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Minireview

The A-loop, a novel conserved aromatic acid subdomain upstream of the Walker A motif in ABC transporters, is critical for ATP binding

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Abstract **ATP-binding cassette (ABC) transporters represent one of the largest families of proteins, and transport a variety of substrates ranging from ions to amphipathic anticancer drugs. The functional unit of an ABC transporter is comprised of two transmembrane domains and two cytoplasmic ABC ATPase domains. The energy of the binding and hydrolysis of ATP is used to transport the substrates across membranes. An ABC domain consists of conserved regions, the Walker A and B motifs, the signature (or C) region and the D, H and Q loops. We recently described the A-loop (*A*romatic residue interacting with the *A*denine ring of *A*TP), a highly conserved aromatic residue ~25 amino acids upstream of the Walker A motif that is essential for ATP-binding. Here, we review the mutational analysis of this subdomain in human P-glycoprotein as well as homology modeling, structural and data mining studies that provide evidence for a functional role of the A-loop in ATP-binding in most members of the superfamily of ABC transporters.**

Keywords: ABC transporter; ATP hydrolysis; Nucleotide-binding domain; Homology modeling; Multidrug resistance; Sequence alignment

1. Introduction

ATP-binding cassette (ABC) transporters constitute one of the largest protein families present in all taxonomic groups [1,2] and play a central role in the transport of a variety of substrates ranging from simple ions to complex toxins and natural product anticancer agents across cell membranes using the energy of ATP hydrolysis [3]. It is thus not surprising that 18 of the 48 human ABC transporters are implicated in the clinical manifestation of diseases [2]. The basic architecture of an ABC protein consists of two nucleotide-binding domains (NBDs) or ABCs and two transmembrane (TM) domains, which are involved in the transport of substrates. Though there is little sequence homology between the TM domains of different ABC transporters, the NBDs are highly conserved and are composed of several distinct sequence motifs [4]. A typical ABC of 200–220 amino acids contains several conserved subdomains, including the Walker A and B motifs, the signature region (also called the LSGGQ motif, linker peptide, or C region) and the D, H and Q loops [5]. Though these motifs were initially recognized on the basis of sequence homology, site-directed mutagenesis and structural studies have elucidated the role(s) of several residues in these subdomains. Moreover, the functional ABC appears to be a 'nucleotide-sandwich dimer' [6] with ATP flanked by the Walker A and B motifs of one ABC and the signature motif and D-loop of the other. A schematic showing the functional catalytic site formed by residues in conserved subdomains in both NBD1 and NBD2 of human P-glycoprotein (Pgp) with ATP at the interphase of both sites is given in Fig. 1. This schematic was generated based on MJ0796 E171Q dimer structure, sequence alignment and the mutational analyses of these residues in NBDs of mammalian Pgps by several groups [6,7].

Several amino acids in these conserved motifs have been shown to play a role in ATP binding [7,8], coordination with Mg^{2+} [9], ATP hydrolysis [8,10,11] and communication between the NBDs and the transport substrate sites [12]. In addition, resolution of structures of some of the ABC proteins by X-ray crystallography shows that a conserved aromatic residue stacks against one side of the adenine ring through π–π interactions. It has not, however, been clear whether there is a conserved subdomain in ABC transporters that facilitates such interactions. Here we review: (i) the NBD structures of ABC proteins and the interactions between the aromatic residue and the adenosine ring of ATP; (ii) recent sequence homology studies that identify a conserved aromatic residue that represents a previously unrecognized, well conserved subdomain in the ABC; (iii) site-directed mutagenesis studies of this aromatic acid ("A-loop") and biochemical characterization of these mutants in Pgp (ABCB1).

