Investigation of the molecular assembly of β-cell K_{ATP} channels

Michael V. Mikhailov*, Ellina A. Mikhailova, Stephen J.H. Ashcroft

Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

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Abstract We have investigated the protein interactions involved in the assembly of pancreatic β-cell ATP-sensitive potassium channels. The channels are a heterooligomeric complex of pore-forming Kir6.2 subunits and sulfonylurea receptor (SUR1) subunits. SUR1 belongs to the ATP binding cassette (ABC) family of proteins and has two nucleotide binding domains (NBD1 and NBD2) and 17 putative transmembrane (TM) sequences. Previously we showed that co-expression in a baculovirus expression system of two parts of SUR1 divided at Pro1042 between TM12 and 13 leads to restoration of glibenclamide binding activity, whereas expression of either individual N- or C-terminal domain alone gave no glibenclamide binding activity [M.V. Mikhailov and S.J.H. Ashcroft (2000) J. Biol. Chem. 275, 3360–3364]. Here we show that the two half-molecules formed by division of SUR1 between NBD1 and TM12 or between TM13 and 14 also self-assemble to give glibenclamide binding activity. However, deletion of NBD1 from the N-part of SUR1 abolished SUR1 assembly, indicating a critical role for NBD1 in SUR1 assembly. We found that differences in glibenclamide binding activity obtained after co-expression of different half-molecules are attributable to different amounts of binding sites, but the binding affinities remained nearly the same. Simultaneous expression of Kir6.2 resulted in enhanced glibenclamide binding activity only when the N-half of SUR1 included TM12. We conclude that TM12 and 13 are not essential for SUR1 assembly whereas TM12 takes part in SUR1 Kir6.2 interaction. This interaction is specific for Kir 6.2 because no enhancement of glibenclamide binding was observed when half-molecules were expressed together with Kir4.1. We propose a model of K_{ATP} channel organisation based on these data.

Key words: Potassium channel; Sulfonylurea receptor; Baculovirus; ABC protein

1. Introduction

A central component of transmembrane signalling in the pancreatic β-cell is the ATP-sensitive potassium (K_{ATP}) channel which couples changes in plasma glucose concentration to insulin secretion and is also the target for the sulfonylurea drugs used to treat non-insulin-dependent diabetes mellitus [2,3] and for diazoxide which inhibits insulin secretion and is used to treat insulinoma and familial persistent hypoglycaemia and hyperinsulinaemia of infants (PHHI) [4]. Metabolic regulation of K_{ATP} channels in response to an elevation of blood glucose is mediated by an increased rate of metabolism of the sugar within the β-cell and a consequent increase in intracellular [ATP]/[ADP] ratio: ATP blocks the channels whilst MgADP activates them [5]. Closure of K_{ATP} channels in response to sulfonylureas and opening of the channels by diazoxide involves direct binding of the drugs to the channel [6].

The β-cell K_{ATP} channel contains two subunits, Kir6.2, an inwardly rectifying K channel, and SUR1, which contains the high-affinity sulfonylurea binding site and whose presence in the complex is essential for regulated channel activity [7,8]. K_{ATP} channel closure is thought to be mediated by effects of ATP on Kir6.2 [9] while SUR1 endows the channel with sensitivity to the inhibitory effects of sulfonylureas and the stimulatory effects of MgADP [10] and K channel openers [11]. Studies on fusion constructs with fixed SUR1:Kir6.2 ratio suggest that the native channel has a $(SUR1 \sim Kir6.2)_4$ stoichiometry [12,13]. A topographical model for SUR1 based on hydrophobicity plots proposes that SUR1 contains an N-terminal hydrophobic region (TMD0) containing five transmembrane helices, and two tandem repeats of six transmembrane helices (TMD1 and TMD2), each set followed by a large cytosolic loop [14]. SUR1 is classified as a member of the ATP binding cassette (ABC) superfamily and the two cytosolic loops, each containing a Walker A and Walker B motif [15], are suggested to function as nucleotide binding domains (NBDs) [16]. Disruption of either NBD results in the unregulated insulin secretion found in PHHI [17,18].

