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Catechol releases iron(III) from ferritin by direct chelation without iron(II) production†

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It has been traditionally considered that catechols release iron from ferritin by reduction to iron(II), which diffuses through the ferritin channels into the intracellular milieu where it participates in the Fenton reaction, producing highly toxic hydroxyl radicals. However, in the present work we have proved that the mechanism of the release of iron from ferritin by catechol does not take place by iron(II) reduction but by direct iron(III) chelation and therefore without iron(II) production. A possible extension of these findings to other catechols is discussed on the basis of the stability with respect to the internal redox reaction of the iron(III)–catechol complexes.

Introduction

Iron is an essential element for living organisms but is highly toxic in excess. Living organisms store iron to provide an appropriate concentration and at the same time to protect themselves against the toxic effects of iron excess. The major intracellular storage form of iron is ferritin, a spherical protein composed of 24 subunits that surround an aqueous cavity capable of accommodating up to 4500 iron atoms as a ferrihydrite iron(III) core.[1–3]

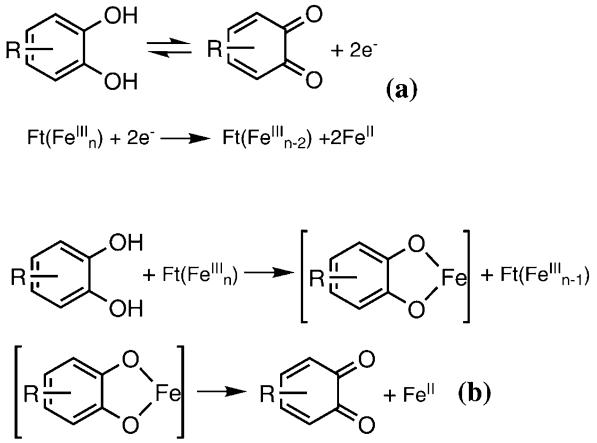
Iron release from ferritin has been extensively studied with the aim of knowing how the iron is mobilized from the ferritin store when required by the cell and furthermore because of its possible role in oxidative stress and in the progression of neurodegenerative diseases.[4,5] In this sense, it is important to consider the fact that increased concentrations of iron have been observed in brain tissue in several neurodegenerative diseases and that most brain iron is stored in the form of ferritin.[4]

Two mechanisms are chemically feasible for removing iron from ferritin: reduction followed by iron(II) mobilization[6] or direct iron(III) chelation.[7] The difference between these two mechanisms becomes crucial *in vivo* because iron, in the free ferrous form, is known to interact with peroxide, *via* Fenton's reaction (eqn. (1)),

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad (1)$$

to produce hydroxyl radicals that are extremely powerful oxidizing agents capable of causing extensive damage to cells. Obviously, this reaction does not take place with chelated iron(III).

It has been traditionally considered that polyphenols and in particular catechols mobilize iron from ferritin by iron reduction, this being the reason for their neurotoxicity.[8] Two mechanistic scenarios have been conceived: for catechols larger than the ferritin channels it has been considered that the redox reaction Fe(III)–Fe(II) could occur by electron tunnelling, without any interaction between catechol and the iron core surface (Scheme 1a).[9] For catechols small enough to traverse the ferritin channels, the reaction takes place in the ferritin cavity, giving rise to iron(II) and quinone (Scheme 1b).[10] This second route has been rigorously shown to occur when 6-hydroxydopamine reacts with ferritin. Both mechanisms 1a and 1b lead to the mobilization of iron(II), which finally diffuses through the ferritin channels into the intracellular milieu where it is able to interact in free radical-producing processes.



Scheme 1

However, we have analyzed the reaction between ferritin and catechol, both in aerobic and anaerobic conditions, and we can conclude that catechol releases iron from ferritin by direct iron(III) chelation and consequently without iron(II) production. The extension of this behaviour to other catechols is discussed on the basis of the stability of their iron(III)–catechol complexes.

Experimental

Horse spleen ferritin (76 mg ml^{-1}, 2200 iron per ferritin) and catechol were purchased from Sigma-Aldrich. Aqueous solutions were prepared using water purified through the Milli-Q system. Ferritin (0.19 mg ml^{-1}, 0.39 mM in iron) in 0.15 M NaCl was incubated at room temperature with catechol at three different concentrations (1.87, 2.81 and 3.75 mM) and varying the pH of the experiment using different buffers: 5.2, 6.8, 7.4 (0.1 M acetate 5.2, 50 mM HEPES 6.8 and 0.1 M TRIS 7.4, 0.1 M NaCl). Blank experiments were performed at the same conditions in the absence of ferritin. All the experiments were also carried out in anaerobic conditions in a glove box and the solutions transferred to screw-cap quartz cuvettes. The development of the iron(III)–catechol complexes were followed by UV-vis spectroscopy using a Thermospectronic UV300 spectrophotometer against reference solutions containing appropriate amounts of buffer-saline and ferritin in the range 400–900 nm. Cycles of 10 minutes were recorded until a total of 20.