2. Interactions between aromatic residues of ABC proteins and the adenine ring of nucleotides

A structure of the NBD domain of histidine permease (HisP) reported in 1998 was the first high-resolution structure of an ABC domain [13]. This structure showed that the major contact between the adenosine ring of ATP and HisP occurred via π–π interactions with a conserved tyrosine residue (Tyr

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Abbreviations: ABC, ATP-binding cassette; CFTR, cystic fibrosis transmembrane regulator; NBD, nucleotide-binding domain; Pgp, P-glycoprotein; TM, Transmembrane domain

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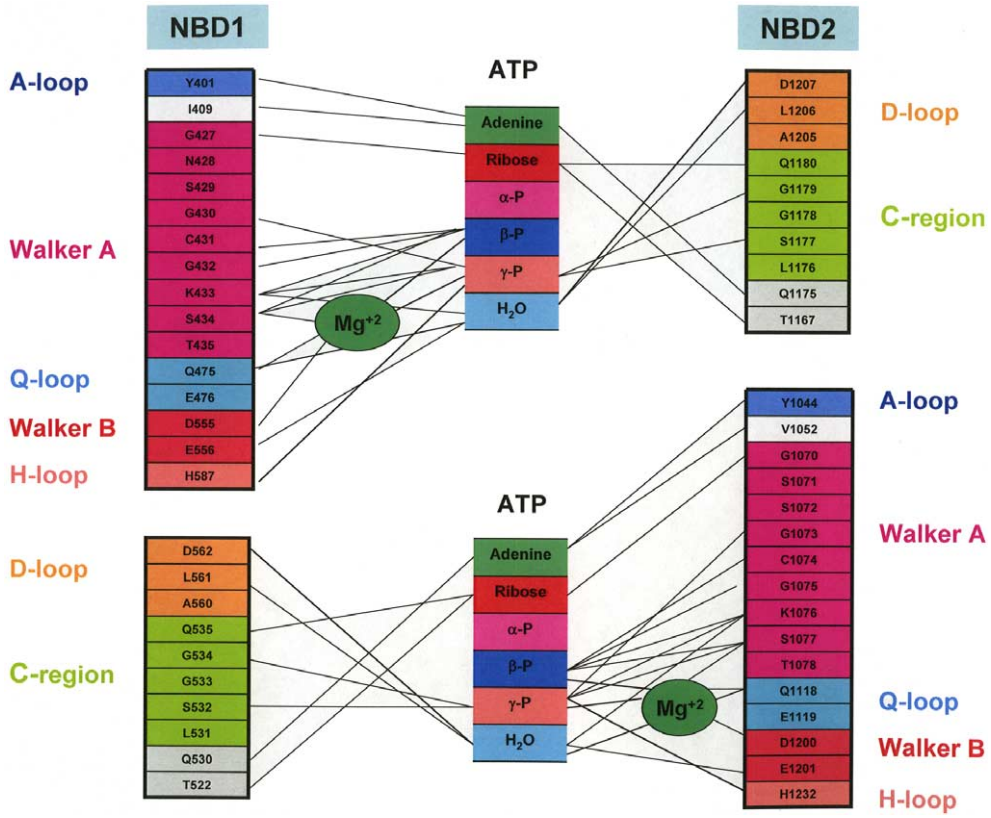

Fig. 1. Schematic showing residues in conserved subdomains of NBD1 and NBD2 of human Pgp interacting with ATP. ATP is sandwiched between the Walker A and B, and A, Q and H loops of the NBD1 and the C (signature) region and D-loop of the NBD2 (top) and vice versa (bottom). The diagram was generated based on the structure of the E171Q mutant MJ0796 NBD dimer [6], sequence alignment, and mutational analyses of a majority of these residues in mammalian Pgps by several groups (Table 1 in [7]).

16). This was consistent with previous mutational studies that demonstrated that transport function and ATP hydrolysis were abolished when Y16 was substituted with S [14]. Subsequently, the crystal structures of both free and nucleotide-bound NBDs of several ABC transporters have been reported. It has been shown that in HlyB [15], MsbA [16], MJ0796 [17] and Tap1 [18] NBDs a tyrosine residue interacts with the adenine ring of ATP. Similarly, structures of MJ1267 [19] and GlcV [20] show that a phenylalanine residue is involved in the recognition of the adenine base. Finally, the structure of the MalK NBD indicates that the adenine base interacts with a tryptophan residue through van der Waals contacts [21]. Thus, analysis of structures of nucleotide-bound ABCs demonstrates that the adenine ring of the nucleotide interacts with an aromatic residue.