It is important to define the regions of SUR1 and Kir6.2 involved in channel assembly and ligand binding. Photoaffinity labelling of both SUR1 and Kir6.2 by radioactive sulfonylurea provided evidence for close association between the two channel subunits [12] and a direct physical association was demonstrated by immunoprecipitation [19]. The proximal C-terminus of Kir6.2 has been shown to be important for interaction with SUR1 [20]. The first transmembrane sequence and the N-terminal region of Kir6.2 have been shown to be important for channel assembly [21]. There is evidence that both N- and C-termini of Kir6.2 cooperate to form the ATP binding site [22]. The C-terminal set of transmembrane domains of SUR1 has been implicated in binding of sulfonylureas [23] and the first five TM domains and the C-terminus have been shown to specify the spontaneous bursting pattern and sensitivity to inhibition by ATP, respectively [24].

We have previously shown that functional K_{ATP} channels can be expressed in *Spodoptera frugiperda* insect cells using baculovirus [25]. In contrast to mammalian cells [8,26,27], co-expression of SUR1 and Kir6.2 is not required in *S. frugiperda* cells for efficient expression of either protein at the cell membrane. We further demonstrated that co-expression in the baculovirus system of two halves of SUR1 divided at Pro1042 between TM12 and 13 leads to restoration of glibenclamide binding activity, whereas expression of either individual N- or

*Corresponding author. Fax: (44)-1865-221834.
E-mail: michail.mikhailov@ndcls.ox.ac.uk

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C-terminal domain shows no glibenclamide binding activity [1]. In the present study we have further examined the ability of various SUR1 half-molecules to self-assemble and give rise to glibenclamide binding activity. We have also examined the influence of co-expression with Kir6.2 on formation of glibenclamide binding activity. We propose a model of K_{ATP} channel organisation based on our data and previous models.

2. Materials and methods

2.1. Cells and viruses

S. frugiperda (Sf21) cells and baculoviruses were maintained as described before [1,25].

2.2. Construction of plasmid DNAs and recombinant baculoviruses

We constructed transfer vectors containing DNA fragments encoding rat SUR1 [7] and mouse Kir6.2 [26] under control of the polyhedrin promoter in the pAcYM1 vector as previously described [1]. pAcNSUR2, pAcNSUR3 and pAcNSUR4 transfer vectors were obtained by cloning in pAcCas1 a PCR copy of SUR1 corresponding to amino acid (aa) sequences 55–984, 55–1125, 55–597, respectively (pAcCas1 contains the portion of the SUR1 gene encoding aa 1–55, a multicloning site and a His_6 tag [1]. pAcNSUR1del transfer vectors were obtained by cloning a PCR copy of SUR1 corresponding to aa sequence 984–1042 in the *Not*I site of pAcSUR4. PCR products encoding transfer vectors pAcCSUR2 and pAcCSUR3 were obtained by cloning PCR products encoding aa 985–1580 and 1126–1580 respectively in pAcYM1. Both PCR products contain sequences encoding a His_6 tag at the 3′ end. Transfer vectors were used for co-transfection of Sf9 cells together with *Autographa californica* nuclear polyhedrosis virus DNA (AcNPV PAK6) [23]. Recombinant baculoviruses AcNPVNSUR2, AcNPVNSUR3, AcNPVNSUR4, AcNPVCSUR1-del, AcNPVCSUR2 and AcNPVCSUR3 were obtained using the corresponding transfer vectors, three times plaque purified and used for infection of Sf21 cells.

2.3. [^{3}H]Glibenclamide binding

Sf21 cells resuspended at a density of 5×10^5 cells/ml in TC100 were incubated at room temperature for 30 min with different concentrations of [^{3}H]glibenclamide (0.2–10 nM) and test substances in a final volume of 400 μl. The incubation was stopped by rapid separation on Whatman GF/C filters soaked in phosphate-buffered saline for 30 min beforehand. Filters were washed and specific binding determined as previously described [28].

2.4. SDS–PAGE and immunoblotting

SDS–PAGE and immunoblotting were performed as previously described [25]. Anti-His_6 tag antibodies (penta-His, Qiagen) and alkaline phosphatase conjugated with anti-mouse antibody were used for detection of His-tagged proteins. Rabbit polyclonal anti-NBD1 antisera (dilution 1:200 000) and alkaline phosphatase conjugated with anti-rabbit antibody were used for detection of recombinant protein containing NBD1. Anti-NBD1 antisera were obtained after immunisation of rabbits with purified NBD1 expressed in *Escherichia coli* [1].