† Electronic supplementary information (ESI) available: Spectral changes accompanying iron removal from ferritin at different catechol concentrations. See http://www.rsc.org/suppdata/dt/b4/b416669h/

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Results and discussion

The study of iron release from ferritin by catechols has been traditionally carried out in the presence of an external iron(II) chelator, usually ferrozine, which acts as an indicator of the iron(II) released.[8,11] However, it has already been pointed out that ferrozine disturbs these studies[12] and therefore, to avoid misleading conclusions, the ideal experiment should be carried out in the absence of ferrozine or any other iron(II) chelating indicator.[13] In this regard, Linert *et al.* studied the release of iron from ferritin by 6-hydroxydopamine in the absence of ferrozine, monitoring the reaction spectrophotometrically by the oxidation of 6-hydroxydopamine to quinone.[10] With the same aim, we have studied the reaction between catechol and ferritin without addition of an iron(II) chelator. This was made possible by following with UV-Vis spectroscopy the direct formation of the catechol–iron(III) complex corresponding to the pH at which the reaction was performed.

Catechols produce strongly coloured complexes with iron(III).[13] This colour arises from a strong ligand-to-metal charge transfer band. Typically, catechols form three different iron(III) complexes as a function of the pH.[13] In particular, aqueous solutions of the catechol (cat) adjusted to pH 6–7 give rise to the bis(catecolate)iron(III) complex $\{Fe(cat)_2\}$, which exhibits a UV-vis maximum at 576 nm. Alternately, the tris(catecolate)iron(III) complex $[Fe(cat)_3]^{3-}$, with a maximum at 483 nm, is obtained at pH > 9.5. A third species, a mono(catecolate)iron(III) complex {Fe(cat)}, can be produced at pH < 5 with a $\lambda_{max} = 700$ nm. In general, the stability of the iron(III)–catecholate complexes depends on the catechol ligand, the extent of the co-ordination to Fe(III) and pH. Thus, in the case of the catechol, the bis- and tris-(catecolate)iron(III) are stable whereas {Fe(cat)} undergoes anaerobic internal electron transfer to produce quinone and iron(II) but only at low pH. This intramolecular redox process occurs because of the similarity in the Fe^{III}/Fe^{II} and quinone/catechol reduction potentials. Catechols with lower reduction potential undergo this decomposition at physiological pH and in extreme cases, as for 6-hydroxydopamine, the reaction with iron(III) gives rise directly to iron(II) and quinone, without the prior formation of an iron(III) complex.[14]

Fig. 1 shows the time dependent release of iron from ferritin in the presence of catechol at pH 7.4 in anaerobic conditions and monitored spectrophotometrically by the formation of the catechol–iron(III) complex. At pH 7.4, the predominant catechol–iron(III) species is $\{Fe(cat)_2\}$ with a small amount of $[Fe(cat)_3]^{3-}$.[13] The electronic spectra exhibit a maximum at 540 nm, which, as expected, is closer to that typically observed for $\{Fe(cat)_2\}$of 576 nm than for $[Fe(cat)_3]^{3-}$ of 483.[13]

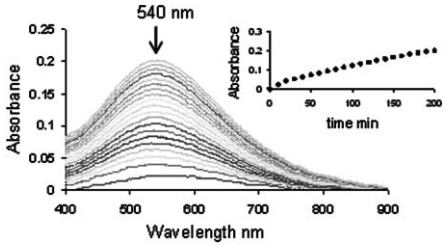

Fig. 1 Spectral changes accompanying iron removal from ferritin by catechol. Anaerobic conditions: [ferritin] = 0.19 mg ml^{-1}, [catechol] = 3.75 mM, pH 7.4, 25 °C. Each spectrum was taken at successive 10 min intervals after the start of reaction. Inset: plot of A^{540} as a function of time.