Recently Mao and coworkers [22] used a data mining and quantum chemical approach to identify residues involved in adenine binding in nucleotide-binding proteins. Their study identified three principal forms of interaction between the adenine base and the nucleotide-binding pocket: (i) hydrogen bonding, (ii) π–π stacking and (iii) cation–π interactions. This study endeavored to quantify the relative importance of these interactions and determined that on average, there are four non-bonded intermolecular interactions required for binding the adenine base to a protein: 2.7 hydrogen bonding interactions, 1.0 π–π stacking interaction, and 0.8 of a cation–π interaction between the adenine base and the protein. Most significant, however, was the conclusion that there is a complementary relationship between hydrogen bonding and the other two forms of non-bonded intermolecular interactions. Stacking interactions that involve aromatic residues from both enzyme and substrate are thus a critical component of the binding of nucleotides to ATP-binding proteins. Based on the individual structures of NBDs and the molecular characteristics for nucleotide-binding, it would be reasonable to expect that a conserved aromatic residue is an integral part of the ABC, and is involved in the recognition of the adenine ring of ATP.

3. A highly conserved aromatic residue subdomain within the ABC

Recently we have taken a comprehensive approach to identifying conserved aromatic residues that may have interactions with the adenine base of ATP in ABC transporters.[1] The first step was to mine the non-redundant protein database for matches to the pattern characteristic of distinct conserved motifs for the ATP-binding site. This search identified a total of 18 514 domains, and matched regions (~25–30 residues upstream of the Walker A motif to ~175 residues downstream) were combined into a multiple alignment. The alignment showed that 15 614 of these domains (84.3%) contain an aro-

[1]Kim, I.W., Peng, X.H., Sauna, Z.E., FitzGerald, P.C., Xia, D., Muller, M., Nandigama, K. and Ambudkar, S.V., submitted for publication.

matic residue at a position 25 residues upstream of the Walker A motif and this number rises to 16 312 or 88.1% if one includes an aromatic residue at a position 25 residues upstream of the Walker A and offset ± 2. Similarly, in a subset of eukaryotic proteins, we identified 3024 domains, 87.6% of which contain an aromatic residue at position 25 ± 2 upstream of the Walker A motif. Though the basic unit of an ABC transporter contains one NBD and one TM domain, the functional protein consists of two units that are either part of a single polypeptide chain or composed of a homo- or hetero-dimer. Table 1 shows the sequence alignment of 23–27 residues upstream of the Walker A motif in the ABCs of all 48 human and selected plant, fungal, and bacterial ABC transporters. Most of these sequences have an aromatic residue located at a position 25 amino acids upstream of the Walker A motif. Among those ABC transporters that contain both NBDs on the same polypeptide chain, the conserved aromatic residue in the C-terminal NBD (NBD2) is almost always a tyrosine, while the N-terminal NBD (NBD1) has either tryptophan or phenylalanine residues in addition to the tyrosine. This is vividly illustrated in a graphical representation of a motif in the form of a Sequence Logo [24,25] generated for this region (Fig. 2). The sequence of the region 23–27 amino acids upstream of the Walker A motif of the NBD1 from all full-length transporters and the NBD of half-transporters (total 59) were grouped together, and the sequence of similar regions from the NBD2 of 37 full-length transporters were used for the generation of the Sequence Logo. It is important to note that the aromatic residues identified as interacting with the adenine moiety of the nucleotide in the X-ray crystallographic studies (see[1] for a complete list), are the ones documented as the highly conserved aromatic residues by large-scale data mining, sequence alignment, and also the Sequence Logo (Fig. 2). Thus, the aromatic residue that stabilizes binding of the adenosine ring of ATP constitutes a highly conserved subdomain with a loop structure located at a position 25 residues upstream of the Walker A motif. This conserved subdomain also appears to mark the amino terminus boundary of the NBD (Fig. 1). To emphasize its importance, we named this conserved subdomain the A-loop (*A*romatic residue interacting with *A*denine base of *A*TP). However, it is important to note that the aromatic acid upstream of the Walker A domain is not conserved in all ABC transporters or in nucleotide-binding protein containing the Walker A (P-loop) motif. Some of the exceptions include members of the Rad50/SMC and MutS/MSH subfamilies, which do not exhibit any transport function but are involved in various DNA repair and maintenance functions [26,27]. In addition, in the human ABCD subfamily the aromatic acid is replaced with either an I or L residue (Table 1). This is not surprising, as different nucleotide-binding proteins interact with the ribose and nucleotide base in a different manner [18,22].