3. Results and discussion

3.1. Generation of recombinant proteins

Fig. 1 illustrates the predicted topology of SUR1 and the regions of SUR1 expressed by vectors used in this study. The NH_2-proximal half-molecule designated NSUR1, which contained the first two sets of putative transmembrane domains (TMD0 and TMD1), NBD1 and the first TM helix of the second six-TM set, TMD2, was as described before [1]. For the present study we constructed further NH_2-proximal half-molecules as follows: NSUR2 (TM1–11+NBD1); NSUR3 (TM1–11+NBD1+TM12–13); NSUR4 (TM1–11). A deletion variant of NSUR1 – NSUR1del – without NBD1 was also constructed. The COOH-proximal half-molecule designated CSUR1, which contained the last five putative TM helices

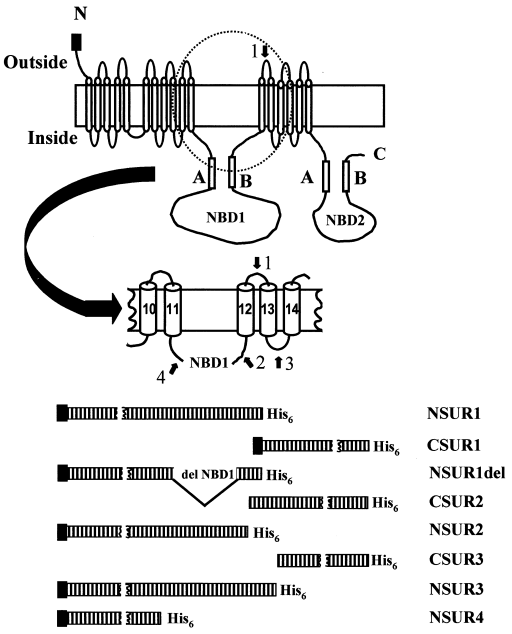

Fig. 1. Predicted topology of SUR1 and design of the recombinant proteins containing regions of SUR1 used in this study. Membrane topologies are based on [14]. Black boxes indicate the SUR1 leader sequence. Stippled lines show SUR1 sequences used in the recombinant proteins whose names are indicated on the right. Arrows indicate sites on SUR1 used for generation of a SUR1 deletion mutant. White boxes (A and B) show the location of Walker A and B motifs that can form nucleotide binding sites in each putative cytosolic nucleotide binding domain (NBD1 and NBD2).

plus NBD2, was described before [1]. For this study we constructed two further COOH-proximal half-molecules containing NBD2 plus either TM12–17 (CSUR2) or TM14–17 (CSUR3). All recombinant proteins were designed to contain a His_6 tag. Fig. 2 shows Western blots (using penta-His antibody and rabbit polyclonal anti-NBD1 antibodies) of Sf21 insect cells expressing the recombinant proteins. The positions of the main bands on the Western blot correspond to those predicted from amino acid sequences. Using monoclonal anti-His antibodies gave a cleaner picture – ordinarily only one band – but the polyclonal antiserum is more sensitive and can be used at dilutions up to 1:1 000 000 (data not shown).

3.2. Glibenclamide binding to insect cells expressing K_{ATP} channel recombinant proteins

As we have previously demonstrated [1,25], *S. frugiperda* insect cells infected with SUR1 express glibenclamide binding activity (Table 1, line 18). The specific binding activity at 10 nM [^{3}H]glibenclamide amounted to approximately 3×10^6 glibenclamide binding sites per cell, more than 1000-fold greater than the density of glibenclamide binding sites in pancreatic β-cells [28]. The glibenclamide binding affinity was reduced nearly 100-fold when Ser in position 1238 was replaced by Tyr (Table 1, line 20), confirming the previously demonstrated importance of the cytosolic loop between TM16 and TM17 for glibenclamide binding [23].

We have previously demonstrated [1] that when SUR1 was divided between TM12 and 13 and either the N-terminal SUR1 half-molecule (NSUR1), or the C-terminal half-molecule (CSUR1) expressed separately in *S. frugiperda* cells, glibenclamide binding was not significantly greater than in cells

infected with parent baculovirus. However, as confirmed here (Table 1, line 1), in cells co-infected with both halves of SUR1 a substantial increase of glibenclamide binding activity is observed. The present study demonstrates that glibenclamide binding activity is also obtained when the SUR1 molecule is divided at other positions. Co-expression of half-molecules corresponding to division of SUR1 between TM13 and TM14 (NSUR3+CSUR3) gave similar glibenclamide activity to NSUR1+CSUR1 (Table 1, line 6). However, co-expression of the half-molecules corresponding to division of SUR1 between NBD1 and TM12 (NSUR2+CSUR2) gave a fourfold increase in glibenclamide binding activity (Table 1, line 4).

