ATP binding site of P2X channel proteins: structural similarities with class II aminoacyl-tRNA synthetases

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Abstract The extracellular loop of P2X channel proteins contains a sequence stretch (positions 170–330) that exhibits similarities with the catalytic domains of class II aminoacyl-tRNA synthetases as shown by secondary structure predictions and sequence alignments. The arrangement of several conserved cysteines (positions 110–170) shows similarities with metal binding regions of metallothioneins and zinc finger motifs. Thus, for the extracellular part of P2X channel proteins a metal binding domain and an antiparallel six-stranded β-pleated sheet containing the ATP binding site are very probable. The putative channel forming H5 part (positions 320–340) shows similarities with the enzyme motif 1 responsible for aggregation of subunits to the holoenzyme.

Key words: Ligand-gated channel; ATP receptor; Purinoceptor; P2X; Aminoacyl-tRNA synthetase

1. Introduction

ATP-gated ion channels (P2X) have been established as one of two main families of purinergic receptors. Today 13 of them have been cloned and sequenced (listed in [1–3]). The channels are permeable to monovalent and divalent cations, and possess two hydrophobic regions that are putative transmembrane domains. These are separated by an extracellular loop containing almost 300 amino acid residues. Sequence homology in this loop region among the various P2X channels is 50–60%. The three-dimensional structure of P2X channels is still unknown; neither X-ray nor NMR analyses have been published.

Aminoacyl-tRNA synthetases catalyse the esterification of a specific tRNA with a particular amino acid. The energy required for this process is obtained by conversion of ATP to AMP and PP_i. These enzymes can be divided into two groups of 10 enzymes each, defined as class I and class II [4–9]. The enzymes of the two groups are principally different by two distinct types of tertiary structures.

Class II synthetases exhibit three homologous sequences called motifs 1, 2 and 3. The latter two are regions of a specific tertiary fold built around a central sheet of six antiparallel β-sheet strands and a long α-helix [5]; they are spatially close and participate in forming the active centre that contains the ATP binding site (compare Fig. 1).

Enzymes specific for the same amino acid but isolated from different species show sequence homology in the range of 20–40% [10–12]. In contrast, homology among enzymes specific for different amino acids from different sources is poor. Within the partial sequences of motifs 1, 2, and 3 they can be estimated to approximately 10% (see [5]).

A series of crystal structures of class II aminoacyl-tRNA synthetases are currently known; these enzymes are specific for serine (*Escherichia coli* [10], *Thermus thermophilus* [13–17]), histidine (*E. coli* [18]), aspartic acid (*T. thermophilus* [19,20], yeast [21–23]), glycine (*T. thermophilus* [24]), phenylalanine (*T. thermophilus* [25–27]), and lysine (LysU from *E. coli* [28]).

In this article similarities between P2X ion channels and class II aminoacyl-tRNA synthetases are described, especially in those regions which may be responsible for ATP binding.

2. Materials and methods

Secondary structure predictions were made by the algorithms given in [29–33] for the individual channel proteins. In Fig. 1, together with the partial sequences displayed, the percentages of secondary structure predictions for β-sheet strands, helices or neither are given.

3. Results

In Fig. 1 aligned regions of P2X channel proteins, for which six β-sheet strands were predicted, are compared with those β-sheet strands of class II aminoacyl-tRNA synthetases found by X-ray analysis as regions of the ATP binding catalytic centre. According to the enzyme structures they are designated β_I–β_{VI}. In the enzymes β-sheet strands β_I and β_{II} belong to motif 2, whereas β_{VI} is a region of motif 3.