4. Mutational studies with tyrosine 401 and 1044 residues in the A-loop of NBDs of Pgp

The conserved tyrosine or other aromatic residues described above have not previously been considered as a conserved subdomain of the ABC. However, several groups have studied the biochemical effects of mutating this residue in several ABC transport proteins. As stated above, the Y16S mutation in the HisP subunit of the bacterial histidine permease was found to abolish the binding of ATP and its transport function [14],

Table 1
Sequence alignment of a region 23–27 amino acids up-stream of the Walker A motif in nucleotide-binding domain of 48 human and selected plant, fungal, and bacterial ABC transporters

ABC transporter	NBD1	NBD2
ABCA sub-family		
ABCA1 (ABC1)[a]	K V **Y** R D	K I **Y** R R
ABCA2 (ABC2)	K V **Y** K D	K V **Y** K S
ABCA3 (ABC3, ABCC)	K V **F** R V	K V **Y** E Q
ABCA4 (ABCR)	K I **F** E P	K I **Y** L G
ABCA5	K T **Y** R K	K E **Y** D D
ABCA6	K E **Y** K G	K E **Y** A G
ABCA7	K R **F** P G	K V **Y** R G
ABCA8	K E **Y** K G	K E **Y** A G
ABCA9	K E **Y** A G	K E **Y** A G
ABCA10	K E **Y** N G	K E **Y** Y E
ABCA12	K I **Y** G S	K T **Y** Q L
ABCA13	K E **Y** E G	K H **Y** R R
ABCB sub-family		
ABCB1 (MDR1)	F S **Y** P S	F N **Y** P T
ABCB2 (TAP1)[b]	F A **Y** P N	
ABCB3 (TAP2)[b]	F A **Y** P N	
ABCB4 (MDR3)	F S **Y** P S	F N **Y** P T
ABCB5	L S **Y** S I	F F **Y** P C
ABCB6 (MTABC2)[b]	F S **Y** A D	
ABCB7 (ABC7)[b]	F E **Y** I E	
ABCB8 (MABC1)[b]	F S **Y** P C	
ABCB9[b]	F T **Y** R T	
ABCB10(MABC1)[b]	F A **Y** P A	
ABCB11 (BSEP)	F H **Y** P S	F T **Y** P S
ABCC sub-family		
ABCC1 (MRP1)	F T **W** A R	L R **Y** R E
ABCC2 (MRP2)	F T **W** E H	V R **Y** R P
ABCC3 (MRP3)	F T **W** A Q	V R **Y** R P
ABCC4 (MRP4)	A F **W** D K	F M **Y** S P
ABCC5 (MRP5)	S P **E** E E	M R **Y** R E
ABCC6 (MRP6)	F A **W** S Q	L R **Y** R P
ABCC7 (CFTR)	L F **F** S N	A K **Y** T E
ABCC8 (SUR1)	F T **W** T P	V R **Y** D S
ABCC9 (SUR2)	F T **W** T P	V R **Y** D S
ABCC10 (MRP7)	F S **W** D P	L A **Y** R P
ABCC11 (MRP8)	G P **E** E E	M K **Y** R D
ABCC12 (MRP9)	G P **E** E Q	M R **Y** R D
ABCD sub-family		
ABCD1 (ALD)[b]	I P **I** V T	
ABCD2 (ALDL1)[b]	V P **I** I T	
ABCD3 (PXMP1)[b]	V P **L** A T	
ABCD4 (PMP69)[b]	V S **I** S A	
ABCE sub-family		
ABCE1 (OABP)[c]	H R **Y** C A	Y K **Y** P G
ABCF sub-family		
ABCF1 (ABC50)[c]	E K **F** S I	F G **Y** Q G
ABCF2[c]	I N **L** S L	F K **Y** T K
ABCF3[c]	E N **F** D V	F Y **Y** D P
ABCG sub-family		
ABCG1 (White)[b]	P W **W** R K	
ABCG2 (MXR, BCRP)[b]	S G **F** L P	
ABCG4 (White 2)[b]	P C **W** R K	
ABCG5 (White 3)[b]	P W **W** D I	
ABCG8 (White 4)[b]	M P **W** T S	
Selected plant, fungal and bacterial ABC transporters		
AtPGP (*A. thaliana*)	F S **Y** P S	F S **Y** P S
AtMRP1 (*A. thaliana*)	F S **W** D S	L R **Y** R P
Ste6 (*S. cerevisiae*)	F S **Y** P S	F A **Y** P S
Pdr5p (*S. cerevisiae*)[d]	R K **F** Q R	L C **Y** E V
Cdr1p (*C. albicans*)[d]	R H **F** Q K	L T **Y** Q V

