Biologically active thiosemicarbazone Fe chelators and their reactions with ferrioxamine B and ferric EDTA; a kinetic study†

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The FeIII abstraction from FeIII/DFO and FeIII/EDTA complex systems by thiosemicarbazone ligands derived from 2-acylpyridine has been studied from a kinetico-mechanistic perspective at relevant pH conditions and at varying temperatures and buffer solutions. The reactions have been found to be extremely dependent on the dominant E/Z isomeric form of the TSC ligands present in the reaction medium. Consequently the isomerisation processes occurring on the free ligands have also been monitored under equivalent conditions. The isomerisation process is found to be acid dependent, despite the absence of protonation under the conditions used, and presumably proceeds via an azo-type tautomer of the ligand. In all cases the existence of outer-sphere interaction processes has been established, both promoting the reactions and producing dead-end complexes. The better oriented forms of the ligands (EZ thiolate) have been found to react faster with the [Fe(HDFO)]+ complex, although for mono-N4 substituted thiosemicarbazones the process is retarded by the formation of a dead-end outer-sphere complex. A comparison with the abstraction of FeIII from [Fe(EDTA)(H2O)]+ has also been conducted with significant differences in the kinetic features that implicate keystone outer-sphere interactions which dominate reactivity, even with isomeric forms that are not the best suited for direct complexation.

Introduction

Heterocyclic thiosemicarbazones (TSC) are potentially tridentate chelating agents bearing both N and S donor centres including an aromatic heterocycle, usually pyridine. Although TSC complexes of many metal ions have been reported,1 their iron coordination chemistry in particular is biologically important.2,3 In previous papers it has been shown that TSC ligands derived from di-2-pyryld ketone,4 2-benzoylpyridine5 and 2-acetylpyridine6 exhibit remarkable anti-proliferative activity against a variety of tumor cells and also that they are well tolerated by healthy cells. There has also been considerable interest in the related TSC Triapine (3-amino-2-pyridinecarbaldehyde thiosemicarbazone)7,8 and this compound has progressed to the clinic as a potential anticancer drug.5,10 Triapine inhibits ribonucleotide reductase (the rate limiting process in DNA replication) and its action is linked with the depletion of the iron within the cell and also to redox activity, as the iron complex of Triapine is more active than the free ligand.2,11,12 So it has emerged that iron is an important target and that the ligands appear to act through a dual mechanism that involves sequestering intracellular Fe and also catalysing Fenton chemistry (eqn (1)) as their Fe complex, leading to intracellular oxidative stress.

\[ \text{[FeIIIL2]}^+ + \text{H}_2\text{O}_2 \rightarrow \text{[FeIII}L_2]^+ + \text{OH}^- + \text{OH}^- \]  

(1)

The complexity and importance of intracellular Fe chelation by small molecules should not be underestimated from an inorganic mechanistic point of view.13 In this respect some studies on Fe(III) speciation between different biologically relevant molecules have already been conducted.14,15 The Fe chelator desferrioxamine B (DFO) has been the ‘gold standard’ in therapeutic Fe chelation for the treatment of severe transfusion-linked Fe overload for more than 30 years. The effectiveness of DFO as an Fe chelator relies upon the high stability of its Fe(III) complex, ferrioxamine B (Scheme 1), in vivo, so any biologically active chelator that is capable of removing Fe from ferrioxamine B must have sufficient thermodynamic stability to also be competitive for labile intracellular Fe. Iron uptake from ferrioxamine B has been observed by some of the thiosemicarbazone ligands discussed in this paper.16–18

An often neglected issue in TSC coordination chemistry is the possibility of E/Z isomerism about the C==N double bond of the Schiff base. No systematic study has been reported on the effect of E/Z isomerisation on complexation behaviour in a biologically relevant medium. In this paper we report the study of the solution behaviour of a series of 2-acylpyridine TSC ligands (HApT, HAp4mT, HAp4pT and HAp44mT; Scheme 1), which show significant changes in their isomeric distribution in different
media. The reaction mechanism operating for isomerisation has been studied and the actuation of proton-assisted outer-sphere interactions has been established for the process. The existence of this solvent-assisted recognition is indicative of what may be occurring during the complexation processes in vivo.