In the β_I region the channel proteins show a relatively well conserved sequence consisting of two hydrophobic residues, and additionally a lysine and an asparagine (ΦΦKN) (Fig. 1). The aligned sequences of the enzymes have the common structure ΦΦRXE. In addition to the hydrophobic residues, K and N of the channel sequences may correspond to R and E of the enzymes. All four columns are completely conserved and thus seem to be important. A consensus sequence may be formulated as $\Phi X_7 \Phi_2$(RK)X(EN). The arginines of the enzyme proteins participate in binding of the α-phosphate moiety of ATP. This function may also very well be fulfilled by the aligned lysine of the channel proteins. At a distance of about six residues towards the C-terminal, the channel proteins exhibit a column consisting of arginine, lysine, and histidine, whereas the aligned enzyme sequences show a column consisting of arginine and histidine. In the enzymes, these residues participate in binding of the γ-phosphate moiety of ATP, and this function may again be fulfilled by the corresponding residues of the channel proteins.

In the β_{II} region, a near constant FRLG sequence common to the channel proteins corresponds to similar sequences of the enzymes (for example FHQLG) (Fig. 1, β_{II}). These stretches are largely followed by hydrophobic and acidic

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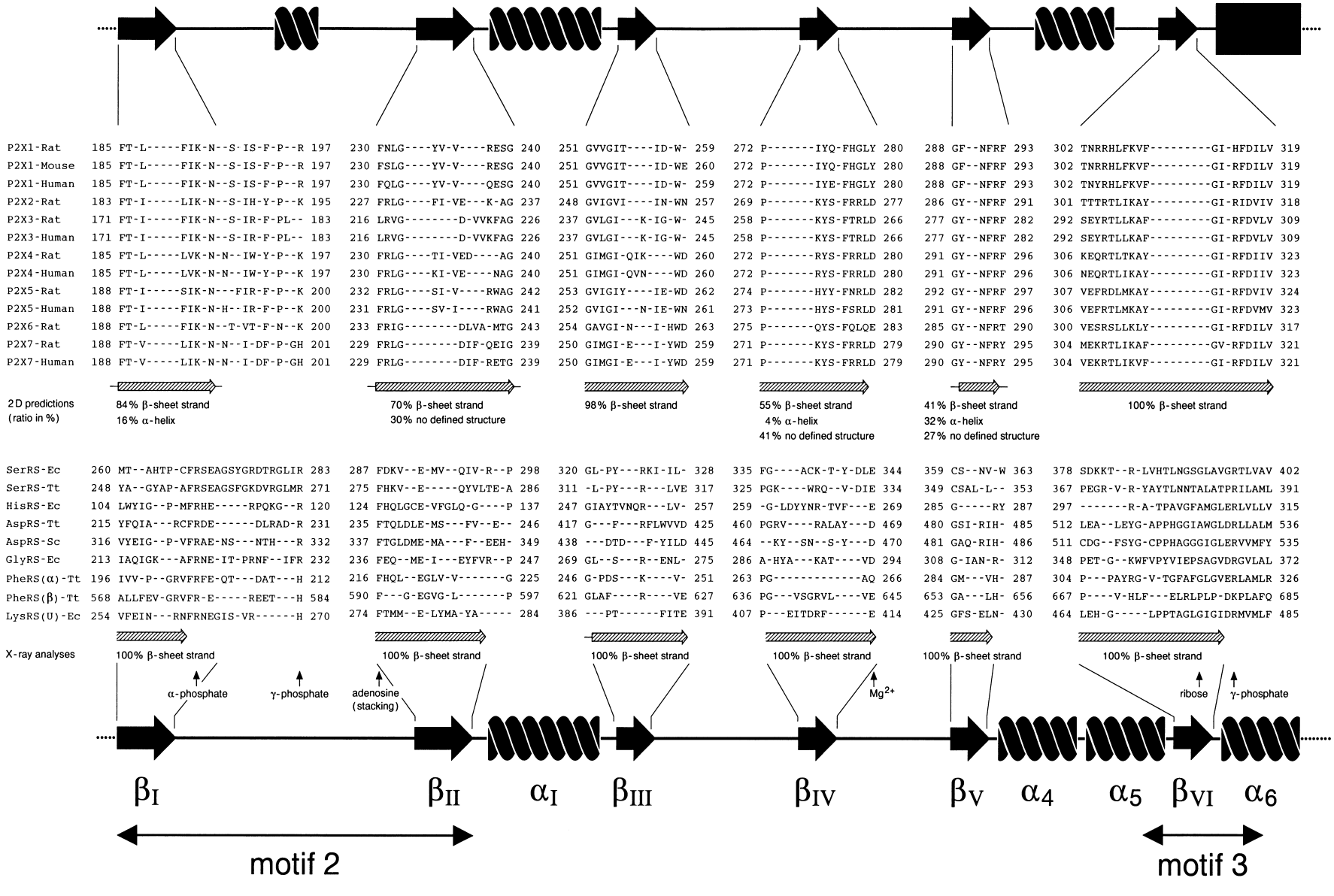
P2X1-Rat 185 FT-L-----FIK-N--S-IS-F-P--R 197 230 FNLG----YV-V----RESG 240 251 GVVGIT----ID-W- 259 272 P-------IYQ-FHGLY 280 288 GF--NFRF 293 302 TNRRHLFKVF---------GI-HFDILV 319
P2X1-Mouse 185 FT-L-----FIK-N--S-IS-F-P--R 197 230 FSLG----YV-V----RESG 240 251 GVVGIT----ID-WE 260 272 P-------IYQ-FHGLY 280 288 GF--NFRF 293 302 TNRRHLFKVF---------GI-RFDILV 319
P2X1-Human 185 FT-L-----FIK-N--S-IS-F-P--R 197 230 FQLG----YV-V----QESG 240 251 GVVGIT----ID-W- 259 272 P-------IYE-FHGLY 280 288 GF--NFRF 293 302 TNYRHLFKVF---------GI-RFDILV 319
P2X2-Rat 183 FT-I-----LIK-N--S-IH-Y-P--K 195 227 FRLG----FI-VE---K-AG 237 248 GVIGVI----IN-WN 257 269 P-------KYS-FRRLD 277 286 GY--NFRF 291 301 TTTRTLIKAY---------GI-RIDVIV 318
P2X3-Rat 171 FT-I-----FIK-N--S-IR-F-PL-- 183 216 LRVG--------D-VVKFAG 226 237 GVLGI---K-IG-W- 245 258 P-------KYS-FTRLD 266 277 GY--NFRF 282 292 SEYRTLLKAF---------GI-RFDVLV 309
P2X3-Human 171 FT-I-----FIK-N--S-IR-F-PL-- 183 216 LRVG--------D-VVKFAG 226 237 GVLGI---K-IG-W- 245 258 P-------KYS-FTRLD 266 277 GY--NFRF 282 292 SEYRTLLKAF---------GI-RFDVLV 309
P2X4-Rat 185 FT-L-----LVK-N-N--IW-Y-P--K 197 230 FRLG----TI-VED----AG 240 251 GIMGI-QIK----WD 260 272 P-------RYS-FRRLD 280 291 GY--NFRF 296 306 KEQRTLTKAY---------GI-RFDIIV 323
P2X4-Human 185 FT-L-----LVK-N-N--IW-Y-P--K 197 230 FRLG----KI-VE----NAG 240 251 GIMGI-QVN----WD 260 272 P-------RYS-FRRLD 280 291 GY--NFRF 296 306 NEQRTLIKAY---------GI-RFDIIV 323
