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Investigation of amino acid containing [FeFe] hydrogenase models concerning pendant base effects

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Dedicated to Prof. Kazuyuki Tatsumi, on the occasion of his 60th birthday.

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ABSTRACT

The present investigations deal with the modeling of the peptide surrounding of [FeFe] hydrogenase using amine containing disulphides to simulate possible influences of the amino acid lysine (K237) on the electrochemical and electrocatalytic properties of biomimetic compounds based on [Fe2S2] moieties. $Fe_3(CO)_{12}$ was reacted with Boc-4-amino-1,2-dithiolane, Boc-Adt-OMe (Adt = 4-amino-1,2-dithiolane-4-carboxylic acid, Boc = *tert*-butoxycarbonyl) and Boc-Adp *tert*-butyl ester (Adp = (*S*)-2-amino-3-(1,2-dithiolan-4-yl)propionic acid) to elongate the Fe···N distance in comparison to the well known $[Fe_2\{(SCH_2)_2NR\}(CO)_6]$ model complexes. Efforts to deprotect the complexes containing Boc-4-amino-1,2-dithiolane with trifluoroacetic acid result in the formation of $[Fe_3(\mu^3\text{-}O)(\mu\text{-}O_2C_2F_3)_6(OC_4H_8)_2(H_2O)]$. The novel [2Fe2S] complexes are characterized using spectroscopic, electrochemical techniques and X-ray diffraction studies.

1. Introduction

For some decades, it has been known that [FeFe] hydrogenase can efficiently form dihydrogen from protons. However, the mechanism for H_2 development has not been established and no model complex reported to date shows electrochemical properties like those observed in natural systems. Since the elucidation of the [Fe2S2] cluster as the active site in [FeFe] hydrogenase [1–3], the question has focused on the nature of the S–S linker and on the catalytic pathway of this organometallic active site and is under investigation of many research groups [4–12]. Based on X-ray analyses of the protein, three different kinds of linkers are possible: 1,3-propanethiolate $[(SCH_2)_2CH_2]$, azadithiolate $[(SCH_2)_2NR]$ and oxadithiolate $[(SCH_2)_2O]$ [13,14]. In particular the amino group containing azadithiolate linker is interesting since DFT calculations and cyclic voltammetry measurements of [FeFe] hydrogenase models have shown that the amino moiety may influence the catalytic mechanism by acting as an initial protonation site [9,15–20].

Subsequently, this proton is directed to the iron centre where it reacts with a hydride bound to the $[Fe^IFe^{II}]$ core. This species is generated by reaction of a proton with a $[Fe^0Fe^I]$ precursor. However, electrochemical investigations of $[Fe_2\{(SCH_2)_2NR\}(CO)_6]$ model compounds do not show a significant positive shift of the redox potential at which H_2 formation is observed. Apart from the influence of the amino moiety in the S–S linker, the protein layer surrounding of the active site of [FeFe] hydrogenase is also expected to impact on the catalytic mechanism and redox potential of the natural enzyme, especially during the transfer of protons. It is known that the alkylic amino function of the amino acid lysine (K237) is only 440 pm away from the distal iron of the [Fe2S2] cluster and can therefore serve as a possible proton source or relay [2,15]. This raises the question of the existence of a possible proton transfer from lysine or the S–S linker to the [Fe2S2] moiety during the catalytic cycle of [FeFe] hydrogenase. So far only little attention has been devoted towards mimicking the enzymatic environment of the H-cluster. To our best knowledge, only two examples are known in literature containing the naturally occurring amino acid cysteine as ligand for [FeFe] hydrogenase model complexes [21,22].