(*continued on next page*)

Table 1 (*continued*)

ABC transporter	NBD1	NBD2
LmrA (*L. lactis*)[b]	F A Y D D	
MalK (*E. coli*)[e]	K A W G E	
HlyB (*E. coli*)[e]	F R Y K P	
MsbA (*E. coli*, *S. typhimurium*)[b]	F T Y P G	
HisP (*S. typhimurium*)[e]	K R Y G G	
MJ0796, LolD (*M. jannaschii*)[e]	K T Y K M	

The sequences of 23–27 residues upstream of the Walker A motif in the NBDs of 48 human and selected plant, fungal, and bacterial ABC transporters were manually aligned (NBD, PSSM-Id: 5341, cd00267; http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=cd00267). Boldface and underlined letters indicate the residues at the equivalent position of Y401 in NBD1 and Y1044 in NBD2 of human Pgp.
[a]The most common alternate name for human ABC transporters is given in parentheses. For transporters from other organisms, the species name is given in italics in parentheses.
[b]Half transporter with one TM domain and one NBD.
[c]Lacks TM domains.
[d]The sequences at a position ~25–29 residues upstream of the Walker A.
[e]Subunit containing only one NBD.

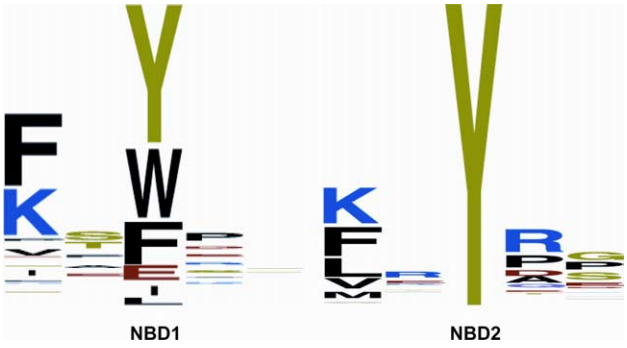

Fig. 2. Sequence Logo of 23–27 residues upstream of the Walker A motif in the nucleotide-binding domain of 48 human and selected plant, fungal, and bacterial ABC transporters. A graphical representation of a motif based on alignment of residues 23–27 upstream of the Walker A in the NBD1 (*left*) and NBD2 (*right*) of all (48) human and selected plant, fungal and bacterial ABC transporters (see Table 1), was generated using WebLogo software (URL: http://weblogo.berkeley.edu/). For the analysis, the sequences of the NBD1 of all full-length transporters and the NBD of half transporters were grouped together (total 59). The sequence of the same region of the NBD2 of 37 full-length transporters was used to generate the Sequence Logo of this site. Each Sequence Logo consists of stacks of symbols, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position [24,25]. Although any one of the aromatic residues (Y, W or F) can be found in the NBD1, only the Y residue is conserved at this position in the NBD2 of all full-length ABC transporters.