In order to determine whether these differences reflected changes in the number of glibenclamide binding sites or in affinity for glibenclamide we measured glibenclamide binding activity at different concentrations of glibenclamide. Typical titration patterns are shown in Fig. 3. A range of different glibenclamide binding activities – from high glibenclamide binding activity (NSUR2+CSUR2) to absence of glibenclamide binding activity (NSUR1+CSUR3) – can be seen. Data were fitted to the equation $B=(B_{max}\times X/(K_d+X)+C\times X$ to estimate the binding affinity (K_d) and number of glibenclamide binding sites (B_{max}). Some of the resulting curves are shown in Fig. 3 and demonstrate good correlation between fitted curves and experimental data. Estimated binding constants and number of binding sites per cell are shown in Table 1. It can be seen that despite varying values of B_{max}, there is no significant difference in K_d between different combinations of expressed proteins. All binding constants are in the range 1.19–1.8 nM, similar to that for binding of glibenclamide to intact SUR1, 1.81 nM (Table 1, line 18). This indicates that self-assembly of half SUR1 molecules leads to the same conformation of the glibenclamide binding site as in native SUR1. Taking in account the bivalent structure of the glibenclamide binding site, we can conclude that the conformation of SUR1 assembled from half-molecules is similar to native SUR1.

3.3. Role of TMD12 and TMD13 in SUR1 assembly

Co-expression of halves of SUR1 divided at three different positions (Fig. 1) – between NBD1 and TM11 (NSUR2 and CSUR2); between TM11 and TM12 (NSUR1 and CSUR1); between TM12 and TM13 (NSUR3 and CSUR3) – all led to restoration of glibenclamide binding activity, but with different efficiency. The largest number of binding sites (863 000) was observed when all six TM regions from the last transmembrane set, TMD2, were present in the COOH-part of SUR1 (NSUR2+CSUR2, Table 1, line 4). The number of glibenclamide binding sites was reduced when only the last four TM helices were present in the COOH-half of SUR1 – 270 000 glibenclamide binding sites per cell for NSUR3+CSUR3 (Table 1, line 6). The increase in binding activity produced when TM12 was present in the C-terminal half-molecule rather than the N-terminal half-molecule suggests that TM12 interactions are of relevance to K_{ATP} channel assembly. However, the number of glibenclamide binding sites remained

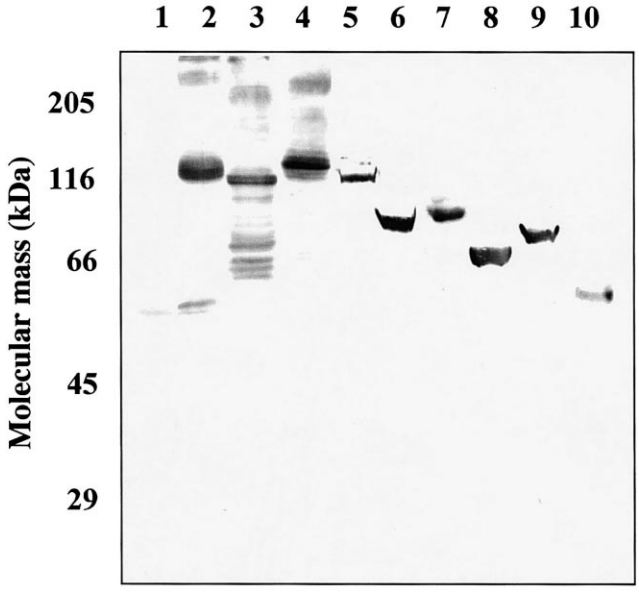

Fig. 2. Western blot of lysed *S. frugiperda* insect cells expressing SUR1 fragments with anti-NBD1 (lanes 1–4) and anti-His_6 (lane 5–10) antisera. Lanes: 1, mock-infected cells; 2, NSUR1; 3, NSUR2; 4, NSUR3; 5, NSUR1; 6, NSUR4; 7, NSUR1del; 8, CSUR1; 9, CSUR2; 10, CSUR3.