The iron abstraction from ferrioxamine B (Scheme 1) has also been studied kinetically with the same HAp4mT and HAp44mT TSC ligands at different pH and temperatures. The results indicate that the more favourably oriented isomer/tautomer of each TSC ligand reacts faster with ferrioxamine B. Nevertheless, a proton-assisted mechanism is also found for the process, indicating the involvement of important outer-sphere recognition reactions en route to the final product. Dead-end outer-sphere complexes appear important for incorrectly oriented isomeric forms of the partially terminal amine substituted HAp4mT ligand. Finally the study has been extended to include reactions with [Fe(EDTA)(H2O)]− (Scheme 1) with the most active TSC HAp44mT ligand. Again, the existence of outer-sphere recognition reactions between the TSC ligands and the iron complex has been established.

Results and discussion

Ligand isomeric distribution in solution

Given the large amount of literature data concerning the possible isomers of tridentate heterocyclic TSC ligands in this study,2,17,19-24 (see Scheme 2 for definitions) we have investigated the E/Z isomeric distribution and interconversion at biologically relevant pH values (where all ligands exist as neutral molecules).4 In all cases the solutions were made up in water/DMSO mixtures (from 3.3:1 to 0.65:1 ratios depending on the ligand) to ensure total solubility of the compounds. The stability and time evolution of this E/Z isomeric distribution was also studied, this being especially relevant due to the rather slow kinetics of iron removal from ferrioxamine B and [Fe(EDTA)(H2O)]− by the TSC ligands in Scheme 1 (see next section).16

The deprotonated EZ thiolate isomer is the one present in most of the metal complexes of TSC ligands (see Scheme 1),6,26 especially those bearing a pyridine thiosemicarbazone tridentate NNS donor set.6,27 Complexation is accompanied by deprotonation (pKα in the 9.5–11 range for the TSC free ligand).4,17 The same thiolate tautomeric form can also be present in the free ligand, being stabilised in solution by hydrogen bonding between the same set of donors.28 For N4-disubstituted TSC ligands the E thione and Z thione tautomers are disfavoured (there being no N4-H present for H-bonding e.g. HAp44mT), and instead the unusual EZ thiolate zwitterionic tautomer (Scheme 2) is found in TSC ligands derived from 2-acetylpyridine.6 For ligands bearing at least one proton on N4 (e.g. HApT, HAp4mT, HAp4pT) intramolecular H-bonding stabilises both the E thione and Z thione forms; N1-HN3 for the Z form and N2-HN4 in all cases.6,19 The corresponding tautomers, ZE thiol and ZZ thiol, are expected to be unimportant once the N1-HN3 H-bonding is lost, despite the possible existence of a new N2-HS interaction. Summarising, even though six different forms of the
Table 1  Isomeric and tautomeric distribution (%) as from Scheme 2 for the different forms possible for the thiosemicarbazone ligands indicated in Scheme 1

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Solvent</th>
<th>E thione</th>
<th>EZ thiolate</th>
<th>Z thione</th>
</tr>
</thead>
<tbody>
<tr>
<td>HApT</td>
<td>DMSO(d6)</td>
<td>95</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>HAp4mT</td>
<td>DMSO(d6)</td>
<td>90</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>DMSO(d6)</td>
<td>85</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>D2O/DMSO(d6)</td>
<td>85</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>after 4 h at 60 °C</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>H2O/DMSO(d6), NaClO4 0.30 M</td>
<td>90</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>H2O/DMSO(d6), (Bu4P)Br 0.30 M</td>
<td>95</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>H2O/DMSO(d6), pH a = 5.4 (PIPPS)</td>
<td>90</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>after 24 h at 50 °C</td>
<td>75</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>H2O/DMSO(d6), pH a = 6.5 (MES)</td>
<td>85</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>H2O/DMSO(d6), pH a = 7.5 (TRIS)</td>
<td>90</td>
<td>10</td>
<td>—</td>
</tr>
</tbody>
</table>

* As measured in the buffer aqueous component of the solvent mixture.

