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Topological Chirality of Iron–Sulfur Proteins

Abstract: *An examination of x-ray structures of single-cluster [4Fe-4S] proteins in the Protein Data Bank has revealed that all redox proteins and the glutamine 5-phosphoribosyl-1-pyrophosphate amidotransferase from* Bacillus subtilis *have a topological configuration arbitrarily designated as D, whereas the DNA repair enzyme endonuclease III from* Escherichia coli *has the opposite topological configuration, L. This is the first example in which both senses of topological chirality have been observed in a class of proteins.* © *1997 John Wiley & Sons, Inc. Biopoly* ***42:*** *411–414, 1997*

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INTRODUCTION

A protein is said to be topologically chiral if its molecular graph cannot be converted into the mirror image (topological enantiomorph) by continuous deformation, such as bending and stretching. We previously showed that single- and double-cluster [4Fe-4S] proteins are topologically chiral.[1] We also observed that the two [4Fe-4S] clusters in ferredoxin from *Peptococcus aerogenes* have quasi-enantiomeric topologies. That is, the two protein substructures obtained by eliminating either one of the two clusters from the molecule belong to heterochiral classes. We concluded that ''the sense of topological chirality is not necessarily an invariant within a given structural series of proteins.'' In the present paper we provide further evidence in support of this conclusion and discuss the correlation between topological chirality and biological functions for a selected class of iron-sulfur proteins.

SINGLE-CLUSTER [4FE-4S] PROTEINS WITH REDOX AND NONREDOX FUNCTIONS

Iron–sulfur proteins[2–7] were first discovered in the late 1950s and early 1960s; these were the ferredoxins and the high potential iron proteins that participate in oxidation-reduction chemistry. It was believed initially that this redox function was the general role played by iron–sulfur clusters, but several important nonredox functions of such clusters were later found in native proteins.[8–13] As a result, iron–sulfur proteins may be historically partitioned into two categories: those possessing a redox function are in one category, and those that do not are in the other.

In the present work, we studied single-cluster [4Fe-4S] proteins in both categories in which the four iron atoms are covalently bonded to cysteine residues of a polypeptide chain. A search of the Brookhaven National Laboratory Protein Data Bank

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(PDB)[14] released on December 30, 1996, disclosed x-ray structures of 18 single-cluster [4Fe-4S] proteins[15–31] (excluding aconitase,[13] where only three iron atoms of the cluster [4Fe-4S] are covalently bound to cysteine residues of a polypeptide chain). Sixteen of these are redox proteins,[15–28] whereas the remaining 2 are nonredox proteins.[29–31] The 18 proteins are listed below, with individual structures identified in boldface by their PDB access codes.

Redox Proteins

Oxidized *Chromatium* high potential iron protein (**1HIP**[15]), oxidized high-potential iron–sulfur protein from *Ectothiorhodospira vacuolata* (**1HPI**[16]), the high-potential iron–sulfur protein from the purple phototrophic bacterium *Rhodocyclus tenuis* (**1ISU**[17]), [4Fe-4S] ferredoxin from *Bacillus thermoproteolyticus* (**2FXB**[18]), the high potential iron–sulfur protein from *Ectothiorhodospira halophila* (**2HIP**[19]), [4Fe-4S] trimethylamine dehydrogenase (**2TMD**[20]), the oxidized high potential iron–sulfur protein from *Chromatium vinosum* (**1NEH**[21]), reduced high-potential iron–sulfur protein from *C. vinosum* (**1HRQ,**[22] **1HRR**[22]), the reduced C77S mutant of the *C. vinosum* high-potential iron–sulfur protein (**1NOE**[23]), the paramagnetic high-potential iron–sulfur protein I from *E. halophila* (**1PIH,**[24] **1PIJ**[24]), the ferredoxin I from *Desulfovibrio africanus* (**1FXR**[25]), oxidized 1[4Fe-4S] *Thermatoga maritima* ferredoxin (**1ROF**[26]), 1[4Fe-4S] ferredoxin from the hyperthermophilic bacterium *T. maritima* (**1VJW**[27]), and the aldehyde ferredoxin oxidoreductase (**1AOR**[28]).

