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Three-dimensional structure prediction of bovine AP lyase, BAP1: prediction of interaction with DNA and alterations as a result of Arg176 → Ala, Asp282 → Ala, and His308 → Asn mutations ☆

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Abstract

BAP1 is an apurinic/apyrimidinic lyase (AP lyase) that plays an important role in the repair of DNA damage. The present study deals with the prediction of the 3D structure of bovine AP lyase based on its sequence homology with human AP lyase. The predicted 3D model of bovine AP1 shows remarkable similarity with human endonuclease in the overall 3D fold. However, significant differences in the model and the X-ray structure were located at some of the important sites. We have analyzed the active center of the enzyme and other sites that are involved in DNA repair. A number of amino acids bind the bases located in the major/minor grooves of DNA. An insertion of Arg176 in the major groove and Met270 in the minor groove caps the DNA bound enzyme's active site, stabilizing the extra helical AP site conformation and effectively locking the protein onto the AP-DNA. Three BAP1 mutants were also modeled and analyzed as regards the changes in the structure. Substitution of Arg176 → Ala leads to the loss of DNA binding whereas mutation of Asp282 → Ala and His308 → Asn leads to a decrease in the enzymatic activity.

Keywords: 3D structure prediction; Homology or comparative modeling; 3D mutation prediction; AP lyase; BAP1

A predisposition to the development of certain specific and familial cancers is associated with the inheritance of a single mutated gene (primary mutation). In the best-characterized cases, this primary mutation is a loss of function consistent with the viability, but resulting in neoplastic change consequent to the acquisition of a second somatic mutation at the same locus. Such genes are referred to as tumor suppressor genes [1]. One of the examples of tumor suppressor proteins is AP lyase. BAP1, an apurinic/apyrimidinic lyase (AP lyase), is a 317 residue protein with a molecular weight of 24 kDa [2]. It is localized on chromosome 3p21.3 [3] and repairs DNA damages in vitro [4]. Although all cells possess machinery for repair of DNA and restoration of the original double helix, some DNA might escape the repair process. In general, DNA damage occurs through misincorporation of deoxynucleotides, deamination of bases during normal genetic functions, X-ray radiation causing nick in the DNA, ultraviolet radiation leading to thymine dimer formation, and interaction of various chemicals with DNA [5,6]. Other DNA damaging agents are anti-tumor drugs such as bleomycin and neocarzinostatin or those that generate oxygen radicals producing a variety of lesions in DNA. Active oxygen species (hydrogen peroxide and hydroxyl radicals) are a continuous threat to cell integrity due to the potential to damage DNA, membranes, and proteins. These species are

☆ The coordinates of the homology models are deposited in the PDB with Accession Nos. 1LYR (wild type) and 1XZR, 1XZS, and 1XZT (mutated models).

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generated by ionizing radiation and cause strand breaks in the DNA. The strand breaks contain 3′ blocking groups such as phosphates and phosphoglycolates which inhibit DNA replication [7–9]. Recent studies indicate that inherited hypersensitivity to ionizing radiation may contribute to human breast carcinogenesis [10]. A variety of oxidative DNA lesions are formed as a consequence of attack on the nitrogenous bases and sugar phosphate backbone. The development of repair mechanism for such lesions aids the success of aerobic life [8,11]. The most common lesion in DNA is the AP site. These sites are formed in DNA by spontaneous hydrolysis of the *N*-glycosidic bond and from the action of DNA glycosylases on the modified bases. The hydrolytic cleavage of the *N*-glycosidic bond to produce apurinic/apyrimidinic sites is quantitatively the most significant structural abnormality to arise in cellular DNA. It has been estimated that a mammalian cell loses up to 10,000 purines per day from its genome [12]. AP sites are both cytotoxic and highly mutagenic due to lack of coding information, and given the stability and frequency of formation, the repair of AP site is essential to cell viability [13]. Any delay in the repair of AP sites provides DNA polymerases the opportunity to induce replication through these non-instructional lesions, resulting in frequent mis-incorporation [14]. Rapid repair of DNA damages is necessary not only due to its lethality to the cell but also in view of its potential to cause mutations resulting in abnormal cell growth [5]. AP sites are specifically corrected by the base excision repair (BER) pathway [15]. These AP sites are recognized by an AP endonuclease, which cleaves the phosphodiester backbone 5′ to the AP site leaving the 3′-hydroxyl nucleotide and 5′ deoxyribose phosphate (5′-dRP) as termini. To complete the repair process, 5′-dRP moiety must be removed. This is frequently accomplished by a β-elimination reaction catalyzed by an AP lyase [16].