TSC ligands studied could be present in solution, only some of them are expected to be observable once thermodynamic equilibrium conditions are attained. For the HApT, HAp4mT and HAp4pT (each bearing at least one H-atom on N4), the preferred forms should be E thione/EE thiol and Z thione/ZE thiol, while for the fully substituted HAp44mT the EZ thiolate is expected to be dominant. The rigidity caused by the H-bonding interactions makes the tautomeric forms distinguishable by 1H NMR, while the slow E ⇌ Z process should make these isomeric forms fully distinguishable also.

1H NMR of freshly prepared DMSO-d6 solutions of HApT, HAp4mT and HAp4pT confirmed, as reported in the literature,6,19–21 that the E thione isomer is dominant. The key resonances for the HApT, HAp4mT and HAp4pT ligands are found at 10.3, 10.3 and 10.7 ppm (N3H) and 8.1, 8.6 and 10.2 ppm (N4H), respectively. Minor downfield signals were associated with the presence of small amounts of EZ thiolate tautomers; 14.1, 14.2 and 14.6 ppm for the N2H proton for the same ligands, respectively (Table 1). For the disubstituted HAp44mT ligand the E thione, EZ thiolate and Z thione forms were all present (9.6 ppm and 15.4 ppm for the N3H proton of the E thione and Z thione forms respectively, and 14.7 ppm for the N2H proton of the EZ thiolate form).20 The relative proportions are in fair agreement with the literature,6,19–21 but only partially support the H-bonding considerations discussed above.

On dissolution of the ligands in aqueous DMSO (with different DMSO contents, see above) only minor changes are seen in the thione/thiolate tautomeric distribution (Table 1), as determined from the associated 1H NMR signals of the aromatic (non-water exchangeable) protons. The thione/thiolate ratios in Table 1 were time-independent in this solvent. However, the relative amount of thiolate tautomer increased slightly at more acidic pH values.17 The E/Z isomeric ratio of these solutions was found to be time dependent. The amount of the Z form increases gradually to a final thermodynamic ratio which is in much better agreement with the distribution expected from the hydrogen bonding interactions discussed above.17 Clearly the E/Z isomeric equilibration reaction appears to be promoted by protons.

In view of the marked changes in the final equilibrium distribution of the different forms occurring in aqueous acidic solutions of these ligands (relative to pure DMSO), a detailed kinetic study of the E ⇌ Z isomerisation process (kE→Z + kZ→E) was pursued at varying temperature, pressure, pH and ionic strength conditions. Time-resolved UV-Vis spectroscopy of the TSC ligands in water/DMSO indicates a neat single step process
Relevant kinetic and activation parameters (pH = 5.5, 0.08 M MES) for the $E \leftrightarrow Z$ isomerisation process observed for the HAp4mT, HAp4mT and HAp4pT TSC ligands under different conditions; $[\text{TSC}] = (1–3\times10^{-5}$ M, $I = 0.27–1.0$ M NaClO$_4$. 

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$T/^{\circ}\text{C}$</th>
<th>$10^3k_{\text{obs}}/\text{s}^{-1}$</th>
<th>$\Delta H^\ddagger/\text{kJ mol}^{-1}$</th>
<th>$\Delta S^\ddagger/\text{J K}^{-1}\text{mol}^{-1}$</th>
<th>$\Delta V^\ddagger/\text{cm}^3\text{mol}^{-1}$</th>
<th>$[\text{H}^+]$-dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAp4mT</td>
<td>60</td>
<td>2.1$^a$</td>
<td>78 $\pm$ 2</td>
<td>$-85 \pm 7$</td>
<td>1.2 $\pm$ 0.4</td>
<td>$k = (61 \pm 4)\times[\text{H}^+]$</td>
</tr>
<tr>
<td>HAp4mT</td>
<td>60</td>
<td>0.073$^a$</td>
<td>not measured</td>
<td>not measured</td>
<td>not measured</td>
<td>not measured</td>
</tr>
<tr>
<td>HAp4pT</td>
<td>62</td>
<td>0.093$^b$</td>
<td>122 $\pm$ 5</td>
<td>16 $\pm$ 15</td>
<td>not measured</td>
<td>$k = (0.8 \pm 0.1)\times[\text{H}^+]$</td>
</tr>
</tbody>
</table>

$^a$ 23% DMSO; $^b$ 62% DMSO.

