Equilibrium Studies of the Iron Exchange Reaction of Ferrioxamine B with 8-hydroxyquinoline-5-sulfonate

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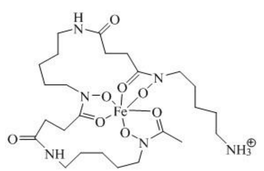
Introduction

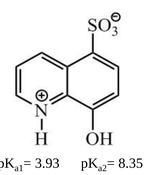
Iron is an essential nutrient for almost all forms of life, however most of the iron in the environment exists in insoluble forms. In order to acquire iron from the environment, many organisms will produce high affinity iron(III) chelators called siderophores. However, once the very stable iron-siderophore complex is formed, it becomes thermodynamically challenging for the iron to be released so that the organism can use it. In order to overcome this challenge, it has been proposed that some organisms may utilize competing ligands to assist in the release of iron through the formation of ternary complexes. These ternary complexes can enhance the release of iron by shifting the reduction potential of the iron complex and facilitating the reduction of iron by physiological reducing agents.[1-3] After reduction the siderophore, which has a much lower binding affinity for iron(II) than iron(III), readily dissociates.

In this study, we will be investigating the ligand exchange reaction between a model siderophore, ferrioxamine B (FeDFB, **I**) and a model competing ligand, 8-hydroxyquinoline-5-sulfonate (H_2QS, **II**). Upon deprotonation, 8-hydroxyquinoline-5-sulfonate forms a stable iron(III) complex (FeQS, **III**) via the aromatic N and hydroxyl O donor atoms. The 8-hydroxyquinoline-5-sulfonate ligand is also biologically relevant as the quinoline functional group is present in flavin mononucleotide, a physiological reductant which has been shown to be capable of reducing ferrixomine B *in vitro*,[4] and in the siderophore quinolobactin.[5] In order to study this reaction and determine the stability constants of the products formed, we performed a series of spectrophotometric measurements in which the pH and H_2QS ligand concentrations were varied. We also determined the stability constants associated with the $H^+ + QS^{2-}$ and $Fe^{3+} + QS^{2-}$ reactions in order to obtain values for these parameters under our experimental conditions. A generalized chemical equation and stability constant expression for the reactions we studied is shown below.

Structures

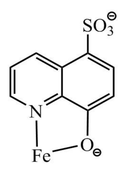


I- Ferrioxamine B (FeDFB)



pK_{a1}= 3.93 pK_{a2}= 8.35

II- 8-hydroxyquinoline-5-sulfonate (H_2QS)



III- 8-hydroxyquinoline-5-sulfonate iron (III) complex (FeQS)

General Reaction and Stability Constant Expression

$$w\,Fe^{3+} + x\,DFB^{2-} + y\,QS^{2-} + z\,H^{+} \rightarrow Fe_w(DFB)_x(QS)_y(H)_z$$

$$\beta_{wxyz} = \frac{[Fe_w(DFB)_x(QS)_y(H)_z]}{[Fe^{3+}]^w [DFB^{2-}]^x [QS^{2-}]^y [H^+]^z}$$

Experimental

Spectrophotometric and potentiometric measurements were collected for all reactions studied. Reaction mixtures were allowed to come to equilibrium at 25°C in a thermostatted flask. A background electrolyte concentration of 0.1 M $NaNO_3$ was added in order to maintain a constant ionic strength. Absorbance measurements were collected using a Shimadzu UV-1601PC spectrophotometer. The pH was monitored using a general purpose Accumet combination electrode with Ag/AgCl reference and an Oakton pH 1100 meter. The pH meter and electrode were calibrated using the GLEE calibration method.[6] The potentiometric and spectrophotometric data was analyzed using pHAB 2003 software.[7]

Results

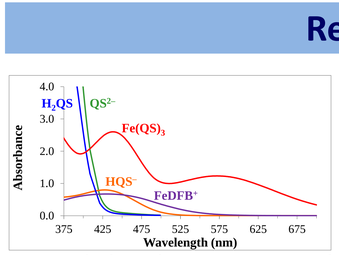


Figure 1: Absorbance of species at concentrations of 0.25 mM Fe^{3+} and 5 mM H_xQS or 0.275 mM DFB^{2-}

Figure 1 shows the absorbance of the species that are present under the conditions of the $FeDFB^+ + QS^{2-}$ reaction series.

- Data in the 425-700 nm range is the useful range for ternary complex analysis.
- Under 425 nm, the absorbance is dominated by the QS ligand species.
- Absorbance in the 525-700 nm range is diagnostic of $Fe(QS)_x$ complexation.