In the present study, we have predicted the 3D structure of bovine AP lyase by means of homology modeling that deals with the structure prediction based on the sequence homologies with the proteins of known structures. We have discussed the active center of enzyme and AP sites involved in DNA repair. We have also modeled three BAP1 mutants and analyzed the changes that take place in the models.

Methods

Sequence retrieval and comparison. Amino acid sequence of bovine AP1 [2,17] was retrieved from SWISS PROT databank [18]. The sequence was submitted for search against Protein databank, PDB [19], using Gapped BLAST and PSI-BLAST algorithm [20]. Human APE1 bound to abasic DNA; pdb id; 1dew [21] showed highest homology (95%) with a large fragment of BAP1 (39–317). This fragment matched with the residues 1–279 of 1dew. For model building of bovine BAP1 (residues 39–317), 1dew was used as the template.

Model building. Three-dimensional model of bovine AP1 was constructed using the crystal coordinates of 1dew. The automated homology model building was performed using the protein structure-modeling program MODELLER 4 [22]. Each model was selected after many runs of the MODELLER (more than 10) in order to achieve the most plausible model satisfying many stereochemical criteria. At each step, the models built by the MODELLER were checked by PROCHECK and the final model was chosen that fits or nearly fits the criteria of an accepted model. The inputs of MODELLER consisted of: (i) the aligned sequences of the target to be modeled and the template, (ii) a command file with co-ordinates of the templates, and (iii) a command file that gives various instructions to the MODELER about model building. The output contained a model for the unknown structures containing all non-hydrogen atoms. Reliability of the predicted homology models was assessed by the ENERGY command of the MODELER. Evaluation was further carried out by the program, PROCHECK [23]. Hydrogen bonding, surface accessibilities, and ion pairs in the homology model were calculated by HBONDS, ACCESS, and ANACON menus of WHATIF [24]. Mutations in the model were performed by the WHAT IF server at http://swift.cmbi.kun.nl/WIWWWI/.

DNA binding. To study the intermolecular interactions like ion pairs, hydrogen bonds, etc., between BAP1 and its bound DNA, the structure of 1dew (protein plus bound DNA) was superimposed on the 3D model of BAP1 generated by MODELLER 4. The structural coordinates of 1dew were then manually deleted. The resultant is the 3D model of BAP1 and DNA in exactly the same orientation as in case of the original X-ray structure. The possible interactions between the two were then observed in terms of hydrogen bonds and ion pairs in order to understand the involvement of specific residues in BAP1–DNA interactions.

Results and discussion

General model description

The schematic representation of the homology model of bovine BAP1 showing the arrangements of secondary structural elements is depicted in Fig. 1. The overall protein fold of BAP1 strongly resembles that of the template. BAP1 is a globular α/β protein consisting of two domains that display similar topologies with each other comprising a six stranded β sheet surrounded by α helices, which pack together to form four layered α/β sandwich. The Ramachandran plot of the model shows that 88.4% residues lie in the most favored region, 11.2% residues in the allowed region, and 0.4% in the generously allowed region. No residue lies in the disallowed region of the plot.

Predicted mechanism of action

Analysis of amino acid sequences of BAP1 and 1dew shows that some of the active site residues are conserved [25,26]. In the 3D model and X-ray structures, the active site is located in a pocket at the top of α/β sandwich and is surrounded by loop regions. In the active site of BAP1 model, the H-bonding pattern is conserved with the imidazole ND1 of His308 interacting with the carboxylate (OD1) of Asp282 (also through ion pair formation).