Table 2

Fig. 1 a) UV-Vis spectral changes obtained on a 2.5 $\times$ 10$^{-5}$ M aqueous (23% DMSO) solution of HAp4mT at 40 $^{\circ}$C (pH = 5.5, MES); total time 24 h. b) Plot of the rate constants observed for the same $E \leftrightarrow Z$ isomerisation process versus $[\text{H}^+]$ ($T = 62^{\circ}$C, $I = 0.27$ M (NaClO$_4$), MES 0.08 M).
For the HAp44mT ligand, the thermal activation parameters are similar to those found for $E \rightleftharpoons Z$ isomerisation processes involving a loss of double bond character by coordination and charge separation stabilisation via a rotational transition state.\textsuperscript{20,30,32,33} Although the value determined for $\Delta^iH^\ddagger$ is rather surprising (expected to be negative in any of the two highly polar solvents used, or in their mixtures), the reactivity indicated in Scheme 3 can easily explain the opposite trends of the entropy and volume of activation. The existence of a hydrogen bonded solvent aggregation due to the presence of a protonated nitrogen in the isomerising species has been consistently held responsible for these opposite trends.\textsuperscript{38}

For the HAp4pT ligand the process is $ca.$ two orders of magnitude slower (as the value for HAp4mT), and the value for $\Delta^iH^\ddagger$ is much more positive while that for $\Delta^iS^\ddagger$ is practically zero ($\Delta^iS^\ddagger$ has not been determined for this very slow process). The data clearly indicates some changes in the mechanistic characteristics of the reaction, which is also shown by the ca. two orders of magnitude difference in the $[H^+]$-dependence of the rate constant. Although an alternative direct inversion process, as indicated for comprehensive studies on azo-derivatives,\textsuperscript{31,32,39,40} would agree with the measured thermal activation parameters for the HAp4pT molecule, the $[H^+]$-dependence of the rate constant in Table 2 does not. The partially substituted character of the N\textsuperscript{a} centre should be related to the overall change in reactivity; the very stable dominant initial $E$ thione form of the ligand encumbers the formation of the azo transition state indicated in Scheme 3 for the $E \rightleftharpoons Z$ isomerisation via N\textsuperscript{a}–N\textsuperscript{a} hydrogen bonding.

**TSC iron uptake from ferrioxamine B**

In view of preliminary observations that indicate that iron is extracted from ferrioxamine B\textsuperscript{13,41} by similar TSC ligands,\textsuperscript{16} and the fact that the process is so slow that equilibration studies are not practical, the kinetics of the iron uptake from ferrioxamine B by the ligands indicated in Scheme 1 was pursued at pH values between 5.5 and 7.5. Lower values were considered biologically irrelevant and higher (pH 8.0–9.0) values produced $[\text{Fe}^{II}(\text{Ap})_2]^+$ complexes (presumably due to the ferric complexes oxidising water being facilitated by alkaline pH).\textsuperscript{4,6,42}

Despite their structural similarity, there was variation in reactivity across the series of TSC ligands. The unsubstituted ligand HApT showed no significant reactivity with $[\text{Fe(HDFO)}]^+$ relative to HAp4pT, HAp4mT and HAp44mT on the same time-scale. Nevertheless, for the HAp4pT ligand the spectral changes indicated that, even in the slightly acidic pH range used, reduction of $[\text{Fe}(\text{Ap4pT})_2]^+$ takes place, which is only prevented at higher acidities (pH $< 5.5$). This observation is consistent with $[\text{Fe}(\text{Ap4pT})_2]^+$ having the highest Fe\textsuperscript{III/II} redox potential of this series;\textsuperscript{6} no further studies were conducted on the $[\text{Fe(HDFO)}]^+$/HAp4pT system as a consequence of this behaviour. For the HAp4mT and HAp44mT systems the reactivity observed leads to final UV-Vis spectra that agree with those expected from the characterized $[\text{Fe}^{III}(\text{Ap4XT})_2]^+$ complexes. The bulk process observed by UV-Vis is complex and shows a kinetic profile that depends on the chosen TSC ligand (Fig. 2).