P2X5-Rat 188 FT-I-----SIK-N---FIR-F-P--K 200 232 FRLG----SI-V----RWAG 242 253 GVIGIY----IE-WD 262 274 P-------HYY-FNRLD 282 292 GY--NFRF 297 307 VEFRDLMKAY---------GI-RFDVIV 324
P2X5-Human 188 FT-I-----FIK-N-H--IR-F-P--K 200 231 FRLG----SV-I----RWAG 241 252 GVIGI---N-IE-WN 261 273 P-------HYS-FSRLD 281 291 GY--NFRF 296 306 VEFRTLMKAY---------GI-RFDVMV 323
P2X6-Rat 188 FT-L-----FIK-N--T-VT-F-N--K 200 233 FRIG--------DLVA-MTG 243 254 GAVGI-N---I-HWD 263 275 P-------QYS-FQLQE 283 285 GY--NFRT 290 300 VESRSLLKLY---------GI-RFDILV 317
P2X7-Rat 188 FT-V-----LIK-N-N--I-DF-P-GH 201 229 FRLG--------DIF-QEIG 239 250 GIMGI-E---I-YWD 259 271 P-------KYS-FRRLD 279 290 GY--NFRY 295 304 MEKRTLIKAF---------GV-RFDILV 321
P2X7-Human 188 FT-V-----LIK-N-N--I-DF-P-GH 201 229 FRLG--------DIF-RETG 239 250 GIMGI-E---I-YWD 259 271 P-------KYS-FRRLD 279 290 GY--NFRY 295 304 VEKRTLIKVF---------GI-RFDILV 321
2D predictions (ratio in %)
84% β-sheet strand
16% α-helix
70% β-sheet strand
30% no defined structure
98% β-sheet strand
55% β-sheet strand
4% α-helix
41% no defined structure
41% β-sheet strand
32% α-helix
27% no defined structure
100% β-sheet strand
SerRS-Ec 260 MT--AHTP-CFRSEAGSYGRDTRGLIR 283 287 FDKV--E-MV--QIV-R--P 298 320 GL-PY---RKI-IL- 328 335 FG----ACK-T-Y-DLE 344 359 CS--NV-W 363 378 SDKKT--R-LVHTLNGSGLAVGRTLVAV 402
SerRS-Tt 248 YA--GYAP-AFRSEAGSFGKDVRGLMR 271 275 FHKV--E-----QYVLTE-A 286 311 -L-PY---R---LVE 317 325 PGK----WRQ--V-DIE 334 349 CSAL-L-- 353 367 PEGR-V-R-YAYTLNNTALATPRILAML 391
HisRS-Ec 104 LWYIG--P-MFRHE-----RPQKG--R 120 124 FHQLGCE-VFGLQ-G----P 137 247 GIAYTVNQR---LV- 257 259 -G-LDYYNR-TVF---E 269 285 G-----RY 287 297 -------R-A-TPAVGFAMGLERLVLLV 315
AspRS-Tt 215 YFQIA---RCFRDE------DLRAD-R 231 235 FTQLDLE-MS---FV--E-- 246 417 G---F---RFLWVVD 425 460 PGRV----RALAY---D 469 480 GSI-RIH- 485 512 LEA--LEYG-APPHGGIAWGLDRLLALM 536
AspRS-Sc 316 VYEIG--P-VFRAE-NS---NTH---R 332 337 FTGLDME-MA---F--EEH- 349 438 ---DTD---F-YILD 445 464 --KY--SN--S-Y---D 470 481 GAQ-RIH- 486 511 CDG--FSYG-CPPHAGGGIGLERVVMFY 535
GlyRS-Ec 213 IAQIGK---AFRNE-IT-PRNF--IFR 232 236 FEQ--ME-I---EYFVR--P 247 269 GL--S---R--ENL- 275 286 A-HYA---KAT----VD 294 308 G-IAN-R- 312 348 PET-G--KWFVPYVIEPSAGVDRGVLAL 372
PheRS(α)-Tt 196 IVV-P--GRVFRFE-QT---DAT---H 212 216 FHQL--EGLV-V-------G 225 246 G-PDS---K----V- 251 263 PG-------------AQ 266 284 GM---VH- 287 304 P---PAYRG-V-TGFAFGLGVERLAMLR 326
PheRS(β)-Tt 568 ALLFEV-GRVFR-E-----REET---H 584 590 F---G-EGVG-L-------P 597 621 GLAF----R----VE 627 636 PG---VSGRVL----VE 645 653 GA---LH- 656 667 P----V-HLF---ELRLPLP-DKPLAFQ 685
LysRS(U)-Ec 254 VFEIN---RNFRNEGIS-VR------H 270 274 FTMM--E-LYMA-YA----- 284 386 ---PT------FITE 391 407 P---EITDRF------E 414 425 GFS-ELN- 430 464 LEH-G-----LPPTAGLGIGIDRMVMLF 485
X-ray analyses
100% β-sheet strand
100% β-sheet strand
100% β-sheet strand
100% β-sheet strand
100% β-sheet strand
100% β-sheet strand
α-phosphate
γ-phosphate
adenosine (stacking)
Mg2+
ribose
γ-phosphate
βI
βII
αI
βIII
βIV
βV
α4
α5
βVI
α6
motif 2
motif 3