biochemical consequences that are consistent with the structure of HisP described above. We recently carried out a comprehensive site-directed mutagenesis of the conserved tyrosine residue in both NBDs of Pgp.[1] In this study, the conserved tyrosine residues in both NBDs were mutated either singly or together and the Y was replaced with a W, F, C or A. The W and F represent alternative aromatic residues, the A represents a non-conservative mutation and the C represents a polar substitution. We systematically evaluated the effect of these mutations on (i) cell-surface expression, (ii) nucleotide binding, (iii) vanadate-induced trapping of nucleotides, (iv) ATP hydrolysis, and (iv) drug transport in intact cells. None of the mutations affected cell-surface expression of the protein, permitting the characterization of the protein–nucleotide interactions for all these substitutions. Substitution of Y401 and Y1044 with C and A at either NBD significantly decreases or abolishes nucleotide binding, trapping and hydrolysis and also affects transport function. On the other hand, the Y → F/W substitutions have no effect on transport function, consistent with previous results that show that substitution with alternative aromatic amino acids are tolerated at this location [6]. Consistent with these findings, it has been demonstrated in wild-type hamster Pgp that the photoaffinity nucleotide, [α-^{32}P] 8-azido-ADP, was crosslinked to the Y398 and Y1041 residues [28]. When the effect of selected mutations on the kinetics of nucleotide binding and/or hydrolysis was also determined, we found that though conservative mutations supported ATP hydrolysis, the kinetic properties were significantly affected. For example, the substitution of Y401 with W resulted in an increase in the K_m (ATP) during ATP hydrolysis and an increase in the K_d (TNPATP) in a binding assay using the fluorescent analog of ATP, TNPATP.[1] Similar results were also reported for the Y477W mutant of bacterial HlyB-NBD [29]. These studies suggest that the Y401 and Y1044 residues in each NBD may be replaced with alternative aromatic residues (F or W) yet continue to retain ATP binding and hydrolysis and transport function. This is because these conservative replacements would permit the molecular interactions described above. However, replacement of Y401 or Y1044 with non-aromatic residues abolishes these interactions, resulting in loss of ATP binding and hydrolysis. The results are thus consistent with our findings from the data mining-sequence alignments and the molecular determinants for ATP-binding based on crystallographic structures and provide the detailed biochemical analysis for the role of a conserved aromatic residue in most ABC transport proteins.

The data in the literature, however, also suggest that the importance of the conserved aromatic residues may also be related to the role of the NBD in which they are located. For example, substitution of F430 with S in the NBD1 of human cystic fibrosis transmembrane regulator (CFTR), does not affect chloride channel function [30]. The tolerance to this mutation may have a structural basis, as F430 does not stack with the adenine ring of the nucleotide but instead makes an edge-to-face contact [31]. In the case of ABC transporters that are ion channels (such as CFTR), the requirement of ATP hydrolysis to power the movement of ions is highly controversial, and recent work suggests that CFTR exhibits adenylate kinase activity (see [32] for a review and a discussion of the energetics of the chloride channel). MRP1 (ABCC1) belongs to the same subfamily (ABCC) as CFTR. Zhao and Chang studied the effect of mutations of the conserved aromatic residues in both NBDs of MRP1 on nucleotide binding and LTC4 transport [33]. They found that like CFTR, the substitution of W653 or Y1302 with C is tolerated in MRP1 but with significant change in the kinetic properties of ATP hydrolysis.

5. Homology model of the NBDs of Pgp: interactions between the conserved subdomain, A-loop and ATP

Although a high-resolution structure of the NBDs of Pgp is currently not available for a verification of the above findings at the structural level, there is a significant degree of conserva-