No dependence of the spectral changes on the concentration of TSC was found, indicating a non-equilibrium reaction under the conditions of the study. In this respect the recent stability constants evaluated for similar systems agree with the complete formation of the $[\text{Fe}^{III}(\text{Ap4XT})_2]^+$ complexes under the conditions of the study.\textsuperscript{17} The data are found independent of ionic strength in the 0.1–0.6 M range. Similarly no dependence on the concentration (0.01–0.16 M) of the buffer was found. The overall transmetallation reaction is faster than the isomerisation process (see above), thus indicating that the reaction is occurring on the initial non-equilibrated isomeric distribution of the TSC ligands used in the study.

For both systems studied (>20-fold excess of ligand HAp4mT or HAp44mT), the changes in the initial spectrum of $[\text{Fe}(\text{HDFO})]^+$ in the visible region (where the ligand isomerisation processes do not interfere) can be modelled by a set of two first order reactions (once the induction period observed for the HAp4mT ligand system has finished). The values of pseudo-first order rate constants derived from aqueous solutions of Fe\textsuperscript{III}/DFO upon addition of the TSC ligands studied (note the different time-scales). $[\text{Fe}(\text{HDFO})]^+ = 5 \times 10^{-4}$ M, [TSC] = 5 \times 10^{-4}$ M, pH = 5.5, $T = 60$ °C. 23% DMSO in aqueous solution.

**Fig. 2**  a) Spectral changes upon the reaction of an aqueous solution of Fe\textsuperscript{III}/DFO with HAp44mT; b) Absorbance versus time spectral changes obtained from aqueous solutions of Fe\textsuperscript{III}/DFO upon addition of the TSC ligands studied (note the different time-scales). $[\text{Fe}(\text{HDFO})]^+ = 5 \times 10^{-4}$ M, [TSC] = 5 \times 10^{-4}$ M, pH = 5.5, $T = 60$ °C. 23% DMSO in aqueous solution.
coordination of the ligand to FeIII ion, the process associated with large errors are involved for such a slow process. DMSO content (not observed for the initial substitution step, favoured in less polar medium).

ESI† The values of the pH value of the medium, as indicated in Fig. 3b and Fig. S1b, systems as a function of the different variables used.

Table 3 summarises the data collected for these systems as a function of the different variables used.

It is clear that while the value of $k_{fast}$ should correspond to a coordination of the ligand to FeIII ion, the process associated with $k_{slow}$ does not correspond to a ligand concentration dependent entry of a TSC to the coordination sphere. An intramolecular process involving the final thiosemicarbazone chelation appears responsible for this second slower step; the alternative DFO final dissociation would be expected to show an increasing rate on acidity. The two-fold increase of $k_{obs}$ obtained on increasing the DMSO content (not observed for $k_{fast}$), agrees with the formation of a relevant $\{[Fe(HDFO)]^+\cdot TSC^\delta\}$ outer-sphere complex prior to the initial substitution step, favoured in less polar medium.

The pH profile (Fig. 3b and Fig. S1b, ESI†) for the values of $k_{fast}$ indicate that a significant acceleration of the process is observed on increasing the acidity of the medium. This is in good agreement with what had been observed for the exchange process between $[Fe(HDFO)]^+$ and ferrichrome A, and was associated with a partial protonation of the FeIII/DFO system (despite a $pK_a$ value for $[Fe(HDFO)]^+$ of ca. 1.0), thus labilising the iron centre. If this is so, the $[Fe(H_{2}DFO)]^+$ species should be extremely reactive in comparison with the major $[Fe(HDFO)]^+$ component of the system in this pH range; the alternative existence of an outer-sphere complex between the neutral TSC ligand and the $[Fe(HDFO)]^+$ species, very sensitive to [H+] as in the studies of the previous section, can also explain the data much more reasonably.