When the reaction was performed at pH 6.8, the maximum was observed at 576 nm (Fig. 2), which corresponds to the expected value for $\{Fe(cat)_2\}$, the only species at this pH.[13]

If finally the pH is lowered to 5.2, the absorbance values significantly decrease and the maximum of the electronic spectra moves to 610 nm (Fig. 3). The spectra correspond to a mixture

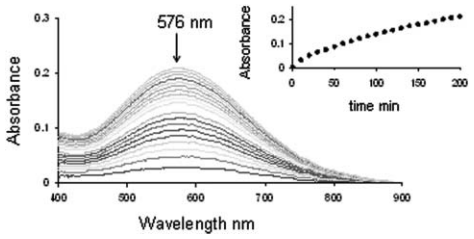

Fig. 2 Spectral changes accompanying iron removal from ferritin by catechol. Anaerobic conditions: [ferritin] = 0.19 mg ml^{-1}, [catechol] = 3.75 mM, pH 6.8, 25 °C. Each spectrum was taken at successive 10 min intervals after the start of reaction. Inset: plot of A^{576} as a function of time.

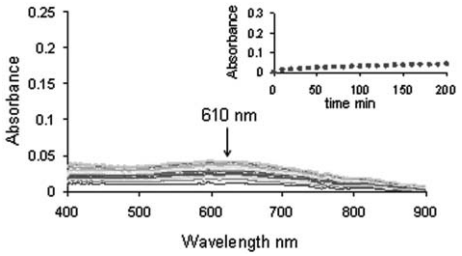

Fig. 3 Spectral changes accompanying iron removal from ferritin by catechol. Anaerobic conditions: [ferritin] = 0.19 mg ml^{-1}, [catechol] = 3.75 mM, pH 5.2, 25 °C. Each spectrum was taken at successive 10 min intervals after the start of reaction. Inset: plot of A^{610} as a function of time.

of $\{Fe(cat)_2\}$ with a small amount of {Fe(cat)} ($\lambda_{max} = 700$ nm), again according to the pH at which the reaction was monitored. The low absorbance values of the UV-vis spectra at this pH are a direct consequence of the lower molar extinction coefficient ε of the {Fe(cat)} complex ($\varepsilon^{700} = 1000$ M^{-1} cm^{-1}) than those of $\{Fe(cat)_2\}$ ($\varepsilon^{576} = 2900$ M^{-1} cm^{-1}) and $[Fe(cat)_3]^{3-}$ ($\varepsilon^{483} = 3700$ M^{-1} cm^{-1}).[13]

It is interesting to note that under the anaerobic conditions in which the experiments were carried out, no oxidation of catechol was observed in the blank experiments, which only contained catechol in buffer (see Experimental section).

From the above results, it can be established that catechol releases iron from ferritin by direct chelation through the formation of an iron(III)–catecholate complex, and its stoichiometry depends on the pH at which the reaction is performed. Furthermore, because the complexes $\{Fe(cat)_2\}$, $[Fe(cat)_3]^{3-}$ and {Fe(cat)} are stable at physiological pHs, no iron(II) is produced and therefore the full iron release from ferritin by catechol does not mobilize free iron(II) but chelated iron(III).

From the absorbance value of 0.209 at 576 nm of the spectrum of Fig. 2, and considering an extinction coefficient of 2900 M^{-1} cm^{-1} for $\{Fe(cat)_2\}$ at 576 nm,[13] a concentration of 7.21×10^{-5} M can be calculated. Taking into account the initial concentration of iron in the ferritin solution of 3.93×10^{-4} M, it can be concluded that a 18.35% of iron has been removed as $\{Fe^{III}(cat)_2\}$after 200 min.

The trend of the release of iron from ferritin by catechol did not change with the catechol concentrations used (1.87, 2.81 and 3.75 mM). The amount of iron removed was ligand concentration-dependent, although the increase was not linear with the concentration of catechol (see ESI†).

If the reaction is carried out in aerobic conditions, the time dependant release of iron from ferritin does not significantly change (Fig. 4). The electronic spectra show a slight shift of the maximum ($\lambda = 535$ nm) and higher absorbance values ($A^{535} = 0.317$). The slight difference in the spectra must be a consequence of the aerobic oxidation of catechol to quinone. In view of this, the conclusions drawn for the ferritin iron removal promoted by catechol in anaerobic conditions can be extended to aerobic ones.

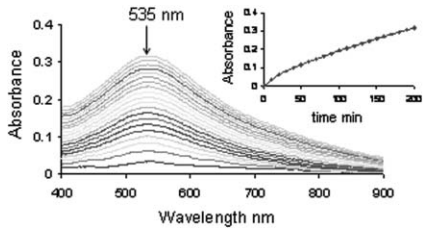

Fig. 4 Spectral changes accompanying iron removal from ferritin by catechol. Aerobic conditions: [ferritin] = 0.19 mg ml^{-1}, [catechol] = 3.75 mM, pH 7.4, 25 °C. Each spectrum was taken at successive 10 min intervals after the start of reaction. Inset: plot of A^{535} as a function of time.

