Multimedia in Biochemistry and Molecular Biology Education

Proteopedia: Rossmann Fold: A Beta-Alpha-Beta Fold at Dinucleotide Binding Sites

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Abstract

The Rossmann fold is one of the most common and widely distributed super-secondary structures. It is composed of a series of alternating beta strand (***β***) and alpha helical (***α***) segments wherein the ***β***-strands are hydrogen bonded forming a ***β***-sheet. The initial beta-alpha-beta (***βαβ***) fold is the most conserved segment of Rossmann folds. As this segment is in contact with the ADP portion of dinucleotides such as FAD, NAD, and NADP it is also called as an "ADP-binding ***βαβ*** fold". The Proteopedia entry on the Rossmann fold (Available at: http://proteopedia.org/w/Rossmann_fold) was generated to illustrate several structural aspects of super families of FAD and NAD(P) binding proteins: (1) The coenzymes FAD and NAD(P) share the basic adenosine diphosphate (ADP) structure. (2) The ***βαβ*** fold motif that is common to both FAD and NAD(P) binding enzymes accommodates the common ADP component of these two coenzymes. (3) In both FAD and NAD(P) binding sites, the tight turn between the first ***β***-strand and the ***α***-helix is in contact with the two phosphate groups of ADP. (4) This hairpin curve includes the first two conserved glycines (Gly-x-Gly) that allow the sharp turn of the polypeptide backbone. (5) The two ***β***-strands of the ***βαβ*** fold may constitute the core of a larger ***β***-sheet that may include up to seven ***β***-strands generally in parallel orientation. (6) The structures of segments between additional strands vary greatly and may be composed of a variety of structures such as multiple short helices or coils. © 2015 by The International Union of Biochemistry and Molecular Biology, 43(3):206–209, 2015.

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In a very large superfamily of dinucleotide binding enzymes, the domains that bind a dinucleotide such as FAD, NAD, and NADP share a common super-secondary structure that is partly composed of a series of alternating beta strand (β) and alpha helical (α) segments wherein the β-strands are hydrogen bonded forming a central β-sheet with helices on either side. There can be up seven strands in one sheet. This domain structure was named "Rossmann fold" after Michael G. Rossmann who first reported it as a common structure in a variety of nucleotide binding proteins, such as lactate dehydrogenase and flavodoxin [1].

The Rossmann fold is one of the five most common structural motifs that appear in a myriad of proteins [2, 3]. The most widely distributed protein folding motifs are thought to be remnants of most ancient protein architectures [2, 3]. In many textbooks, the Rossmann fold is defined as a $\beta\alpha\beta\alpha\beta$ structure in accordance with the original report [1]. However, elucidation of the structures of many dinucleotide binding enzymes revealed a great diversity in the overall structure of the dinucleotide binding domains. The major differences among dinucleotide binding domains of proteins include extensive divergence of the primary structure, variable number of β-strands in the complete β-sheet that forms the Rossmann fold, and large variations in the length and secondary structure of the segment that connects the second and third β-strands.

The diversity of the Rossmann fold can be illustrated by two enzymes as examples. In dogfish lactate dehydrogenase the NAD binding motif, $\beta\alpha\beta\alpha\beta$ fold, is a continuous 60 residues long segment (PDB ID: 3LDH) [4]. In contrast, in yeast D-amino acid oxidase, the FAD binding domain includes only the initial $\beta\alpha\beta$ fold in a continuous segment of 30 residues, but between the second and third β-strands there is long stretch of 121 residues that includes four helical segments and three β-strands located in a different region of the enzyme (PDB ID: 1C0I) [5].