As for the values of $k_{slow}$, they are smaller than those found for the $E \rightleftharpoons Z$ isomerisation process indicated in the previous section (Table 2) (and this isomerisation cannot be observed at the wavelengths shown in Fig. 2a), which definitively agrees with the slow step observed being the final chelation of the TSC ligands. Summarising, the substitution of DFO by the incoming TSC ligand seems to be governed by the sequential entry, as found for similar systems, and partial coordination ($k_{fast}$) followed by the complete tridentate coordination ($k_{slow}$) of one of the TSC ligands. After the first initial coordination, DFO dissociates from the ternary FeIII/TSC/DFO complex and fast entry of a second TSC ligand completes the reaction.

In this respect, the dramatic differences in the values of the rate constants determined for the two studied ligands could be tentatively attributed to the different amounts of the $EZ$ thiolate (Scheme 2) form present in solution for the two ligand systems; this form being the reactive one for formation of the final $[Fe(TSC)]^+$ complexes. Effectively, a definite decrease in the reaction rate constant for the fast step ($k_{fast}$) is obtained for the reaction of $[Fe(HDFO)]^+$ with HAp4mT at pH 5.5 after isometric equilibration (according to the data collected in the previous section), which is fully compensated once the change from a 40% to a 20% $EZ$ thiolate form of the ligand is considered (Table 1, Fig. S2, ESI†). Nevertheless, this can only explain a factor of three in the rate constant increase from HAp4mT to HAp44mT, and by no means the induction period observed for the spectral changes on HAp4mT transmetallation (Fig. 2b, 2.5 h at 60 °C and pH = 5.5). Given the fact that the basicities of the pyridine rings in HAp4mT and HAp44mT are similar, and should not lead to any significant differences in reactivity according to the facts indicated in the previous section, the degree of substitution on the N4 atom, has to be considered as responsible. The fast initial formation of a very favourable outer-sphere aggregate of HAp4mT with the $[Fe(HDFO)]^+$ complex, that does not evolve directly to the substituted complex (i.e. a dead-end species), can easily explain the
The time resolved spectral changes in all cases showed the characteristics of the EDTA complex without any redox chemistry; the final spectral changes, as well as the spectral changes associated, correspond within concentration profiles (Fig. 4). One of the reaction rate constants, three identifiable reaction steps with well defined spectra and changes in the 400–700 nm range using SPECFIT produces the final \([\text{Fe(Ap4mT)}_2]^+\) complex. Furthermore, the rate constants \(k_{\text{obs1}}, k_{\text{obs2}}, \text{and } k_{\text{obs3}}\) processes monitored correspond to an associative process, which is in good agreement with a simple chelation sequence from a \([\text{Fe}^{2+}/\text{EDTA}]/\text{TSC}\) ternary system to produce the final \([\text{Fe(Ap44mT)}_2]^+\) complex. Furthermore, the simple substitution processes on the \([\text{Fe(EDTA)(H}_2\text{O)}]^+\) complex has been established as having a well defined dissociative activation character.