Nonredox Proteins

The glutamine 5-phosphoribosyl-1-pyrophosphate (PRPP) amidotransferase from *Bacillus subtilis* (**1GPH**[29]) and the DNA repair enzyme endonuclease III from *E. coli* (**2ABK**[30,31]).

All 18 aforementioned proteins are topologically chiral.

Except for [4Fe-4S] trimethylamine dehydrogenase,[20] which catalyzes the oxidative *N*-demethylation of trimethylamine to form dimethylamine and formaldehyde, and aldehyde ferredoxin oxidoreductase,[28] which catalyzes the reversible oxidation of aldehydes to their corresponding carboxylic acids, the 16 redox proteins are electron transfer proteins.[15–28] The [4Fe-4S] cluster in any of the redox proteins serves predominantly as an electron carrier by participating in oxidation-reduction chemistry:

$$[4\text{Fe-4S}]^{3+} + e^- \Leftrightarrow [4\text{Fe-4S}]^{2+}$$

$$[4\text{Fe-4S}]^{2+} + e^- \Leftrightarrow [4\text{Fe-4S}]^{1+}$$

where electron paramagnetic resonance spectroscopy has been widely used to distinguish between the 3+ and 1+ redox states of the [4Fe-4S] cluster.

The glutamine PRPP amidotransferase from *B. subtilis*, a homotetramer of subunits, each of which contains a [4Fe-4S] cluster, catalyzes the first committed step of de novo purine nucleotide biosynthesis.[29] The [4Fe-4S] cluster in this enzyme, however, does not have a catalytic but a structural function that ''helps orient the groups in the active site appropriately for catalysis''[12] and a regulatory role[12,29] or physiological[8] function, namely, ''the relation between the rate of purine synthesis and growth appears to be mediated by the effect of O_2 on the Fe-S cluster of this protein''[12] or ''provision of a mechanism for selective inactivation and proteolytic degradation of the enzyme during starvation.''[8]

The DNA repair enzyme endonuclease III exhibits both apurinic/apyrimidinic endonuclease activity and DNA *N*-glycosylase activity.[12,30–32] The [4Fe-4S] cluster in this enzyme, which does not involve redox chemistry and is inactive in catalysis, plays a structural role[12,32] in DNA binding. That is, the iron–sulfur cluster in the enzyme endonuclease III is essential for organizing the structure of the DNA binding loop.[30]

TOPOLOGICAL CHIRALITY AND BIOLOGICAL FUNCTION

Topologically chiral single-cluster [4Fe-4S] proteins can only have two configurations, arbitrarily denoted D and L for the purpose of the present paper. Figure 1 shows the two configurations represented by two abstracted single-cluster [4Fe-4S] proteins. Figures 1a and 1b are said to have an opposite sense of topological chirality even though the polypeptide residues may have different constitutions.

A one-to-one mapping of topological configurations and biological functions cannot be expected because there are only two configurations but at least five biological functions performed by iron–sulfur proteins: redox, catalysis, regulation, stabilization of protein structure, and biological sensor.