Fig. 1. Alignments of 13 characteristic sequence regions of P2X channel proteins (above) and nine class II aminoacyl-tRNA synthetases (below) which include partly or completely the β-sheet strand regions. The diagrams at the top and bottom indicate: arrow for β-sheet strand, helical structure for α-helix and box for transmembrane domains. Amino acid residues involved in binding of ATP by class II aminoacyl-tRNA synthetases are marked by arrows. The ratios of β-sheet strand or α-helix predictions obtained by five different prediction algorithms are given as percentage below the P2X channel sequences. β-Sheet regions of class II aminoacyl-tRNA synthetases are taken from crystal structures of the nine enzyme sequences. The Swiss-Prot and PIR database registration numbers are: P2X1-Rat P47824; P2X1-Mouse P51576; P2X1-Human P51575; P2X2-Rat P49653; P2X3-Rat P49654; P2X4-Rat P51577; P2X4-Human Q99571, O00450; P2X5-Rat P51578, Q64613; P2X5-Human Q93086, Q93087; P2X6-Rat P51579; P2X7-Rat Q64663; P2X7-Human Q64663; SerRS-Ec P09156, SerRS-Tt P34945, HisRS-Ec P04804, AspRS-Tt P36419, AspRS-Sc P04802, PheRS(α)-Tt P27001, PheRS(β)-Tt P27002, LysRS(U)-Ec P14825 (all Swiss-Prot) and GlyRS-Tt S58522 (PIR) (Ec = *Escherichia coli*, Tt = *Thermus thermophilus*, Sc = *Saccharomyces cerevisiae*).
←