### TSC iron uptake from Fe\(^{3+}/\)EDTA

In view of the data determined in the previous section and the historical importance of the EDTA competition studies to assess the quality of a chelator system,\(^{41}\) the kinetics of iron removal from ferric EDTA was pursued. The reactions were studied with the HAp44mT ligand at pH values of 5.5 and 6.5, where the precursor complex exists as a heptacoordinate \([\text{Fe(EDTA)(H}_2\text{O})]^+\) pentagonal bipyramidal (Scheme 1)\(^{48,49}\) and the TSC ligands exist dominantly as neutral molecules.\(^{4}\) Under these conditions no Fe\(^{3+}/\)TSC signature at 640 nm was observed,\(^{50,51}\) which confirms the fact the TSC ligand removes Fe\(^{3+}\) directly from the EDTA complex without any redox chemistry; the final UV-Vis spectra showed in all cases the characteristics of the \([\text{Fe(Ap44mT)}_2]^+\) complex. The time resolved spectral changes are very complex, nevertheless, the fitting of the full spectral changes in the 400–700 nm range using SPECFIT\(^{50}\) produces three identifiable reaction steps with well defined spectra and concentration profiles (Fig. 4). One of the reaction rate constants, as well as the spectral changes associated, corresponds within error limits to the previously determined \(E\rightleftharpoons Z\) isomerisation process, \(k_{\text{obs1}}\) from Table 2, while the other two, \(k_{\text{obs2}}\) and \(k_{\text{obs3}}\), do not show any dependence on the concentration of the ligand or pH, and have spectral changes which correspond to the formation of the final substituted complex. The relevant kinetic and thermal activation parameters collected in Table 4 clearly indicate that these two \(k_{\text{obs1}}, k_{\text{obs2}}\) processes monitored correspond to an associative process, which is in good agreement with a simple chelation sequence from a Fe\(^{2+}/\)EDTA/TSC ternary system to produce the final \([\text{Fe(Ap44mT)}_2]^+\) complex. Furthermore, the simple substitution processes on the \([\text{Fe(EDTA)(H}_2\text{O})]^+\) complex has been established as having a well defined dissociative activation character.\(^{48,49}\)

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{pH} & 10^{3}\times k_{\text{obs1}}/s^{-1} & 10^{3}\times k_{\text{obs2}}/s^{-1} & \Delta H^\ddagger /\text{kJ mol}^{-1} & \Delta S^\ddagger /\text{J K}^{-1}\text{mol}^{-1} & 10^{3}\times k_{\text{obs3}}/s^{-1} & \Delta H^\ddagger /\text{kJ mol}^{-1} & \Delta S^\ddagger /\text{J K}^{-1}\text{mol}^{-1} \\
\hline
5.5 & 2.1 (2.1)\text{a} & 1.6 & 48 \pm 5 & -176 \pm 15 & 1.7 & 79 \pm 1 & -101 \pm 1 \\
6.5 & 0.18 (0.18)\text{a} & 1.8 & \text{not determined} & \text{not determined} & 1.2 & \text{not determined} & \text{not determined} \\
\hline
\end{array}
\]

* In brackets are the values of the \(E\rightleftharpoons Z\) isomerisation process indicated in Table 2.

**Fig. 4** UV-Vis spectral changes observed for the reactions of \([\text{Fe(EDTA)(H}_2\text{O)}]^+\) with HAp44mT. Inset: Kinetically fitted concentration profile of the different species for the same reaction. \([\text{HAp44mT]} = 6 \times 10^{-2}\text{ M, [Fe(EDTA)(H}_2\text{O)}] = 2.2 \times 10^{-4}\text{ M, pH = 5.5, T = 70 °C, 40% DMSO in aqueous solution.}

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**Table 4** Relevant kinetic (average for 3–5 runs at different TSC concentration at 60 °C) and thermal activation data for the processes observed upon reaction of HAp44mT with \([\text{Fe(EDTA)(H}_2\text{O)}]^+\) 40% DMSO in aqueous solution.
HAp44mT is related to the fact that, at pH = 5.5, the catalysed reversion to the E thione and EZ thiolate forms of the ligand competes favourably with the substitution of EDTA by the Z thione form of the HAp44mT ligand.

Conclusions

The freshly prepared thiosemicarbazones of the HApT family used in this study show a dominant presence of the isomeric $E$ thione and EZ thiolate tautomers that equilibrate in protic medium with a mixture increased with presence of the Z thione form. The relative thione/thiolate tautomeric ratios are dependent on the degree of substitution on the N4 terminal amino group. The $E \xrightleftharpoons{\text{Z}}$ isomerisation reaction has been kinetically studied at variable temperatures and pressures and indicates an acid-assisted isomerisation mechanism.