In light of these results we can conclude (i) first, that reduction of iron within the ferritin cavity is not a pre-requisite of its release, in contrast to the usual assumption. In fact, ferritin iron removal by direct iron(III) chelation was previously observed for a series of bidentate hydroxypyridinone and hydroxamate molecules.[7,15] (ii) Second, the first step in the release of iron from ferritin by catechols capable of penetrating the ferritin core involves the formation of the catechol–iron(III) complex corresponding to the pH at which the reaction is carried out. If this complex is stable, as occurs in the case of catechol, the process of iron removal from ferritin takes place through direct iron(III) chelation and consequently without iron(II) mobilization. However, if the iron(III)–catechol complex undergoes an internal redox reaction giving rise to iron(II) and quinone, as occurs in the case of other catechols, such as 6-hydroxydopamine,[14] the release of iron from ferritin produces iron(II). Therefore the stability of the iron(III)–catechol formed determines whether the release of iron from ferritin produces free iron(II) or chelated iron(III), which is crucial for the *in vivo* effects of catechols.

Acknowledgements

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References

1 G. C. Ford, P. M. Harrison, D. W. Rice, J. M. A. Smith, A. Treffery, J. L. White and J. Yariv, *Philos. Trans. R. Soc. London, Ser. B*, 1984, **304**, 551–565.
2 P. M. Harrison, S. C. Andrews, P. J. Artymuik, G. C. Ford, J. R. Guest, J. Hirzmann, D. M. Lawson, J. C. Livingstone, J. M. A. Smith, A. Treffery and S. J. Yewdall, *Adv. Inorg. Chem.*, 1991, **36**, 449–486.
3 A. K. Powell, in *Metal Ions in Biological Systems*, ed. A. Sigel and H. Sigel, Marcel Dekker, New York, 1998, vol. 35, pp. 515–561 and refs. therein.
4 M. Gerlach, D. Ben-Shachar, P. Riederer and M. B. H. Youdim, *J. Neurochem.*, 1994, **63**, 793–807.
5 (*a*) K. Jellinger, W. Paulus, I. Grundke-Iqbal, P. Riederer and M. B. H. Youdim, *J. Neural Transm. Parkinson's Dis. Dementia Sect.*, 1990, **2**, 327–340; (*b*) W. Linert and G. N. L. Jameson, *J. Inorg. Biochem.*, 2000, **79**, 319–326.
6 N. D. Chasteen, *J. Biol. Chem.*, 1982, **257**, 7672.
7 R. C. Hider, and A. D. Hall, in *Perspectives on Bioinorganic Chemistry*, ed. R. W. Hay, J. R. Dilworth and K. B. Nolan, 1991, vol. 1, pp. 209–254.
8 K. L. Double, M. Maywald, M. Schmittel, P. Riederer and M. Gerlach, *J. Neurochem.*, 1998, **70**, 2492–2499.
9 (*a*) R. F. Boyer, H. M. Clark and A. P. LaRoche, *J. Inorg. Biochem.*, 1988, **32**, 171–181; (*b*) G. D. Watt, D. Jacobs and R. B. Frankel, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7457–7461.
10 G. N. L. Jameson, R. F. Jameson and W. Linert, *Org. Biomol. Chem.*, 2004, **2**, 2346–2351.
11 (*a*) H. P. Monteiro and C. C. Winterbourn, *Biochem. Pharmacol.*, 1989, **38**, 4177–4182; (*b*) M. J. Hynes and M. O. Coinceanainn, *J. Inorg. Biochem.*, 2002, **90**, 18–21; (*c*) S. Ahmad, V. Singh and G. S. Rao, *Chem.-Biol. Interactions*, 1995, **96**, 103–111; (*d*) R. Agrawal, P. K. Sharma and G. S. Rao, *Toxicology*, 2001, **168**, 223–230.
12 J. P. Laulhere and M. Fontecave, *Biometals*, 1996, **9**, 10–14.
13 M. J. Sever and J. J. Wilker, *Dalton Trans.*, 2004, 1061–1072 and refs. therein.
14 G. N. L. Jameson and W. Linert, *J. Chem. Soc., Perkin Trans. 2*, 2001, 569–575.
15 N. Gálvez, B. Ruiz, R. Cuesta, E. Colacio and J. M. Domínguez-Vera, submitted for publication.