Figure 2 shows a series of $FeDFB^+$ solutions at pH 6.0 with varying amounts of HQS^- ligand.

- Fe-QS complexation is demonstrated by increasing absorption band at 590 nm.
- A Schwarzenbach analysis at 590 nm indicates at least two light absorbing species in addition to $FeDFB^+$ are present. This is confirmed by a Principal Component Analysis in pHAB.
- Evidence suggests presence of one or more ternary complexes.

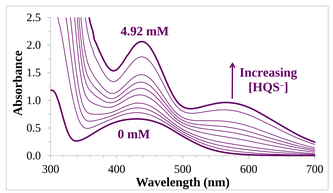


Figure 2: Reaction of 0.246 mM $FeDFB^+$ with 0 mM to 4.92 mM H_xQS at pH 6.0 in 0.05 M MES buffer

Figures 3A and 3B show a series of $FeDFB^+$ solutions with a fixed concentration of H_xQS at varying solution pH. From pH 9.5 to 6.5, an increase in absorbance is observed in absorption bands at 440 and 570 nm followed by a decrease in absorbance from pH 6.5 to 3.0.

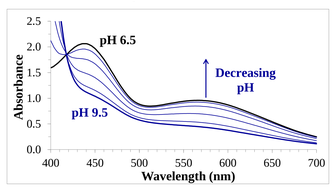


Figure 3A: Reaction of 0.246 mM $FeDFB^+$ with 4.92 mM H_xQS in basic solution (pH 9.5 to 6.5)

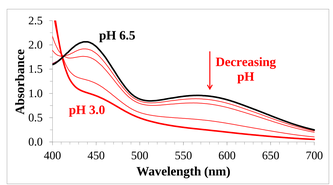


Figure 3B: Reaction of 0.246 mM $FeDFB^+$ with 4.92 mM H_xQS in acidic solution (pH 6.5 to 3.0)

Analysis and Discussion

- Table 1 shows the stability constants determined in this work. β_{1122} is determined from the data shown in Tables 2 and 3. β_{0011}, β_{0012} and β_{1030} were determined from separate spectrophotometric titration experiments (not shown).
- β_{1122} for the formation of $Fe(DFB)(QS)_2(H)_2^-$ was determined using conditions of 1.26–4.92 mM QS ligand and pH 4.5–8.0 assuming $FeDFB^+$ and $Fe(QS)^{3-}$ were also present. For conditions outside this range, an other light absorbing complex, presumably $Fe(DFB)(QS)_1(H)_1$, was present and caused poor fitting results.
- Addition of the $Fe(DFB)(QS)_1(H)_1$ to the model resulted in ill-defined and unstable β_{1111} and molar absorptivities parameters. In order to determine β_{1111}, additional experiments would need to be formed under conditions where this complex is present in greater quantities.
- Based upon previous ternary complex studies, the protons in the ternary complex are added to the deferrioxiamine B ligand to give $Fe(H_2DFB)(QS)_2^-$.
- Figure 4 shows a comparison of the fraction of $FeDFB^+$ converted into ternary complex for the QS ligand and previously reported ternary complexes. $Fe(DFB)(QS)_2(H)_2^-$ clearly has a much greater stability than the other ternary complexes at neutral pH values.

Product	β	Log β (Std Dev)
HQS^- (pka_2)	β_{0011}	8.35 (±0.02)
H_2QS ($pKa_1 + pKa_2$)	β_{0012}	12.28 (±0.02)
$Fe(QS)_3^{3-}$	β_{1030}	31.901 (±0.004)
$Fe(DFB)(QS)_2(H)_2^{3-}$	β_{1122}	52.641 (±0.003)

Table 1: Stability constants for the $FeDFB^+ + QS^{2-}$ reaction.

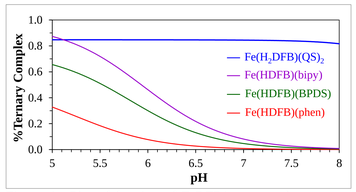


Figure 4: Plot of fraction of $FeDFB^+$ converted to ternary complex under conditions of 0.25 mM $FeDFB^+$ and 5.0 mM ligand

Conclusions

- 8-Hydroxyquinoline-5-sulfonate (QS) forms a ternary complex with ferrioxamine B (FeDFB) with the stoichiometry of $Fe(H_2DFB)(QS)_2$.
- At pH 6.0-8.0, the $Fe(H_2DFB)(QS)_2$ ternary complex is more stable than any of the previous ferrioxamine B ternary complexes reported.

References

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