sequences and a final G or P. Whereas the first region of this block shows convincing similarities, sequences are less similar in the second region. Nevertheless, a consensus sequence can be derived as follows:
F(RH)-X(ΦG)$X_4Φ_2$XΦ(EDQ)$Φ_3$(RK)X_2(GP). In the enzymes, the conserved phenylalanine side chain is responsible for stacking interactions with the adenine of the ATP molecule, and a counterpart of this hydrophobic and aromatic residue can again be seen in P2X channel proteins.

In all tertiary structures of class II aminoacyl-tRNA synthetases a structure immanent α-helix part was found between $β_{II}$ and $β_{III}$; this structure is also predicted for all P2X channel proteins at the corresponding positions (not shown).

For the $β_{III}$ region three characteristic parts may be mentioned (Fig. 1, $β_{III}$): in the channel proteins a conserved GΦΦG stretch appears which is less well conserved in the enzymes. In the central part (NQ), (RK), and hydrophobic columns are present, though incomplete in both groups. Finally, at the end of this region columns of acidic residues in both protein groups are preceded by hydrophobic residues. In summary, although there is no residue conserved in all proteins, the general character of this region seems to be similar for both groups. This is also indicated by the suggested consensus sequence G$Φ_2$(PG)X_2(NQ)X(RK)XΦX$Φ_2$(DE).

In the $β_{IV}$ region, the channel proteins again show more homology than the aminoacyl-tRNA synthetases, although they are not as well conserved as in the $β_V$ and $β_{VI}$ regions (Fig. 1, $β_{IV}$). Commonly, both families start with P or (PG), and end with a D or a (DE) column. The central section comprises (KRH), (YST) and hydrophobic stretches, and the consensus sequence PX_7(RKH)X(FY)X_2Φ(ED) can be given.

