Refolding of Cytochrome $b_{562}$ and Its Structural Stabilization by Introducing a Disulfide Bond

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The packing mechanism of the secondary structures (4-α-helices and 3_10-helix) of cytochrome $b_{562}$ is simulated by the “island model,” where the formation of protein structure is accomplished by the growth-type mechanism with the driving force of packing of the long-range and specific hydrophobic interactions. Packing proceeds through the formation of the structure at the nonhelical part, where a lot of hydrophobic pairs are distributed. Consequently, conformation, nearly similar to the native one, is successfully obtained. With the help of this result, the theoretical prediction of the possibility of forming this disulfide mutant (N22C/G82C) of $b_{562}$ can be performed prior to the experiments by our geometrical criterion (“lampshade”). This criterion is expected to be a significant principle for introducing possible disulfide bonds into a protein to be engineered.

KEY WORDS: Cytochrome $b_{562}$; disulfide mutant; island model; site-directed mutagenesis; 4-α-helical packing.

1. INTRODUCTION

In recent biotechnology, the design of proteins such as mutation or chemical modification has been attempted to produce useful proteins, since the higher-order structures of protein molecules specify the biological activities [Gray and Matthews (1987) and Matthews et al. (1987) for increasing thermal stability; Zhang et al. (1991) for increasing helix stability; and Eriksson et al. (1992) for regulating hydrophobic effect]. It is, however, considered to be extremely difficult to predict that the modified amino acid sequence can fold properly to yield the desired activity, and the design of proteins and their structural analyses have been carried out rather intuitively and empirically, requiring much time. For these reasons, it is desirable that the higher-order structures of protein molecules can be theoretically predicted in advance of creating proteins of the modified amino acid sequences. On the other hand, the “island model” was proposed by us to simulate the protein folding based on its mechanism, as seen in our previous works for proteins of known tertiary structure (Saitô, 1982, 1989; Saitô et al., 1988; Yoshimura et al., 1991; Watanabe et al., 1991; Kobayashi et al., 1992). This simulation method was also applied to a protein of unknown tertiary structure (Ota and Saitô, 1992). In the present paper, we study, following the island model, the refolding of cytochrome $b_{562}$ and the structure of its single disulfide mutant. We propose the principle for introducing possible disulfide bonds into a protein to be engineered.

Cytochrome $b_{562}$ is a heme protein composed of 4-α-helices (Weber et al., 1981). The function of $b_{562}$ is not yet fully known, but the information on its structure has already been obtained (Mathews et al., 1979; Lederer et al., 1981). Fortunately, the newest atomic coordinates of $b_{562}$ have just been registered in the Brookhaven Protein Data Bank by Hamada et al.
According to the X-ray analysis, four nearly parallel α-helices pack together, 310-helix is formed at the turn part, and the heme group is located among the helices (Fig. 1). Thus, this structure is relatively stable. Methods have been attempted that will allow us to fashion this protein into an organized material with the aid of this advantage. One method is to produce a stable two-dimensional crystal of b562 or one-molecule-thick films of b562 with crystalline order. Even if a monolayer is formed at the air/water interfaces, the proteins would tend to denature at the air/water interfaces and their higher-order structures might be loosen. For direct spreading of b562 molecules onto a two-dimensional surface, some modification of the protein itself for stabilizing the tertiary structure will be necessary to avoid the denaturation at the air/water interfaces. We expect that b562 can be stabilized by introducing a disulfide bond between the appropriate sites and then can be crystallized at the two-dimensional interface. Site-directed mutagenesis is now attempted for b562. Disulfide mutants were also attempted by other researchers (Perry and Wetzel, 1984, 1986; Sauer et al., 1986; Pantoliano et al., 1987; Matsumura et al., 1989; Mitchinson and Wells, 1989; Nishikawa et al., 1990) for several proteins other than heme proteins.

Our task is to find two optimal sites for introducing a disulfide bond into b562 on the computer modeling and then to examine the possibility of forming properly a disulfide bond between the two sites by the packing simulation. Before this task, however, we should understand the mechanism of packing of the native b562, which is a typical 4-α-helical protein. The 4-α-helical protein is also a suitable sample from the side of the de novo design of structurally stable proteins (Othendorf et al., 1987). In particular, an approach aimed at the design of a 4-α-helix bundle protein has recently been experimentally attempted (Regam and DeGrado, 1988). Following the mechanism of packing of the secondary structures of the native b562, we will simulate the packing of those of the mutated b562 (N22C/G82C) in order to examine the possibility of forming a disulfide mutant of b562. This mutated b562 has already been produced by means of gene technology according to our prediction, and its experimental studies are now in progress. We expect that in the near future the formation of a disulfide bond will be confirmed experimentally. In this paper, however, we focus our attention on the theoretical side of this investigation.

In the second section, we discuss theoretically the refolding of the native b562 following the method of the island model, which is studied by Saitō and collaborators. See Saitō (1982, 1989), Saitō et al. (1990), Saitō et al. (1992) for reviews, Saitō et al. (1988) for myoglobin, Yoshimura et al. (1991) for lysozyme and phospholipase, and Kobayashi et al. (1992) for bovine pancreatic trypsin inhibitor. In the third section, we suggest the optimal sites for introducing a disulfide bond into b562 by making use of the graphical criterion ("lampshade") proposed by Watanabe et al. (1991).