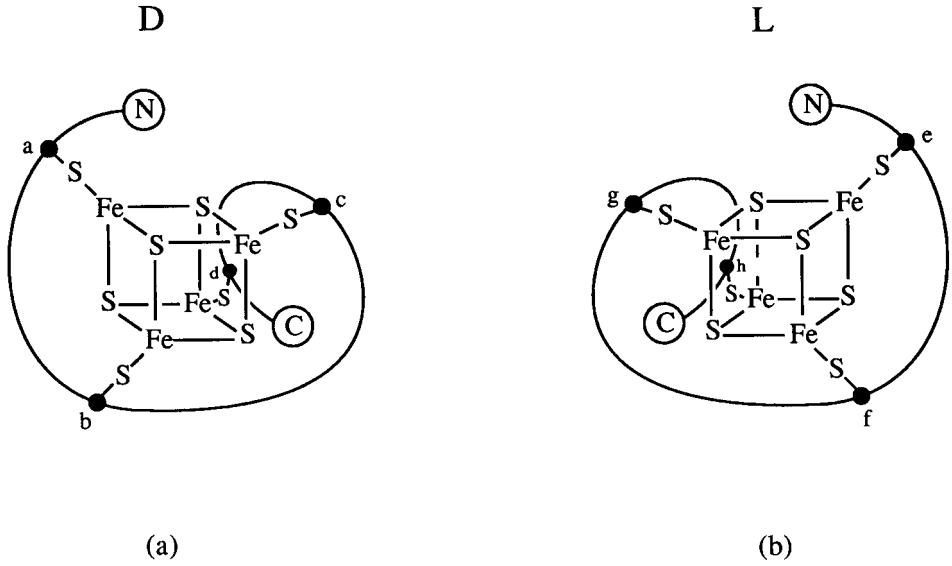

FIGURE 1 The condensed schematic representation of the two configurations of topologically chiral single-cluster [4Fe-4S] proteins. (a) D configuration. The α-carbons of the four amino acid residues that are covalently bonded to the four iron atoms in [4Fe-4S] are represented by solid circles. The residue numbers a, b, c, and d satisfy $0 < a < b < c < d$. (b) L configuration. The α-carbons of the four amino acid residues are represented by solid circles. The residue numbers e, f, g, and h satisfy $0 < e < f < g < h$.

Let us suppose that there are three topologically chiral proteins, each of which performs a different biological function. Since each structure can only belong to either D or L, at least two of three proteins must share the same topological configuration. Furthermore, two proteins sharing the same biological function may have opposite topological configurations. Thus, in general, there exists a many-to-two mapping between biological functions and topological configurations, which makes it impossible to draw a general relationship between them. For example, the glutamine PRPP amidotransferase from *B. subtilis* performs two functions—a structural function and a regulatory function (see the preceding section), yet only one configuration can obviously be assigned to this protein. In this case, the configuration is D (see Figure 1a in which a = Cys236, b = Cys382, c = Cys437, and d = Cys440). The DNA repair enzyme endonuclease III from *E. coli,* which *also* has a structural function (see the preceding section), has the L configuration (see Figure 1b where e = Cys187, f = Cys194, g = Cys197, and h = Cys203).

All 16 single-cluster [4Fe-4S] proteins in the redox category have a topological D configuration. For example, the configuration of the oxidized *Chromatium* high potential iron protein[15] is given by Figure 1a, in which a = Cys43, b = Cys46, c = Cys63, and d = Cys77. In general, Figure 1a is an abstract representation of all the remaining redox proteins. Note that the structure of C77S mutant of the *C. vinosum* high-potential iron-sulfur protein[23] is given by Figure 1a, in which a = Cys43, b = Cys46, c = Cys63, and d = Ser77, with the sulfur atom at d replaced by oxygen. As we saw, the glutamine PRPP amidotransferase from *B. subtilis* also has a topological D configuration. The only deviation from this pattern is the other nonredox protein, the DNA repair enzyme endonuclease III from *E. coli,* whose topological configuration is L. A possible explanation for this unique exception may be found in the fact that the amino acid sequence motif around the [4Fe-4S] cluster in this protein is remarkably compact as compared to other [4Fe-4S] proteins. More specifically, ''the [4Fe-4S] cluster is bound entirely within the carboxyl-terminal loop with a ligation pattern (Cys-X_6-Cys-X_2-Cys-X_5-Cys) distinct from all other known [4Fe-4S] proteins [including the glutamine PRPP amidotransferase from *B. subtilis*]. . . . Overall, the structure reveals an unusual fold and a new biological function for [4Fe-4S] clusters.''[31]

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