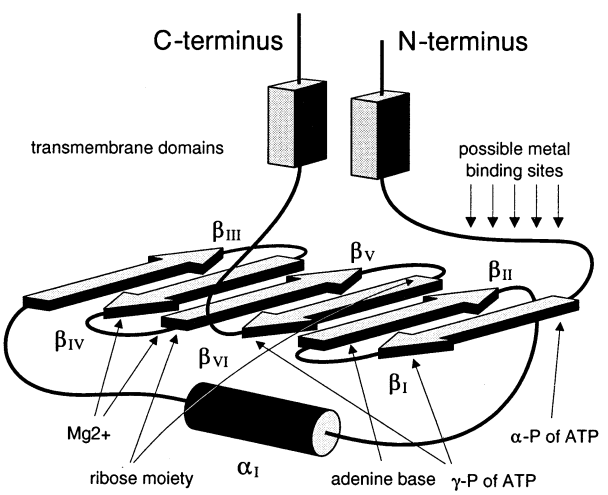

Fig. 2. Proposed structure for P2X channel proteins containing two transmembrane domains, a six-stranded antiparallel β-pleated sheet and metal binding sites. The ATP binding region may be constructed in an analogous fashion as those of class II aminoacyl-tRNA synthetases. Regions responsible for binding of the different parts of the ATP molecule, and Mg^{2+} ion, and eventually for a further metal ion are indicated by arrows.

The acidic residue at the end of the $β_{IV}$ region has an important function in complexing of the magnesium ion of an $[ATP\text{-}Mg]^{2-}$ which is the actual substrate for the enzymes and probably also the agonist for P2X channels (Fig. 1, $β_{IV}$). A second acidic residue responsible for the same function and also for contacting the ribose moiety is situated between β-sheet regions $β_{IV}$ and $β_V$ of class II aminoacyl-tRNA synthetases. Again, corresponding acidic residues are found in the P2X sequences (not shown).

In the region of sheet strand $β_V$ the aminoacyl-tRNA synthetases each possess a slightly differing sequence whereas the P2X sequences are nearly constant [GYNFRF] (Fig. 1, $β_V$). Common to both protein families are a G column in the first position, an N column and an R or (HR) column all followed by residues of predominantly hydrophobic character. For both protein families the consensus sequence G(SY)X_2(NR)Φ(HR)Φ suggests a similarity.

The most evident analogy occurs in the C-terminal region of the $β_{VI}$-sheet strand and its counterpart of the channel proteins (Fig. 1, $β_{VI}$). In both protein families three typical features can be recognised: the first position of these stretches is largely occupied by G followed by a column of hydrophobic residues, in the central region conserved columns of R and D are clearly observed, and 3–5 aminoacyl residues with hydrophobic side chains occur at the end (Fig. 1, $β_{VI}$). Thus, a consensus sequence GΦXRΦX$Φ_3$ is clearly indicated with the first X position occupied by acidic residues in the enzymes and the second X position by D in all channel sequences. For the N-terminal part of the $β_{VI}$ region a consensus sequence X(DE)X(KR)XΦX(KR)ΦΦ can be postulated. Altogether, channel and enzyme proteins exhibit relatively conserved sequences within this $β_{VI}$ strand region and both protein families correspond well.

Class II aminoacyl-tRNA synthetases use the conserved arginine of the C-terminal part of the $β_{VI}$ region for the interaction with the γ-phosphate moiety of the ATP molecule. Hydrogen bonds are formed by the enzymes with the ribose part of ATP by a glycine of motif 3. These functions may be easily performed in the P2X channel proteins by the conserved arginines and glycines of the P2X channels in the C-terminal sequence.