The kinetic studies carried out for the iron abstraction from the Fe$^{III}$/DFO system with the fully substituted HAp44mT ligand indicate a dominant activity of its EZ thiolate form (the one matching the conformation of the final bis-thiosemicarbazone complexes), which is acid-assisted via the formation of an outer-sphere complex. When the mono-N4-methylated ligand HAp4mT is used instead, the above mentioned outer-sphere complexation leading to a dead-end complex implies a definite induction period for the formation of the final [Fe(Ap4mT)$_{2}$]$^{+}$ complex with consequently longer reaction times.

Unexpectedly, the HAp44mT iron uptake from the Fe$^{III}$/EDTA system is much more complex than that observed for the Fe$^{III}$/DFO. In this case the substitution-active TSC ligand isomerisation mechanism.

Experimental

Compounds

All the thiosemicarbazone ligands used in this study were prepared according to literature methods. [Fe(EDTA)(H$_2$O)]$^{3+}$ is commercially available and was used without any further treatment; [Fe(HDFO)]$^{3+}$ (ferrioxamine B) was also prepared according to the literature.

The buffer solutions used in this study were selected according to their inability to act as competing ligands for the Fe$^{III}$ complexes in this study as well as their $K_s$ values. MES, PIPPS, TRIS, CAPS have been used in all cases at concentrations that were capable of buffering all the relevant species in the medium. The final pH was adjusted by adding perchloric acid or sodium hydroxide to the final solutions. Their final ionic strength was always adjusted to 0.25–0.30 M with NaClO$_4$, unless stated.

Instruments

$^1$H NMR spectra were recorded on a Varian VNMRS 400 MHz instrument at the Serveis Científico-Tècnics de la Universitat de Barcelona. pH values were measured on a Crison pH & ION-Meter GLP 22+ instrument using a standard glass combined electrode. UV-Vis spectra were recorded on Cary50, HP8453 or J&M TIDAS instruments.

Kinetics

All the solutions to be used for monitoring the kinetic processes were prepared by adding the calculated amounts of the different ligands dissolved in water or DMSO, according to their solubility in the final media and ionic strength, to stock buffered aqueous solutions. In all cases the maximum volume percentage of DMSO was 65%. The final ionic strength was set normally at 0.27 M with NaClO$_4$, although changes from 0.15 to 1.0 M do not produce any significant difference in the processes observed.

All the kinetic measurements for the different systems carried out at ambient pressure were followed by UV-Vis spectroscopy in the full 300–800 nm range inside the thermostatic multicell compartments of a Cary50 or HP8453 instrument. For the monitoring of reactions at variable pressure, an already described pressurising setup connected to an J&M TIDAS instrument has been used. For the isomerisation studies the values of the concentrations of the thiosemicarbazone ligands were set within the (1–3)×10$^{-4}$ M range, while for all the substitution systems the limiting [Fe$^{III}$] values were within the (1–5)×10$^{-5}$ M range, unless stated, and the uptaking ligands in excess were in at least 10 or 20-fold floating factor (depending on the stoichiometry of the final complex). The general kinetic technique has already been described. The time-dependence of the spectral changes were fitted to single ($k_{\text{obs}}$) or double ($k_{\text{obs}}$ plus $k_{\text{back}}$) or triple ($k_{\text{obs}}$ plus $k_{\text{back}}$ plus $k_{\text{react}}$) exponential dependence by the use of the SPECFIT software. For the simpler processes, fitting was conducted on a single wavelength basis in the range where no interference of other secondary reactions was observed; in these cases no dependence of the value of the observed rate constants with the wavelength was observed. Nevertheless, for systems where the process was more complex, the fitting of the full spectral changes was also conducted with good results. Table S1, ESI collects all the values of $k_{\text{obs}}$ determined for the different systems as a function of temperature and pH. All post run fittings were carried out by least-squares methods using standard commercial software. The typical error limits for the values of $k_{\text{obs}}$ are within 15% of the value indicated in Table S1, ESI.

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References
