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学位（専攻分野）	博士（理学）
学位記番号	総研大甲第77号
学位授与の日付	平成6年3月24日
学位授与の要件	数物科学研究科 構造分子科学専攻 学位規則第4条第1項該当
学位論文題目	Stabilization of Superoxidized Form of Synthetic Fe ₄ S ₄ Cluster as the First Model of High-Potential Iron-Sulfur Proteins under Aqueous Conditions
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論文内容の要旨

4-Fe iron-sulfur proteins function as electron transfer catalysts in various biological redox reactions such as photosynthesis, nitrogen fixation, nitrite reduction and so on. Ferredoxins (Fd) and high-potential iron-sulfur proteins (HiPIP) containing Fe₄S₄ cores exhibit the [Fe₄S₄]^{2+/-+} and [Fe₄S₄]^{3+/-2+} redox couples, respectively. Most of synthetic Fe₄S₄ clusters show a stable [Fe₄S₄]^{2+/-+} redox couple in both organic and aqueous solutions, but the [Fe₄S₄]³⁺ state of synthetic Fe₄S₄ clusters is unstable in polar solvents. In accord with this, the [Fe₄S₄]³⁺ core of HiPIPOx is believed to be protected from water attack by hydrophobic protein environments around the Fe₄S₄ core, and stabilization of the [Fe₄S₄]³⁺ core of synthetic Fe₄S₄ clusters in polar organic solvents has been achieved by using sterically encumbered terminal ligands.

In this work, electrochemical behavior of a series of [Fe₄X₄(YAd)₄]²⁻ (X, Y = S, Se; Ad = adamantane) was examined in both DMF and aqueous solutions, and stabilization of the superoxidized form [Fe₄X₄(YAd)₄]⁻ was succeeded as the first HiPIP model in H₂O.

In Chapter III-1, redox behavior of [Fe₄X₄(YAd)₄]²⁻ (X, Y = S, Se) was described. Those clusters showed not only the [Fe₄X₄(YAd)₄]^{2-/-3-} but the [Fe₄X₄(YAd)₄]^{-/-2-} couples in DMF. The stability of the superoxidized forms in DMF decreases in the order; [Fe₄S₄(SAd)₄]⁻ > [Fe₄S₄(SeAd)₄]⁻ > [Fe₄Se₄(SAd)₄]⁻ > [Fe₄Se₄(SeAd)₄]⁻. The superoxidized form [Fe₄X₄(YAd)₄]⁻ was much more subject to hydrolysis than [Fe₄X₄(YAd)₄]³⁻ and [Fe₄X₄(YAd)₄]²⁻ in the presence of H₂O in DMF, since the cyclic voltammogram of [Fe₄S₄(SAd)₄]²⁻ in DMF containing a small amount of H₂O (1 vol%) showed a strong irreversible oxidation current at potentials more positive than the anodic wave of the [Fe₄S₄(SAd)₄]^{-/-2-} couple, while the cathodic and anodic waves of the reversible [Fe₄S₄(SAd)₄]^{2-/-3-} couple were observed at the same potentials as those in dry DMF. This fact implies that the affinity of H₂O to the [Fe₄S₄]³⁺ core is much stronger than that to the [Fe₄S₄]²⁺ and [Fe₄S₄]⁺ ones. On the other hand, the cyclic voltammogram of (Et₄N)₂[Fe₄S₄(SAd)₄] in the presence of a 30 molar excess of free AdSH displayed both the [Fe₄S₄(SAd)₄]^{2-/-3-} and [Fe₄S₄(SAd)₄]^{-/-2-} couples even in H₂O/DMF (23 vol%). The distinct difference in the stability of [Fe₄S₄(SAd)₄]⁻ in the absence and presence of free AdSH in H₂O/DMF is reasonably explained by depression of dissociation of the AdS⁻ ligand from the [Fe₄S₄]³⁺ core. Surprisingly, the stable [Fe₄X₄(YAd)₄]^{-/-2-} redox couple was observed in aqueous PDAH (PDAH = Poly[2-(dimethylamino)hexanamide]) or Triton X-100 solutions even in the absence of free AdYH. The stability of [Fe₄X₄(YAd)₄]⁻ in aqueous solution is explained by depression of dissociation of the AdS⁻ ligand from the [Fe₄S₄]³⁺ core because of the insolubility of the ligands.

In Chapter III-2, the protonation behavior of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{n-}$ ($n = 1, 2, 3$) was described. Both cathodic and anodic peak potentials of the $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{2-}/^{3-}$ and $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}/^{2-}$ redox couples were shifted by -0.06 V/pH between pH = 6 to 11 of aqueous PDAH solution. This observation suggests that those redox reactions are accompanied by reversible protonation of core and/or terminal sulfur. Such a reversible protonation of the cluster is explained by penetration of H_2O or H_3O^+ to the $[\text{Fe}_4\text{S}_4]^{3+}$ core through PDAH. The pKa values of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{3-}$, $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{2-}$, and $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ determined electrochemically were essentially consistent with the values obtained from pH titration and electronic absorption spectra in the same media.