4. Discussion

Aminoacyl-tRNA synthetases functioning as important parts of the machinery which translates the genetic information into protein structures must have been present in their

primordial forms in the earliest stages of biological evolution (compare [34]). If P2X channel proteins are related to class II aminoacyl-tRNA synthetases, high sequence homology cannot be expected. Additionally, those regions of the enzyme molecules which are responsible for recognition of tRNAs and amino acids may have been lost during evolution of channel proteins. There are indeed some indications for this assumption which will be discussed in a later more detailed paper. Although primary structures of proteins imply common features of tertiary structures, the latter ones are far more stable than primary structures in homologous protein series [35]. Therefore more similarities in tertiary and basic secondary structures of class II synthetase and P2X channels should be expected than in sequence homologies.

The sequence stretches of aminoacyl-tRNA synthetases aligned for any β-sheet strand normally exhibit a general common structure together with individual variations, whereas the channel proteins are more conserved. In many cases the sequence stretches are even completely identical. This may be due to a different degree of evolution, and is also typical for sequences important for the functions of the respective protein.

The sequence of the channel proteins, which may form a β-pleated sheet consisting of six strands (positions 170–330, about 160 residues, placed between the cysteine-rich region (110–170) and the second transmembrane domain), has a length sufficient for such a structural feature. The N-terminal cysteine-rich region exhibits the general sequence: $CX_{8-10}CX_5CX_{14-16}CX_{9-10}CX_5C$. Searches carried out with a GCG software package in protein data bases show a considerable high sequence similarity of this sequence region with metallothioneins. As a second possibility some of the conserved cysteine residues can also be regarded as regions of zinc finger motifs in which stretches of amino acids are folded around a zinc ion. The side chains of four amino acids, cysteine or histidine residues, are always coordinated with the zinc ion [36]. Similar zinc binding motifs are also found in both classes of aminoacyl-tRNA synthetases (listed in [37]), and the P2X channel protein may very well possess a metal binding site in the mentioned region (Fig. 2).

For all ATP binding motifs of the synthetases, corresponding sequences can be found in the channel proteins (Figs. 1 and 2): in the β_I region an arginine may bind to the α-phosphate moiety. In the β_I and β_{VI} regions, arginine, lysine, or histidine may interact with the γ-phosphate group. In the β_{II} region a phenylalanine may be responsible for stacking interactions with the adenine base, a glycine in β-sheet strand β_{VI} for interaction with the ribose, and a glutamate at the end of β_{IV} may bind the magnesium cation of an $[ATP\text{-}Mg]^{2-}$ complex, together with a second glutamate or aspartate between β_{IV} and β_V (not shown in Fig. 1), which is also involved in interaction with the ribose moiety.

Remarkably, the channel region around position 150, which corresponds to synthetase motif 1, does not show very convincing analogies (not shown). In the enzymes the sequences of motif 1 are responsible for aggregation of two or four protein chains to the holoenzyme. The region around position 330 (termed H5 or P-region) has been suspected to be involved in channel pore formation and is characterised by a sequence motif FX_8GX_5P [38,39]. It exhibits certain similarity to motif 1 of the synthetases which is characterised as $G(FY)X_5P$. The motif 1-like structure may possibly be used by the channel proteins for aggregation of subunits and formation of a ion-pore structure. For example, in the crystal structure of phenylalanyl-tRNA synthetase from *T. thermophilus* [26] four subunits $(\alpha\beta)_2$ aggregate with their motif 1 regions to a 'quasi barrel'. Possibly, the primordial motif 1 occurred again by gene duplication mechanisms during evolution of P2X channels behind motif 3 and was used for construction of ion pores. In this context it should be mentioned that voltage-gated ion channels have a tetrameric structure, confirmed by the first published crystal structure of a K^+ channel [40].

Although the sequence similarities indicate perhaps a six-stranded β-pleated sheet for the ATP binding domain of the channel proteins, details of their tertiary structures may deviate from that of the synthetase core. However, our hypothesis may be helpful for future research including X-ray analyses of channel protein crystals, NMR spectroscopy and modelling of the P2X receptor ATP binding site, or affinity labelling experiments.

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