flasks; this was followed by addition of 3 ml of KCl–HCl buffer (pH 3) and bidistilled water to the mark. After shaking for 5 min the absorbance was measured at 390 nm in 1.00 cm light-path silica cuvettes against a blank prepared in the same way but without the ligand.

Spectrophotometric measurements were carried out with a Perkin Elmer spectrophotometer model 124.

Solid Fe(III)–DFT complex was prepared by mixing the methanolic solutions of Fe(III) nitrate and ligand in a molar ratio of 1:3 with a slight excess of ligand. The solution was stirred for 48 h and the small quantity of undissolved residue was removed by filtration. To this solution the same volume of n-hexane was added; the brown oil was separated in the bottom of the flask. After centrifugation the solvent was removed and the residue was evaporated and dried in a desiccator over silica gel.

The composition of the solid was determined by standard microanalytical methods. The Fe(III) content was determined by atomic absorption spectroscopy; the sodium content was measured using flame photometer. The deduced formula for the Fe(III)–DFT complex is $Fe(C_{15}H_{20}NO_8)_3(NaNO_3)$. Anal. calc.: C, 46.26; H, 5.18; N, 4.80; Fe, 4.79; Na, 1.97; O, 37.00%. Found: C, 46.78; H, 6.42; N, 5.28; Fe, 4.28; Na, 1.40%.

The FTIR spectra were recorded on a Perkin Elmer 2000 spectrometer coupled with a PC IR Data Manager program using KBr techniques. Figure 3 shows the FTIR spectrum of ligand DFT and the difference spectrum Fe(III)–DFT versus $NaNO_3$.

The ^{13}C NMR spectra of DFT and the Fe(III)–DFT complex after dissolution in D_2O were measured on a Varian Gemini 300 Fourier transform spectrometer at room temperature in 5 mm outer diameter (O.D.) tubes at 75 MHz. The spectral width was 19 000 Hz, pulse width 6.0 μs. Chemical shifts were measured relative to that of internal standard 1,4-dioxane set at 67.4 ppm downfield from that of Me_4Si.

Results and discussion

Solutions containing a mixture of Fe(III) and DFT have an absorbance maximum at 390 nm. The measurements at 390 nm were performed using a series of solutions with changing Fe(III) and DFT molar ratios. The plot of the absorbance versus DFT mole fraction X, where X = [DFT] / [DFT] + [Fe(III)] shows the maximum at X = 0.75 (Figure 2), indicating that Fe(III) forms a complex with DFT in the stoichiometric ratio of Fe(III):DFT = 1:3. On the basis of this result the complex was prepared as described and one molecule of $NaNO_3$ was found to be bonded to Fe(III)–DFT. Sodium in solution originated as the impurity in the ligand.

The ^{13}C NMR chemical shifts of the ligand DFT and its complex with Fe(III) are tabulated in Table 1. The chemical shifts are assigned according

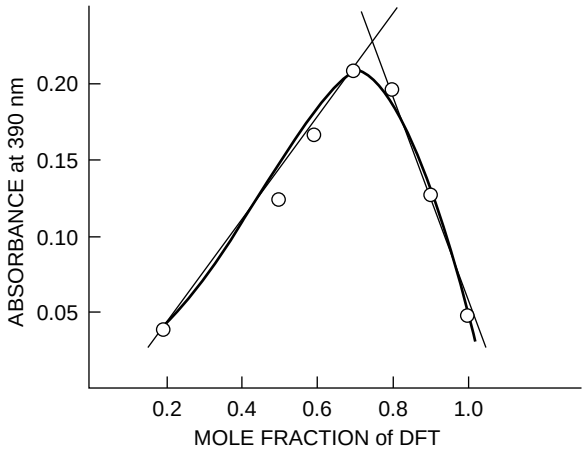

Figure 2. Determination of the composition of the Fe(III)–DFT complex by Job's method.

Table 1. ^{13}C NMR chemical shifts (ppm) for DFT and Fe(III)–DFT

Carbon	DFT[a]	Fe(III)–DFT
C-1	53.44	53.90
C-2	95.94	95.91
C-3	71.03	71.18
C-4	70.07	70.02
C-5	69.65	69.60
C-6	64.69	64.69
C-1′	171.83	–
C-2′	63.11	61.90
C-3′	35.07	35.73
C-4′	126.25	127.44
C-5′	131.63	131.68
C-6′	116.75	116.72
C-7′	155.98	155.79

[a] β-D-Pyranose form.

to the data reported earlier (Röper *et al*. 1983). The aqueous solution of DFT contains 73% β-pyranose, 14% α-furanose and 13% β-furanose. The ^{13}C NMR spectrum of Fe(III)–DFT in D_2O) was measured over a long period amounting to the accumulation of 36 000 transients. The NMR spectra were taken at comparable pD: 4.26 and 3.83 for DFT and Fe(III)–DFT, respectively, measured according to Covington *et al*. (1968). This would mean that the observed differences in the NMR spectra of the ligand and its complex with Fe(III) are not a pH effect of protonation/deprotonation of the carboxylate group and secondary amine which could influence ^{13}C-resonances of C-1, C-2′, C-3′ and C-4′ atoms; the shifts of these are essential points in the interpretation of the data. From the data in Table 1 it is evident that Fe(III) forms a complex with DFT in its β-pyranose form. The presence of the Fe(III) ion causes an upfield shift on the C-2′ atom of the tyrosine moiety in DFT ($\Delta\delta$ – 1.21 pm), whereas on C-1, C-3′ and C-4′ atoms a deshielding effect was observed. The data obtained indicate that the complex formation involves the amino acid moiety of the Amadori compound. It is interesting that the signal of the carboxylate group is missing from the spectrum of the Fe(III)–DFT complex; a similar effect was observed in the complexes of Fe(III) with bleomycin and tallysomicin, and with a peptidoglycan monomer (Dabrowiak *et al*. 1979, Tonković *et al*. 1989).