In Chapter III-3, the stability of $[\text{Fe}_4\text{X}_4(\text{YAd})_4]^{-}$ ($\text{X}, \text{Y} = \text{S}, \text{Se}$) was discussed. The X-ray crystal structure of $(\text{Ph}_4\text{As})_2[\text{Fe}_4\text{S}_4(\text{SAd})_4]$ revealed that the $[\text{Fe}_4\text{S}_4]^{2+}$ core has enough space for coordination of H_2O to Fe. Based on extreme lability of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ in $\text{H}_2\text{O}/\text{DMF}$ (1 vol%), the size of the AdS^{-} ligand is not enough to prevent the coordination of H_2O to the $[\text{Fe}_4\text{S}_4]^{3+}$ core. The effective enhancement of the stability of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ by an addition of free AdSH in $\text{H}_2\text{O}/\text{DMF}$ (23 vol%) strongly indicates that the hydrolysis of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ results from the replacement reaction of AdS^{-} ligated on Fe by H_2O . The stability of $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ in aqueous PDAH solutions even in the absence of free AdSH may, therefore, be associated with insolubility of AdS^{-} (or AdSH) in H_2O . Thus, the present work reveals that the $[\text{Fe}_4\text{S}_4]^{3+}$ core can be stabilized by depression of dissociation of terminal thiolate ligands from the core rather than hydrophobic spheres around the Fe_4S_4 core.

In Chapter III-4, redox behavior of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ ($\text{R} = \text{Ph}, t\text{-Bu}, \text{CPh}_3, \text{C}_6\text{H}_4\text{-}p\text{-}t\text{-Bu}$) in aqueous solutions is discussed. From the view points that the stability of the superoxidized form $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ on aqueous media depends on the solubility of the terminal thiolate in solvents, the oxidation behavior of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ with a water insoluble RS^{-} ligand ($\text{R} = \text{Ph}, t\text{-Bu}, \text{CPh}_3, \text{C}_6\text{H}_4\text{-}p\text{-}t\text{-Bu}$) was examined in aqueous micellar solution. In accord with the results in chapter III-3, those clusters show the stable redox couple $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{-}/^{2-}$ in aqueous solution. Especially, an aqueous solution prepared by an addition of a DMF solution of $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SAd})_4]$ to H_2O showed the stable $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}/^{2-}$ redox couple even in the absence of any surface-active agents. Although the observation that superoxidized $[\text{Fe}_4\text{S}_4(\text{SAd})_4]^{-}$ exists in H_2O is not consistent with the generally believed view that the $[\text{Fe}_4\text{S}_4]^{3+}$ core is protected from H_2O attack by hydrophobic spheres formed by polypeptide chains, the redox potentials of some of HiPIP are also reported to be shifted by -0.060 V/pH.

論文の審査結果の要旨

本学位論文は生体機能と関連して重要な $[\text{Fe}_4\text{S}_4]$ 骨格をもつクラスターを合成し、cyclic voltammetry を中心に x 線構造解析、電子スペクトル測定などの測定手段を用いてその安定性を論じたものである。合成されたクラスターは $[\text{Fe}_4\text{X}_4(\text{YAd})_4]^{2-}$, (Ad=1-adamantane)で、X, YとしてS およびSeの組み合わせが用いられた。酸化還元状態の測定の結果、DMF 中では $[\text{Fe}_4\text{X}_4(\text{YAd})_4]^{n-}$ の $n=1\sim 3$ が安定であるが、少量の水を添加すると加水分解する。一方 AdS^- イオンを加えておくと安定である。また界面活性剤を使い水のみで溶かすと安定である。これらの結果と、構造解析の結果から、クラスター、とくに超酸化状態($n=1$)のクラスターの水中での安定性は、従来提案されていた立体的に嵩高い疎水性の置換基による水分子の排除効果よりも、むしろ配位子である Ads^- が水に不溶であることがより重要であると推定した。この推論にもとづき、Adを他の疎水性基に置換したクラスターについても議論している。また、酸化還元と平行して起こるプロトン付加平衡についてもくわしく研究し、 pKa の値を求めた。

この研究はこのような新しい合成 Fe_4S_4 クラスターのいろいろな酸化状態における安定性を組織的かつ系統的に調べ、十分に意義のある結果を得ていると評価される。とくに、このクラスターが $n=1$ の超酸化状態において水中でも安定であることをはじめて見出し、その原因に迫った点を高く評価できる。

面接による試験は学生本人に約45分間にわたって論文内容についての発表を行わせ、ひきつづき、約2時間にわたって試験を行った。発表は学位論文の研究内容を要領よくまとめており、また、研究内容に関する質問にもほぼ適確に答えていた。一般的学力についても水準をクリアしていると判断される。本論文は英文で書かれており、文章、表現等、語学力についても問題はなかった。

また、公開発表会における発表、質疑応答についても問題はなかったので本学位論文は学位授与に値するものと判断した。