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Electrochemical and ligand binding studies of a de novo heme protein

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Abstract

Heme proteins can perform a variety of electrochemical functions. While natural heme proteins carry out particular functions selected by biological evolution, artificial heme proteins, in principle, can be tailored to suit specified technological applications. Here we describe initial characterization of the electrochemical properties of a de novo heme protein, S824C. Protein S824C is a four-helix bundle derived from a library of sequences that was designed by binary patterning of polar and nonpolar amino acids. Protein S824C was immobilized on a gold electrode and the formal potential of heme–protein complex was studied as a function of pH and ionic strength. The binding of exogenous N-donor ligands to heme/S824C was monitored by measuring shifts in the potential that occurred upon addition of various concentrations of imidazole or pyridine derivatives. The response of heme/S824C to these ligands was then compared to the response of isolated heme (without protein) to the same ligands. The observed shifts in potential depended on both the concentration and the structure of the added ligand. Small changes in structure of the ligand (e.g. pyridine versus 2-amino pyridine) produced significant shifts in the potential of the heme–protein. The observed shifts correlate to the differential binding of the N-donor molecules to the oxidized and reduced states of the heme. Further, it was observed that the electrochemical response of the buried heme in heme/S824C differed significantly from that of isolated heme. These studies demonstrate that the structure of the de novo protein modulates the binding of N-donor ligands to heme.

Keywords: Protein design; Binary patterning; Cyclic voltammetry; Redox protein; Heme protein

1. Introduction

Proteins have the ability (i) to bind target compounds with high affinity and exquisite selectivity, and (ii) to signal such binding events by a variety of mechanisms. These properties render proteins ideally suited for use in a variety of sensing applications. Redox proteins are particularly attractive as sensors because changes induced by ligand binding can be detected optically and/or electrochemically, thereby obviating the need for additional detection reagents [1–4].

Naturally occurring proteins, however, have several drawbacks that can limit their usefulness in technological applications. Natural proteins were selected by evolution to perform specific functions in particular biological environments. Because these functions–and the environments in which they evolved–may differ substantially from those required by technological applications, the 'evolutionary baggage' associated with natural proteins may render them unsuitable for incorporation into sensors or other devices. For these reasons, we have begun to explore the electrochemical properties of artificial redox proteins.

Our initial studies, reported herein, focus on the de novo protein S824C. This protein is a variant of sequence S824, which was chosen from a library of 102-residue proteins designed to fold into four-helix bundles [5,6]. This library was designed using the 'binary code' strategy, in which each position in an amino acid sequence is designed to be either polar or nonpolar, but the exact identity of each polar and nonpolar residue is not specified, and is varied combinatorially [7–9]. Initial characterization of several proteins chosen arbitrarily from this library showed that the majority of them form well-folded and/or native-like structures [5,6].

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The de novo proteins devised by the binary code strategy were not explicitly designed to bind heme. Nonetheless, protein S824 and several other proteins from the libraries do in fact bind heme [10]. We have shown previously that these de novo heme proteins (i) readily cycle between oxidized and reduced states; (ii) bind reversibly to small ligands (e.g. CO); and (iii) in some cases catalyze redox (e.g. peroxidase) chemistry [11–13].

The 3-dimensional structure of protein S824 was solved at high resolution and shown to be a well-ordered four-helix bundle [6]. Because this structure was determined in the absence of heme, the structural details of heme binding are not known. However, given the well-packed core of apo-S824, binding of the heme macrocycle must produce some structural rearrangements. In the resulting heme–protein structure, the heme is unlikely to be fully sequestered in the hydrophobic core. The resulting access to water presumably accounts for the relatively low reduction potential of S824 [13].

The reduction potential of heme/S824 was shown previously to be −0.374 V (vs. Ag/AgCl). (The value was reported as −0.174 V versus NHE [13].) This suggests that histidine (rather than methionine) binds the heme iron [13]. (Cysteine can be excluded because it does not occur in the sequence of S824.) The sequence of S824 contains 12 histidines, and these polar side chains are exposed to solvent in the NMR structure of the apo-protein (Fig. 1). Upon heme binding, structural reorganization presumably moves histidines side chains into positions consistent with binding to the partially buried macrocycle.