The FTIR spectra (Figure 3) of the DFT ligand and its complex with Fe(III) exhibit broad bands at 3350 cm^{-1} originating from O–H stretching vibrations caused by the hydroxy groups of the fructose part of the ligand. This band remains unchanged by complexation. In the range 3000–2300 cm^{-1} the spectrum of DFT displays several bands which can be assigned to C–H and N–H stretching vibrations, but it is difficult to differentiate them. The bands are shifted or disappear from the spectrum of the complex. The band of the carbonyl or normal carboxyl group in the range 1740–1700 cm^{-1} is present in the spectrum of the ligand only as a weak shoulder. This implies that the amino acid part is present mainly in the zwitterion state in the solid and that the fructose part is not present in its open chain form in a considerable amount. The band at 1740 cm^{-1} appears in the spectrum of the Fe(III)–DFT complex. The appearance of such a band in the infrared spectra of Pd(II) and Pt(II) complexes with Amadori compounds was mentioned by Chen *et al*. (1989) and was attributed to carbonyl absorption due to the open chain form of the ligand in these complexes. It appears, therefore, that the open chain form of DFT is present in the solid Fe (III) complex. The asymmetric and symmetric modes of vibration of the carboxylic group at 1617 and 1392 cm^{-1} in the free ligand are shifted to 1615 and 1385 cm^{-1}, respectively in the complex showing the difference of 230 cm^{-1}. The separation between the asymmetric and symmetric frequencies suggests monodentate coordination of carboxylate group (Nakamoto 1978). The band at 1522 cm^{-1} in the ligand assigned to N–H deformation motion is shifted to 1517 cm^{-1} in the complex, indicating complexation through the amino group (Koegel *et al*. 1955). The bands in the region 1020–1260 cm^{-1} assigned to $-CH_2$, –COH, –CCH bending vibrations,

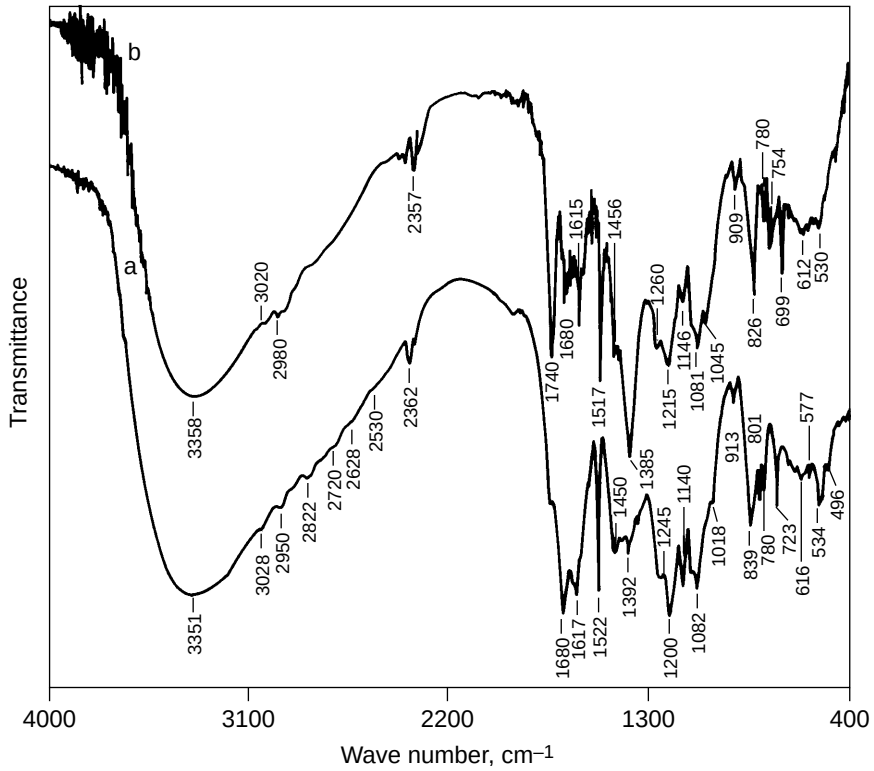

Figure 3. FTIR spectra taken in KBr pressed pellets: (a) DFT; (b) difference spectrum Fe(III)–DFT versus $NaNO_3$.

C–O stretching and O–H bending vibrations are shifted and weakened upon complexation (Williams & Fleming 1973).

Different analyses of the Fe(III)–DFT complex suggest that Fe(III) coordination involves the carboxylate group and nitrogen atom from the tyrosine part, while the fructose part of the Amadori compound does not participate in complex formation. In such a way the octahedral coordination geometry of Fe(III) can be achieved. The analogous coordination through the carboxylate group and nitrogen atom of the amino group was proposed in the complexes of Amadori compounds with Pd(II) and Pt(II) (Chen *et al.* 1989).

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