Redox Properties of 4Fe–4S Clusters with Cys-containing Dipeptide Ligands as a Model of High Potential Iron–Sulfur Proteins

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Abstract

An electrochemical oxidation study of 4Fe–4S clusters with dipeptides containing Cys and a hydrophobic amino acid residue revealed the stabilization of the oxidized high potential iron–sulfur protein by hydrophobic groups of surrounding polypeptides and the regulation of its redox potential by electronic properties of coordinated cysteinyl groups.

Introduction

Biologically relevant 4Fe–4S clusters have the function of electron transfer [1]. Much effort to synthesize high potential iron–sulfur protein (HP) model complexes resulted in the mere detection of their $[Fe_4S_4]^{3+/2+}$ redox couples by electrochemical oxidation [2–7]. Such results led to the conclusion that the $[Fe_4S_4]^{3+}$ core had a limited stability. The causes of the instability of the $[Fe_4S_4]^{3+}$ core and the stability of the oxidized HP have both remained unresolved. Our recent electrochemical study of 4Fe–4S clusters with alkane- and arenethiolate ligands revealed the high water sensitivity of the $[Fe_4S_4]^{3+}$ core as well as the effective lowering of such sensitivity by hydrophobic groups bound to the α-carbon of coordinated thiolate ligands [8]. The results prompted us to study how hydrophobic groups of the surrounding polypeptides stabilize an oxidized HP core.

Experimental

$[n\text{-}Bu_4N]_2Fe_4S_4(Z\text{-}Cys\text{-}X\text{-}OEt)_4]$ clusters with X = Ile, Leu, Val, Ala and Gly (**1**) were prepared from $[n\text{-}Bu_4N]_2[Fe_4S_4(S\text{-}t\text{-}Bu)_4]$ and Z-Cys-X-OEt by the method reported by Bobrik *et al.* [9]. $[Et_4N]_2[Fe_4S_4(SR)_4]$ clusters with R = *p*-substituted phenyls (**2**) and alkyls (**3**) were prepared by the method reported by Christou and Carter [10]. $[Ph_4P]_2[Fe_4S_4(2,4,6\text{-triisopropylbenzenethiolato})_4]$ (**4**) was prepared by the ligand exchange method as mentioned above for the synthesis of **1** [4]. Cyclic voltammograms (CV) were measured with a three-electrode system under an argon atmosphere. The working electrode was a glassy carbon disc and a Pt wire served as the counter electrode. A saturated calomel electrode (SCE) was used as the reference electrode. Solutions consisted of 2 mM of sample and 100 mM of $n\text{-}Bu_4NClO_4$ as the supporting electrolyte. The solvents employed (dichloromethane, acetonitrile, isobutylronitrile and *N,N*-dimethylformamide) were distilled according to the reported procedures for electrochemical measurements [11], dried over activated 0.4 nm molecular sieves, degassed and stored under an argon atmosphere. Water contents of the solvents were less than 0.01 wt.%. The stability of the oxidation product during a $[Fe_4S_4]^{3+/2+}$ redox couple was evaluated by the i_{pc}/i_{pa} ratio of CV. The diffusion peak current was corrected for the baseline drift due to the solvent.

Results and Discussion

In a previous electrochemical study [8], we reported that the $[Fe_4S_4]^{3+}$ core was more stable in non-polar solvents such as CH_2Cl_2 than in polar solvents. The decomposition rate of the $[Fe_4S_4]^{3+}$ core in the presence of water probably depends on the polarity of the solvents. Actually, in CH_2Cl_2, even 4Fe–4S clusters with benzenethiolate and *prim*- and *sec*-alkanethiolate ligands displayed one-electron oxidation reactions, though they were reported to exhibit multi-electron oxidation reactions in polar solvents [12]. Such a finding makes it possible to examine the relation between the stability of the $[Fe_4S_4]^{3+}$ core and the ligand structure.

CV of **1** was recorded in CH_2Cl_2 to evaluate the stabilizing influences of hydrophobic groups of peptide ligands on the $[Fe_4S_4]^{3+}$ cores (Table 1). Clusters with dipeptides containing Ile, Leu and Val exhibited discrete one-electron oxidation reactions, while those with Ala and Gly showed multi-electron

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TABLE 1. Electrochemical oxidation data for $[n\text{-}Bu_4N]_2[Fe_4S_4(Z\text{-}Cys\text{-}X\text{-}OEt)_4]$ in CH_2Cl_2, scan rate 100 mV/s

X	E_{pa} (V vs. SCE)	E_{pc}	$E_{1/2}$	i_{pc}/i_{pa}
Ile	0.10	−0.01	0.06	0.15
Leu	0.10	−0.01	0.06	0.15
Val	0.14	0.00	0.07	0.10
Ala	[a]	[a]	[a]	[a]
Gly	[a]	[a]	[a]	[a]

[a]Multi-electron oxidation process.

oxidation reactions due to the deficiency of hydrophobicity. From the i_{pc}/i_{pa} ratio the order of hydrophobic amino acid residues to stabilize a $[Fe_4S_4]^{3+}$ core is Ile = Leu ≥ Val > Ala = Gly. The order is consistent with hydrophobicity scales for amino acid residues [13]. The low i_{pc}/i_{pa} ratios observed in one-electron oxidation reactions are attributed to the separation of the hydrophobic groups from the core by a cysteinyl group [8]. The results indicate that the HP core is protected from water molecules by the hydrophobic side chains of the amino acid residues in the direct vicinity of the core or by the hydrophobic core environments shielded from the bulk water. Such a speculation is consistent with the finding that upon one-electron oxidation of the reduced HP (a $[Fe_4S_4]^{2+}$ core) the oxidized species ($[Fe_4S_4]^{3+}$) is unstable, especially in Me_2SO solution where the original peptide conformation is not retained [14].