For the current studies, protein S824 was attached to a gold electrode via an engineered Gly–Gly–Cys linker at the C-terminal end. (The terminally modified protein is called S824C.) Heme was bound to the protein and the electrochemical response of this immobilized heme–protein was studied as a function of pH and ionic strength. Further, the electrochemical response of heme/S824C to added N-donor ligands was measured for a series of imidazole derivatives and pyridine derivatives. To assess the role of the protein structure on the properties of the bound heme, we compared our electrochemical and ligand binding results for heme/S824C with those obtained for isolated heme (without protein) immobilized on a gold electrode.

2. Results

2.1. Immobilization and characterization of de novo heme protein S824C

2.1.1. Attachment of the heme protein to a gold electrode

To facilitate attachment of the protein to a gold electrode, we modified the original sequence S824 by elongating the gene to encode a Gly–Gly–Cys tripeptide at the C-terminus of the protein. The new protein is called S824C. Our method for attaching S824C to the gold electrode is shown schematically in Fig. 2. Initially, the electrode was coated with a cystamine monolayer. The coated electrode was then reacted with the bifunctional reagent *N*-succinimidyl-3-maleimidopropionate to yield a maleimide functionalized electrode [14]. The resulting electrode was then reacted with S824C to yield the immobilized four-helix bundle. Finally, the immobilized protein was treated with Fe (III) protoporphyrin IX to generate the immobilized heme protein, as shown in Fig. 2.

2.1.2. Cyclic voltammetry of the immobilized heme protein

As shown in Fig. 3, cyclic voltammetry of heme/S824C immobilized on a gold electrode revealed a single redox wave with a formal potential (vs. Ag/AgCl/KCl) of $E°=-0.350$ V. The formal potential was calculated as the average of the cathodic and anodic peak potentials in the cyclic voltammogram (CV).

The observed formal potential for heme/S824C immobilized on the gold electrode is in close agreement with the

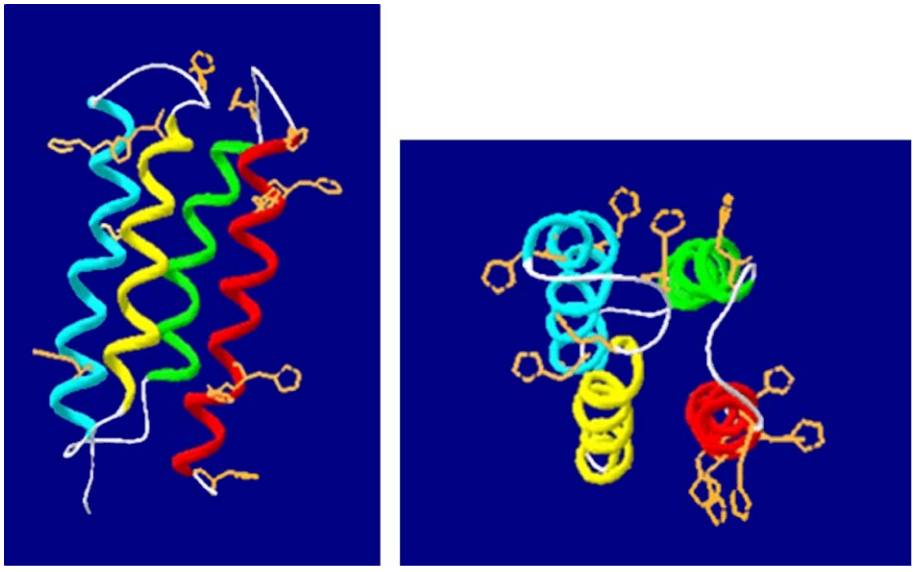

Fig. 1. (a) Ribbon diagram of the four helix bundle protein S824 showing the placement of histidine residues. (b) Head-on view of the structure showing the 12 histidine residues in orange. Note that most of the histidine residues in the apo-protein are exposed to the solvent, as specified by the binary code strategy. The NMR structure was determined in the apo form by Wei et al. [6].