Table 1
Glibenclamide binding to Sf21 insect cells expressing K_{ATP} channel proteins and their fragments

Line	Expressed protein	K_d (nM)	B_{max} (binding sites/cell×1000)
1	NSUR1+CSUR1	1.52 ± 0.51	108 ± 22
2	NSUR1+CSUR1+Kir6.2	1.32 ± 0.42	211 ± 33
3	NSUR1+CSUR1+Kir4.1	1.47 ± 0.35	81 ± 17
4	NSUR2+CSUR2	1.43 ± 0.52	863 ± 41
5	NSUR2+CSUR2+Kir6.2	1.19 ± 0.24	428 ± 35
6	NSUR3+CSUR3	1.80 ± 0.51	260 ± 19
7	NSUR3+CSUR3+Kir6.2	1.21 ± 0.32	170 ± 26
8	NSUR2+CSUR1	1.39 ± 0.45	110 ± 21
9	NSUR2+CSUR1+Kir6.2	1.51 ± 0.35	91 ± 15
10	NSUR2+CSUR3	1.38 ± 0.52	253 ± 41
11	NSUR2+CSUR3+Kir6.2	1.54 ± 0.44	229 ± 35
12	NSUR1+CSUR3		No glibenclamide binding
13	NSUR1+CSUR3+Kir6.2	1.6 ± 0.37	77 ± 21
14	NSUR4+CSUR2		No glibenclamide binding
15	NSUR4+CSUR2+Kir6.2		No glibenclamide binding
16	NSUR1del+CSUR2		No glibenclamide binding
17	NSUR1del+CSUR2+Kir6.2		No glibenclamide binding
18	SUR1	1.81 ± 0.53	2990 ± 95
19	SUR1+Kir6.2	1.52 ± 0.43	1470 ± 65
20	SUR1(S1238Y)	> 100	2500 ± 500

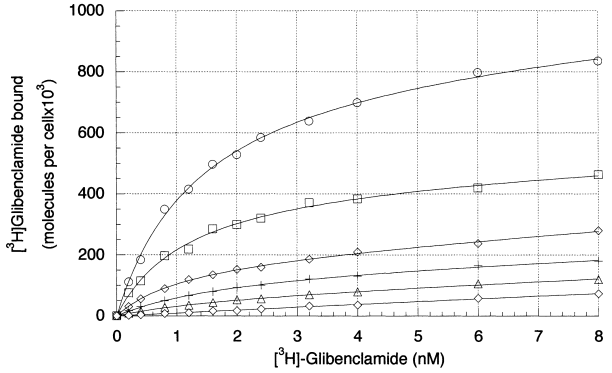

Fig. 3. Glibenclamide binding to *S. frugiperda* insect cells expressing different recombinant proteins. [^{3}H]Glibenclamide binding (dpm) was measured as described in Section 2. Data were fitted to the equation $B=(B_{max}\times X/K_d+X)+C\times X$, where B = number of bound glibenclamide molecules per cell, X = concentration of glibenclamide (nM), B_{max} = total number of glibenclamide binding sites per cell, K_d = constant of binding (nM), C = coefficient characterising unspecific binding to filter. ○, NSUR2+CSUR2; □, NSUR2+CSUR2+Kir6.2; ×, NSUR1+CSUR1+Kir6.2; +, NSUR1+CSUR1; △, NSUR1+CSUR3+Kir6.2; ◇, NSUR1+CSUR3.

unchanged when TM12 and TM13 regions were omitted from the NH_2-half of SUR1 – 239 000 glibenclamide binding sites per cell for NSUR2+CSUR3 (Table 1, line 10) – indicating that TM12 and TM13 are not essential for half SUR1 molecule assembly; and TM12 and TM13 interactions with the last four TM helices of SUR1 do not contribute to SUR1 assembly. The number of glibenclamide binding sites was less when half-molecules divided between TM11 and TM12 were co-expressed – 108 000 glibenclamide binding sites per cell for NSUR1+CSUR1 (Table 1, line 1). The number of glibenclamide binding sites remained nearly the same – 110 000 glibenclamide binding sites per cell for NSUR2+CSUR1 (Table 1, line 8) – when we removed TM12 from the NH_2-half of SUR1. However, no binding was seen when TM13 was removed from the COOH-half of SUR1 (NSUR1+CSUR3, Table 1, line 12). We interpret these data to indicate that TM12 is not tightly positioned when present in the NH2-part of SUR1 (NSUR1) and can abolish half-molecule interaction (NSUR1+CSUR3). The presence of TM13 in the COOH-half of SUR1 (CSUR1) can stabilise TM12 and leads to restoration of SUR1 assembly (NSUR1+CSUR1). These data indicate strong interactions between TM12 and TM13.