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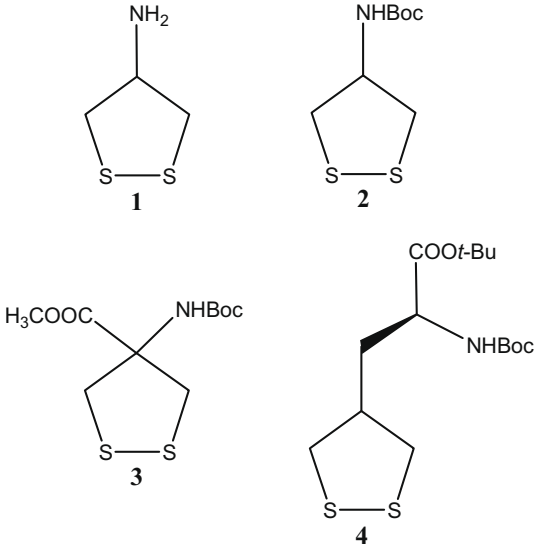
Herein we report about novel [FeFe] hydrogenase model complexes where the Fe···N distance is increased with respect to the value of 345 pm reported by Rauchfuss et al. [23] for complexes of the type $[Fe_2\{(SCH_2)_2NR\}(CO)_6]$ to reflect the structural and electrochemical features of lysine (K237). This modification as aimed at investigating, whether the proton relay is established by an assumed azadithiolato linker or a pendant amino moiety of lysine. [Fe2S2] clusters are reported containing 4-amino-1,2-dithiolane and its Boc protected analogue (**1** and **2**) [24], Boc-Adt-OMe (Adt = 4-amino-1,2-dithiolane-4-carboxylic acid) (**3**) [25] and Boc-Adp *tert*-butyl ester (Adp = (*S*)-2-amino-3-(1,2-dithiolan-4-yl)propionic acid) (**4**) [26] (Scheme 1). The structural and catalytic properties of the compounds were investigated by spectroscopic, X-ray diffraction and electrochemical studies.

2. Experimental

2.1. Materials and methods

Toluene and THF were dried over KOH and distilled from sodium/benzophenone. Chemicals were received from Fluka or Acros and used without further purification. 4-Amino-1,2-dithiolane hydrochloride (**1**·HCl) [24], Boc-Adt-OMe (**3**) [25] and Boc-Adp *tert*-butyl ester (**4**) [26] were synthesized following literature procedures. All reactions were carried out under an argon atmosphere. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (detection under UV light at 254 nm) and FC (flash chromatography) on Fluka silica gel 60. ^{1}H NMR and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker AVANCE 200 MHz or 400 MHz spectrometer, whereby the splitting of proton resonances are defined s (singlet), d (doublet) and m (multiplet). Infrared spectra were obtained from KBr pellets with a Perkin–Elmer 2000 FT-IR instrument. The intensity of the signals is assigned as vs (very strong), s (strong), m (medium) and w (weak). EPR spectra were recorded on a Bruker ESP 300E EPR-spectrometer. Electron impact mass spectrometry was carried out at 70 eV with a Finnigan SSQ710 using desorption electron ionisation (DEI) mode. Expected and experimental isotope distributions were compared.



Scheme 1. Ligand molecules for mimicking of lysine (K237) in the [FeFe] hydrogenase.

2.2. Synthesis of ligands and complexes

2.2.1. tert-Butyl 1,2-dithiolan-4-yl carbamate (*2*)

4-Amino-1,2-dithiolane hydrochloride (527 mg, 3.36 mmol) was suspended in THF (10 mL) and di-*tert*-butyl dicarbonate (879 mg, 4.03 mmol), dissolved in THF (20 mL) and pyridine (20 mL), was slowly added at 0 °C. After 1 h at 0 °C the solution was stirred at room temperature for additional 24 h, followed by the addition of 10 mL water. The solution was extracted with dichloromethane and the combined organic phases were separated, dried with sodium sulphate, evaporated to dryness and recrystallized from dichloromethane/pentane (1:10). Yield: 310 mg (42%) as a pale yellow solid. Anal. calcd. for $C_8H_{15}O_2S_2N{\cdot}0.3$ THF: C, 45.48%; H, 7.22%; N, 5.76% S, 26.39%. Found: C, 45.7%; H, 7.1%; N, 5.8%; S, 26.8%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.94 (m, 2H, N*H* and C*H*), 3.24–3.05 (m, 4H, CH_2), 1.43 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 56.4 (CH_2CHCH_2), 44.9 (CH_2CHCH_2), 28.3 ($C(CH_3)_3$). MS (DEI): *m/z* 221, $[M]^+$, 166 $[M-t\text{Bu}]^+$. IR spectrum in KBr, cm^{-1} (selected bands): 3355 (s), 2925 (s), 2854 (m), 1679 (vs), 1523 (s), 1368 (s), 1168 (s).