tion in the NBD sequence of Pgp and other ABC transporters, including the following whose crystal structures can be found in the Protein Data Base: MJ1267 and MJ0796 from *M. jannaschii*, MalK, MsbA, BtuD and HlyB of *Escherichia coli*, MalK of *T. litoralis*, HisP from *S. typhimurium*, MsbA from *V. cholera*, human TAP1, and mouse CFTR [6,13,15,16,18,19,21,31,34–37]. Conservations in the NBD sequences are most pronounced for the Walker A and B motifs, and in the signature LSGGQ motif. These conserved sequence segments could provide strong constraints for modeling. Since a number of NBD structures were already available ranging from prokaryotes such as *E. coli* to eukaryotes including humans, these structures were aligned to generate a more precise, structure-based sequence alignment, against which the N-and C-terminal NBDs of Pgp sequences were aligned. With this alignment, additional conserved sequence motifs such as the A-, Q-, D-, and the H-motifs could be precisely modeled. By homology modeling, we mapped the sequence of the Pgp N-and C-terminal NBDs onto the two subunits of the dimeric E171Q mutant of the MJ0796 ABC [6]. The amino acid sequence of NBD1 and NBD2 of human Pgp is 29.3% and 25.7% identical and 48.9% and 45.1% similar, respectively, to the sequence of the NBD of MJ0796. The model was then manually adjusted for side-chain clashes and for accommodation of deletions and insertions. The resulting dimeric NBD was subjected to a few cycles of molecular dynamics in the CNS program to remove unfavorable interactions present in the initial model and to conform to the standard stereochemistry values of bond angles, bond lengths, and dihedral angles. The final NBD model was checked with the program PROCHECK. The positions of ATP molecules and Mg^{2+} ions were derived from the consensus positions determined by a multiple structure alignment (although in dimeric structure of E171Q MJ0796 NBD Na^{+} is present instead of Mg^{2+}, we have modeled Mg^{2+} based on biochemical studies with E556/1201Q double mutant of Pgp, which is similar to E171Q mutant of MJ0796, failed to trap nucleotide in the presence of Na^{+}; Sauna and Ambudkar, unpublished data). The atomic model of dimeric NBDs of Pgp (Fig. 3) was built with the program O [38]. As shown in Fig. 3, ATP is present at the inter-phase of the nucleotide-sandwich dimer formed by the Walker A and B motifs of NBD1 and the signature region and the D-loop in NBD2. It is important to note that though two bound ATP molecules are shown to be similar to the E171Q mutant MJ0796 dimer, convincing experimental evidence for Pgp even with the E556/1201Q mutation is still lacking. The biochemical evidence supports the binding or occlusion of one molecule of ATP or ADP in both wild-type and mutant protein. The signature motif in each NBD of Pgp has been shown to be in close proximity to the Walker A motif of the other NBD [39]. Analysis of the conserved residues which form the ATP-binding pocket (see expanded panel in Fig. 3) clearly indicates that Y401 in NBD1 stacks against the adenine base of ATP, possibly through π–π interactions. The Y401 residue is stabilized by T435 in the Walker A motif, most likely through van der Waals contacts (the distance between OH of Y401 and C of T435 is 4.1 Å). Consistent with this model, T435 in the Walker A motif is highly conserved in avian and mammalian Pgps as well as in other ABC transporters. The adenine ring of ATP on the other side is in close contact with the Q1175 residue (distance 3.8–4.2 Å), which is located just upstream of the signature region of NBD2. Both Q530 in NBD1 and Q1175 in