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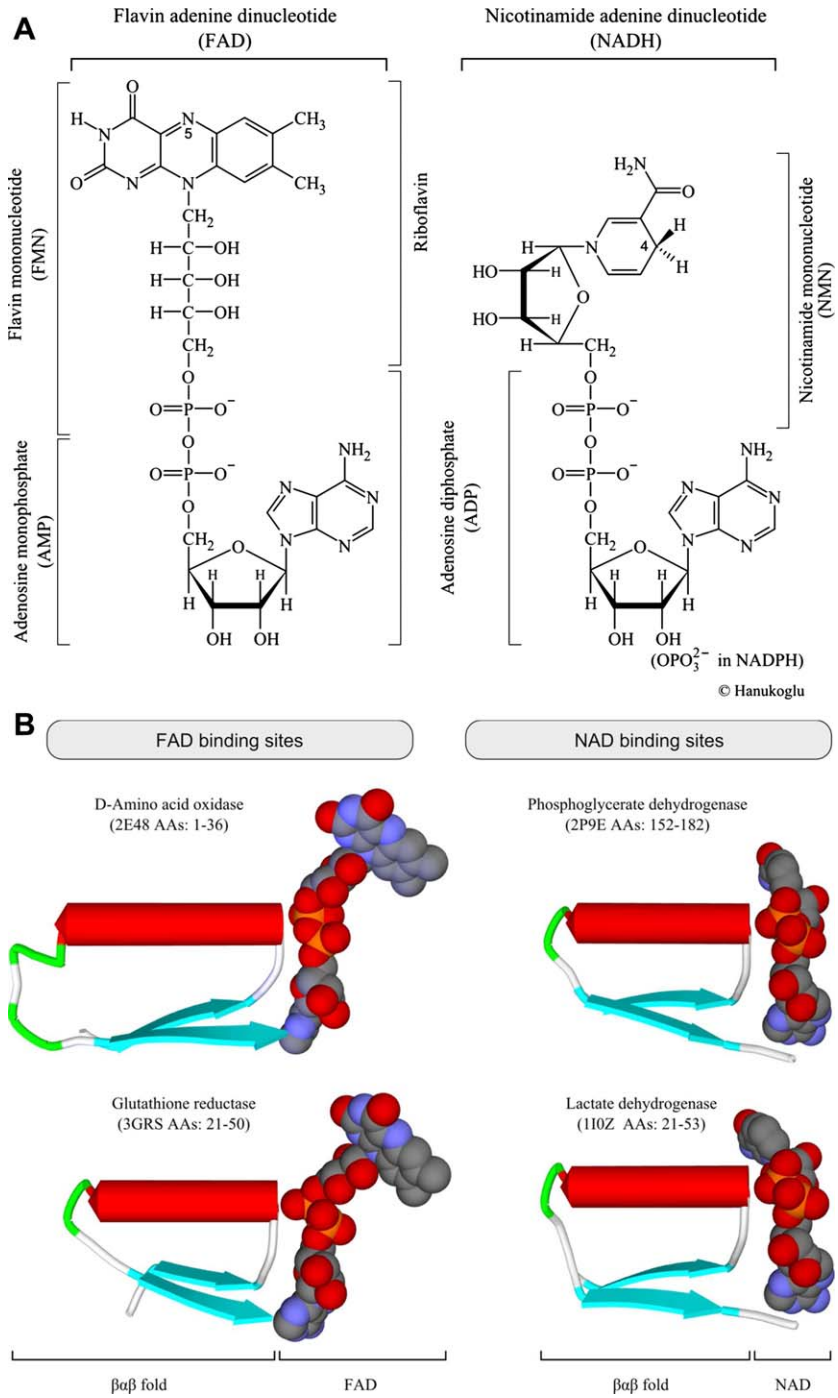

FIG 1

(a) Structures of FAD and NADH in a vertical orientation similar to that in the Rossmann folds that are shown in Fig. 1b. FAD can be viewed as a hybrid of adenosine monophosphate (AMP) + FMN or as adenosine diphosphate (ADP) + riboflavin. Note that the ADP portion is common to both FAD and NAD(P). NADP differs from an NAD by the presence of a phosphate group instead of the 2′ hydroxyl group in the adenosine ribose ring as shown at the bottom. Two atoms, N5 of FAD and C4 of NAD are marked with their number as these are the atoms that are involved in electron transfer from NADH to FAD. (b) FAD and NAD binding sites in representative enzymes. For both FAD and NAD binding sites only about 30 residues are shown (residue numbers in parentheses on the figure). These residues include the first two β-strands and the α-helix that is in between these two strands. FAD and NAD structures are shown in CPK format. Atom colors: C: gray; O: red, P: orange and N: purple. To facilitate comparison of the structures, in all four examples the helix was positioned horizontally with the β-strands below it. Example proteins are D-amino acid oxidase [12], glutathione reductase [13], phosphoglycerate dehydrogenase [14], and lactate dehydrogenase [15]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