2.2.2. $[\{(\mu\text{-}SCH_2)_2CHNHBoc\}Fe_2(CO)_6]$ (*5*)

tert-Butyl 1,2-dithiolan-4-yl carbamate (**2**) (25 mg, 0.11 mmol) and $Fe_3(CO)_{12}$ (57 mg, 0.11 mmol) were dissolved in THF (30 mL) and refluxed for 1 h. After removing the solvent under reduced pressure, the crude product was purified by FC (diethylether/hexane = 1:1). Yield: 31 mg (55%, R_f = 0.3) as a red powder. Anal. calcd. for $C_{14}H_{15}Fe_2O_8S_2N{\cdot}0.1$THF: C, 34.03%; H, 3.13%; N, 2.76% S, 12.62%. Found: C, 34.4%; H, 2.7%; N, 2.6%; S, 11.9%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.49 (m, 1H, N*H*), 3.09 (m, 1H, C*H*), 2.74 (d, ^2J = 11 Hz, 2H, 2 × CH_AH_B), 1.39 (m, 11H, CH_3 and 2 × CH_AH_B). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 207.3 (C≡O), 153.7 (*C*(O)O), 80.4 ($C(CH_3)_3$), 53.0 (CH_2CHCH_2), 29.7 (CH_2CHCH_2), 28.2 ($C(CH_3)_3$). MS (DEI): 445 $[M-2CO]^+$, 417 $[M-3CO]^+$, 389 $[M-4CO]^+$, 361 $[M-5CO]^+$, 333 $[M-6CO]^+$. IR spectrum in KBr, cm^{-1} (selected bands): 3442 (s), 2982 (s), 2077 (m), 2077 (vs), 2000 (vs), 1687 (s), 1369 (s), 1166 (s).

2.2.3. $[\{(\mu\text{-}SCH_2)_2C(C(O)OCH_3)(NHBoc)\}Fe_2(CO)_6]$ (*6*)

Boc-Adt-OMe (**3**) (16.3 mg, 0.06 mmol) and $Fe_3(CO)_{12}$ (30 mg, 0.06 mmol) were dissolved in dry toluene (20 mL). The solution was heated at reflux for 30 min followed by the removal of the solvent under reduced pressure. The remaining material was purified by FC (THF/hexane = 1:3). Yield: 30 mg (92%) as a red solid. Anal. calcd. for $C_{16}H_{17}Fe_2O_{10}S_2N{\cdot}0.8$THF: C, 37.39%; H, 3.82%; N, 2.27% S, 10.40%. Found: C, 38.0%; H, 3.4%; N, 2.3%; S, 10.0%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.47 (s, 1H, N*H*), 3.67 (s, 3H, OCH_3), 2.94 (d, ^2J = 13.6 Hz, 2H, 2 × CH_AH_B), 2.27 (d, ^2J = 14.4 Hz, 2H, 2 × CH_AH_B), 1.40 (s, 9H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 207.2/206.8 (C≡O), 171.7 (*C*(O)OCH_3), 154.4 (NH*C*(O)), 81.1 ($C(CH_3)_3$), 61.2 (CH_2CCH_2), 53.3 (OCH_3), 28.1 ($C(CH_3)_3$), 27.1 (CH_2CCH_2). MS (DEI): 503 $[M-2CO]^+$, 475 $[M-3CO]^+$, 447 $[M-4CO]^+$, 419 $[M-5CO]^+$, 391 $[M-6CO]^+$. IR spectrum in KBr, cm^{-1} (selected bands): 3442 (s), 2929 (m), 2076 (vs), 2038 (vs), 2000 (vs), 1709 (s).

2.2.4. $[\{(\mu\text{-}SCH_2)_2CCH_2C(C(O)Ot\text{-}Bu)(NHBoc)\}Fe_2(CO)_6]$ (*7*)

Boc-Adp *tert*-butyl ester (**4**) (33 mg, 0.09 mmol) and $Fe_3(CO)_{12}$ (48 mg, 0.09 mmol) were dissolved in dry toluene (20 mL) and refluxed for 2 h. After evaporation of the solvent under reduced pressure the crude product was purified via FC (ethyl acetate/hexane = 1:2). Yield: 35 mg (63%, R_f = 0.7) as an orange powder. Anal. calcd. for $C_{21}H_{27}Fe_2O_{10}S_2N{\cdot}$THF: C, 42.81%; H, 5.03%; N, 2.00% S, 9.14%. Found: C, 43.0%; H, 5.2%; N, 2.1%; S, 9.9%. Although the analyses data for "S" are somewhat unsatisfactory, the MS (DEI) results and other spectroscopic analysis data are consistent