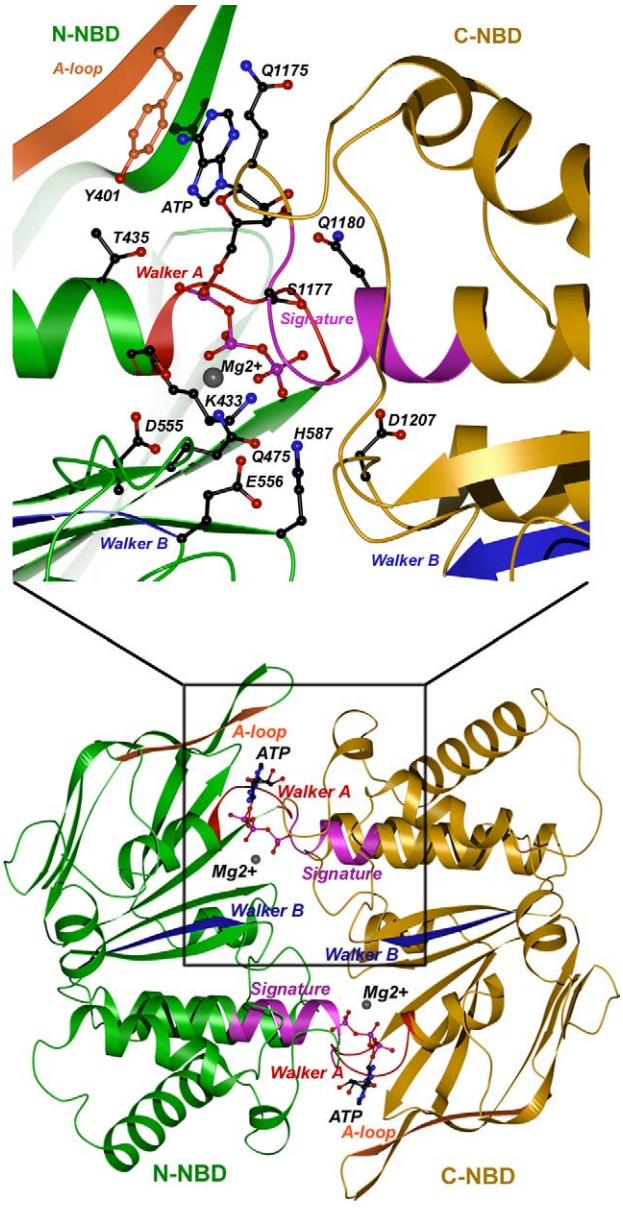

Fig. 3. Model structure of the dimeric Pgp nucleotide-binding domain showing conserved residues involved in ATP-binding. The atomic model of the dimeric NBDs of Pgp was based on the coordinates from the crystal structure of the MJ0796 E171Q dimer (PDB code: 1L2T) [6] by mapping the N- and C-terminal NBD sequence to either subunits, respectively. The model was built with the program O [38]. The resulting dimeric NBD was subjected to a few cycles of molecular dynamics in the CNS program to remove unfavorable interactions present in the initial model and to conform to the standard stereochemistry values of bond angles, bond lengths, and dihedral angles and the final dimeric NBD model was checked with the program PROCHECK. The N-terminal NBD is depicted as a ribbon diagram in green; and the C-terminal NBD is shown in gold. Functionally important sequence motifs are highlighted in different colors. The Walker A motif appears in red, the Walker B motif blue, the signature motif magenta and the A-loop brown. The modeled ATP is shown as a ball-and-stick model with carbon atoms in black, oxygen red, nitrogen blue and phosphorous magenta. The Mg^{2+} ion is shown as a metallic ball. The conserved residues that are part of the ATP-binding environment are shown in the expanded panel. The Y401 residue of the conserved subdomain, A-loop (shown in brown) stacks against the adenine ring of ATP. The figure was created with GLR software (www.convent.nci.nih.gov/glr).

NBD2, which are located just upstream of the L residue in the LSGGQ signature region (531–542 and 1176–1187) (Fig. 1), are highly conserved in avian and mammalian Pgps.

6. Summary

We have described here a conserved, previously undescribed subdomain of the ABC, which we named the A-loop (*A*romatic residue interacting with the *A*denine ring of *A*TP). This aromatic residue, 25 ± 2 residues upstream of the Walker A motif, is highly conserved in a set of 18 514 non-redundant ABC domains (except for Rad50/SMC and MutS/MSH subfamily members). Biochemical and site-directed mutagenesis studies demonstrate that this residue is critical for nucleotide binding and hydrolysis. The structures of NBDs of ABC proteins in a nucleotide-bound form solved to date all show interaction between this conserved aromatic acid and the adenine moiety of ATP through π–π interactions, hydrogen bonding or van der Waals contacts. Homology modeling of the NBDs of Pgp based on the structure of a dimer of the E171Q mutant MJ0796 NBD supports the view that the tyrosine (or Phe or Trp in other ABC proteins) residue interacts with the adenine base of ATP through π–π interactions. Furthermore, this residue lies on a loop – hence the term A-loop – and this subdomain marks the consensus boundary at the amino-terminal end of the NBD.

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