In spite of the different kinds of hydrophobic amino acid residues, redox potentials of **1** with X = Ile, Leu and Val were observed near 0.06 V (versus SCE). The result implies that redox potentials of **1** are regulated by the electronic property of the cysteinyl group. To confirm whether electronic properties of the ligands have a regulating influence on the $[Fe_4S_4]^{3+/2+}$ redox potentials or not, CV of **2** and **3** were recorded in CH_2Cl_2. Figure 1 shows the correlation of redox potentials of **2** and **3** with Hammett σ_p and Taft σ^* constants [15]. The electronic properties of the ligands evidently control the $[Fe_4S_4]^{3+/2+}$ redox potentials. Potentials become more positive as the electron-releasing tendency of the substituents decreases. A similar correlation of the $[Fe_4S_4]^{2+/+}$ redox potentials of 4Fe–4S clusters with arene- and alkanethiolate ligands with Hammett σ_p and Taft σ^* constants was reported by DePamphilis *et al.* [2]. The observed potential of **1** (0.06 V versus SCE) gives a σ^* value for a $CH_2CH(NHCOO-)CONH-$ group through the correlation in Fig. 1. The known σ^* value of a CH_2CH_2Cl group (0.385) is similar to the observed σ^* value. Apparently, such a group has a strongly electron-withdrawing CONH group comparable to that of a Cl group at the α-carbon. This result leads to the conclusion that the redox potentials of **1** are regulated by the electronic property of the cysteinyl group.

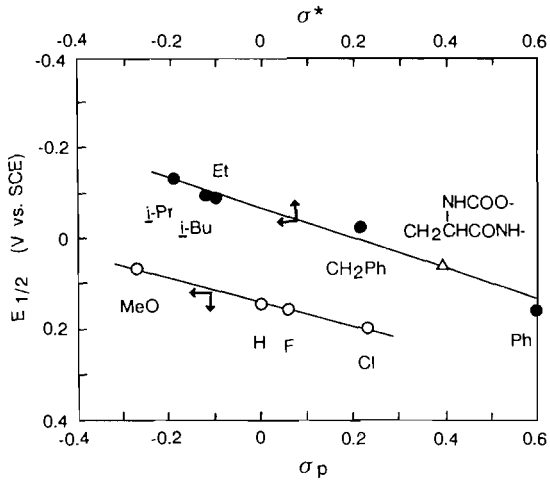

Fig. 1. Correlation of $[Fe_4S_4]^{3+/2+}$ redox potentials with σ_p and σ^* values in CH_2Cl_2, scan rate 100 mV/s. ○, $[Fe_4S_4(SC_6H_4\text{-}p\text{-}X)_4]^{2-/-}$; ●, $[Fe_4S_4(SR)_4]^{2-/-}$ (R = alkyl).

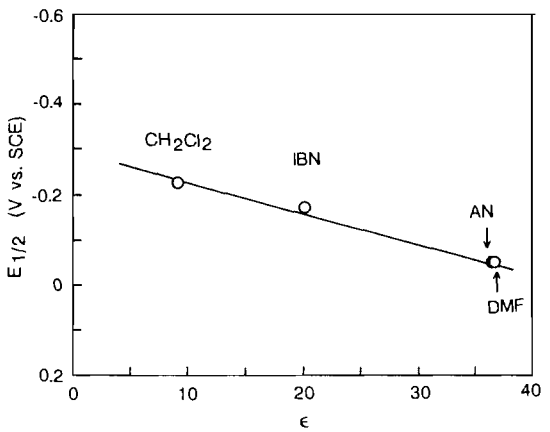

Fig. 2. Solvent effects on the redox potentials of $[Fe_4S_4(2,4,6\text{-triisopropylbenzenethiolato})_4]^{2-/-}$. Scan rate, 100 mV/s. AN, acetonitrile; IBN, isobutyronitrile.

In order to clarify the influence of the dielectric property of the solvent on the $[Fe_4S_4]^{3+/2+}$ redox potential, the redox potential of **4** was measured in various solvents (Fig. 2). The potential becomes more negative in proportion to the decrease in the dielectric constants of solvents. The result indicates that in hydrophobic solvents the redox potential of **1** is about 0.0 V (versus SCE), which is a similar value to that of HP (0.11 V versus SCE). Therefore, the redox potential of HP is mainly regulated by the electronic properties of their cysteinyl ligands, whereas that of 4Fe–4S ferredoxin is controlled by the electronic properties of cysteinyl ligands as well as the NH---S bonds formed by the specially folded conformation of peptide ligands [16, 17].

The electrochemical oxidation study of 4Fe–4S clusters with dipeptides containing Cys and a hydrophobic amino acid residue provided a useful piece of information about both the redox potential control of HP and the stability of its oxidized one.

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