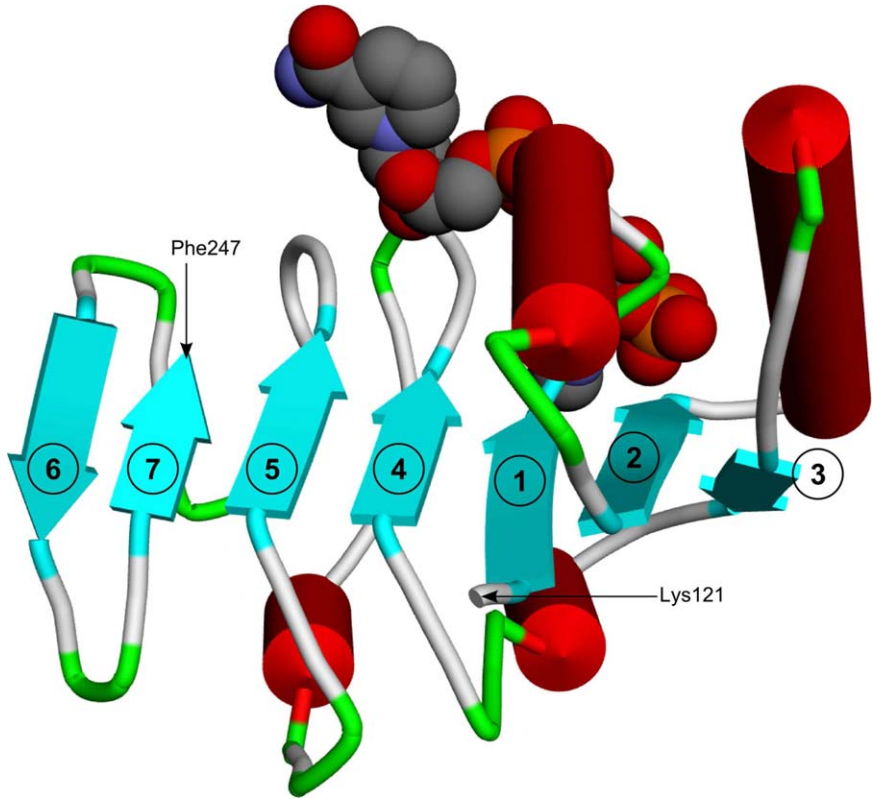

FIG 2

The orientation of the β-strands in the Rossmann fold of the NADP binding site of methylene-tetrahydromethanopterin dehydrogenase (PDB ID: 1LUA). The structure of only a continuous segment from residue Lys121 to Phe247 is shown. Note that the β-sheet is composed of seven strands. The order of the strands is 3214576 (the strand closest to the N-terminus is numbered 1 and the rest of the strands are numbered consecutively). Strand 6 is in antiparallel orientation to the other six strands. Note that the helix between strand 1 and 2 is in contact with the phosphate group of the NADP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The two examples above also illustrate a general characteristic that the initial $\beta\alpha\beta$ fold is the most conserved segment of Rossmann folds across diverse families of enzymes. Schulz et al. examining FAD binding domains of four enzymes noted that the $\beta\alpha\beta$ fold structure was associated with a specific consensus sequence of Gly-*x*-Gly-*x*-*x*-Gly (wherein *x* represents any amino acid) at the region of the tight loop between the first β-strand and the α-helix [6]. Examination of the sequence of a series of NADP binding proteins led to the discovery that in many NADP-dependent enzymes this structure is characterized by a specific consensus sequence (briefly: Gly-*x*-Gly-*x*-*x*-Ala) that differs from the NAD binding site by one residue, with an alanine instead of the last glycine [7]. Based on this finding glutathione reductase coenzyme specificity was re-engineered from NAD to NADP [8].

The Proteopedia entry on the Rossmann fold was generated to illustrate several structural aspects of super families of FAD and NAD(P) binding proteins: (1) The coenzymes FAD and NAD(P) share the basic adenosine diphosphate (ADP) structure (Fig. 1*a*). (2) The $\beta\alpha\beta$ fold motif that is common to both FAD and NAD(P) binding enzymes accommodates the common ADP component of the coenzymes (Fig. 1*b*). (3) In both FAD and NAD(P) binding sites, the tight turn between the first β-strand and the α-helix is in contact with the two phosphate groups of ADP (Fig. 1*b*). (4) This hairpin curve includes the first two conserved glycines (Gly-*x*-Gly) that allow the sharp turn of the polypeptide backbone. (5) The two β-strands of the $\beta\alpha\beta$ fold may constitute the core of a larger β-sheet that may include up to seven β-strands generally in parallel orientation. (6) The structures of segments between additional strands vary greatly and may be composed of a variety of structures such as multiple short helices or coils.

The β-strands that make up the β-sheet of the Rossmann fold are generally in a parallel orientation, and the carboxy end of each strand faces the dinucleotide. However, at the edge of the β-sheet, in some enzymes there may be a β-strand in anti-parallel orientation. For example, in the NAD binding domain of homoserine dehydrogenase (PDB ID: 1EBF) there are six strands that are ordered 321456 (the strand closest to the N-terminus is numbered 1 and the rest of the strands are numbered consecutively). Strand 3 at the edge of this sheet is anti-parallel to the rest

[9]. Another example is the NADP binding site of methylene-tetrahydromethanopterin dehydrogenase [10]. In the NADP binding site of this enzyme, the β-sheet is composed of seven strands (ordered 3214576). While six strands are parallel, only strand 6 at the edge of the sheet is in antiparallel orientation to the other six strands (Fig. 2).

The oxidoreductase enzymes that include at least one $\beta\alpha\beta$ fold to bind FAD or NAD(P) represent a large superfamily with thousands of members, e.g. [11]. Many of these proteins hardly share sequence homology e.g. [7]. But, the conservation of the canonical $\beta\alpha\beta$ fold motif in these proteins provides evidence for a common evolutionary origin of these proteins. Thus, comparison of the Rossmann fold in diverse proteins is a useful exercise to show conservation of structure despite great divergence of sequences across many families of proteins. Identification of $\beta\alpha\beta$ folds is also useful for predicting structure and function of novel proteins that are identified by genomic sequencing.

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