We therefore examined the sequence linking TM12 and TM13. Hydropathy plots [29] of the $SUR1_{1031-1060}$ sequence show that this region contains a markedly hydrophobic domain flanked by two hydrophilic regions (Fig. 4). A number of proteins, including Kir6.2, contain a sequence similar to the P (pore) or H5 loop first identified in the voltage-gated K channel family and have been shown to function as part of the K selectivity filter. The recent determination of the structure of the transmembrane domains of a related protein, the KcsA channel of *Streptomyces lividans* [30], has confirmed this region does indeed form the pore. Fig. 4 shows hydropathy plots for the pore region of the K channels KcsA, Kv1.1 and Kir6.2 and for the water pore protein aquaporin [31]. Each contains a 30-aa sequence containing a similar hydrophilic–hydrophobic–hydrophilic motif to $SUR1_{1031-1060}$. We suggest, therefore, the possibility that the loop connecting TM12 and TM13 in SUR1 may not be entirely extracellular but may function as a pore-like region. Interestingly, this motif is also present in DSUR, a *Drosophila* homologue of SUR1 [32], but the hydrophobic domain is deleted in SUR2 [33].

3.4. Essential role of NBD1

There was no glibenclamide binding activity observed when two half-molecules lacking NBD1 were co-expressed (NSUR4+CSUR2, Table 1, line 14). Nor was glibenclamide binding activity expressed when CSUR1 was co-expressed with NSUR1del, a variant of NSUR1 lacking NBD1 (Table 1, line 16). Thus NBD1 plays a key role in SUR1 self-assembly.

3.5. SUR1–Kir6.2 interaction

Consistent with our previous results [25], simultaneous expression of SUR1 and Kir 6.2 in *S. frugiperda* cells leads to a decrease in number of glibenclamide binding sites – from 2 990 000 to 1 470 000 (Table 1, lines 18 and 19). This effect is very different from what is observed in mammalian cells where trafficking signals prevent membrane targeting of Kir6.2 or SUR1 alone [27]. In *S. frugiperda* cells we have shown that both Kir6.2 and Sur1 are independently capable of reaching the plasma membrane [25]. Since the expression level of SUR1 remained the same compared with expression of SUR1 alone or co-expression of SUR1 and Kir6.2 (data not shown), the decrease in B_{max} can be attributed to interference between Kir6.2 and Kir6.2 SUR1 or SUR1 insertion in insect cell membranes. A similar decrease in number of

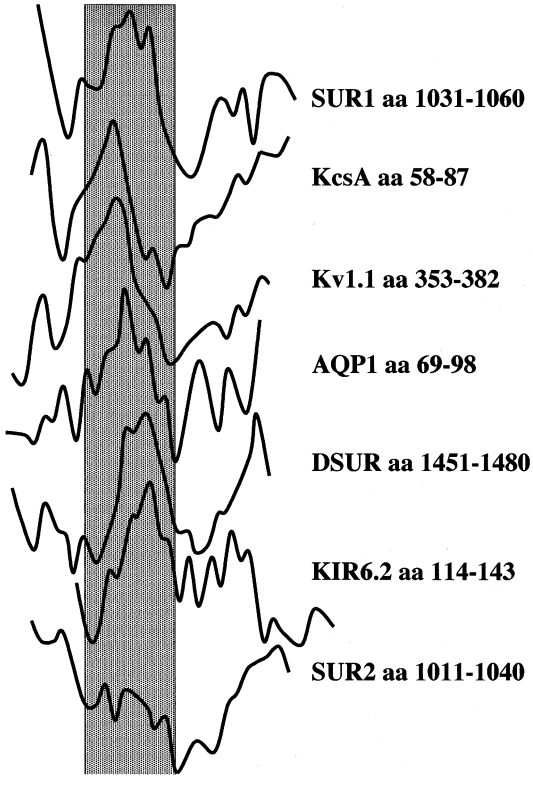

Fig. 4. Hydropathy plots for $SUR1_{1031-1060}$, a putative pore-like region. In addition to $SUR1_{1031-1060}$, the figure shows hydropathy plots [29] for the pore region of the K channels KcsA, Kv1.1, and Kir6.2 as well as the water pore protein, aquaporin. The figure also shows hydropathy plots for the corresponding regions of DSUR and SUR2.

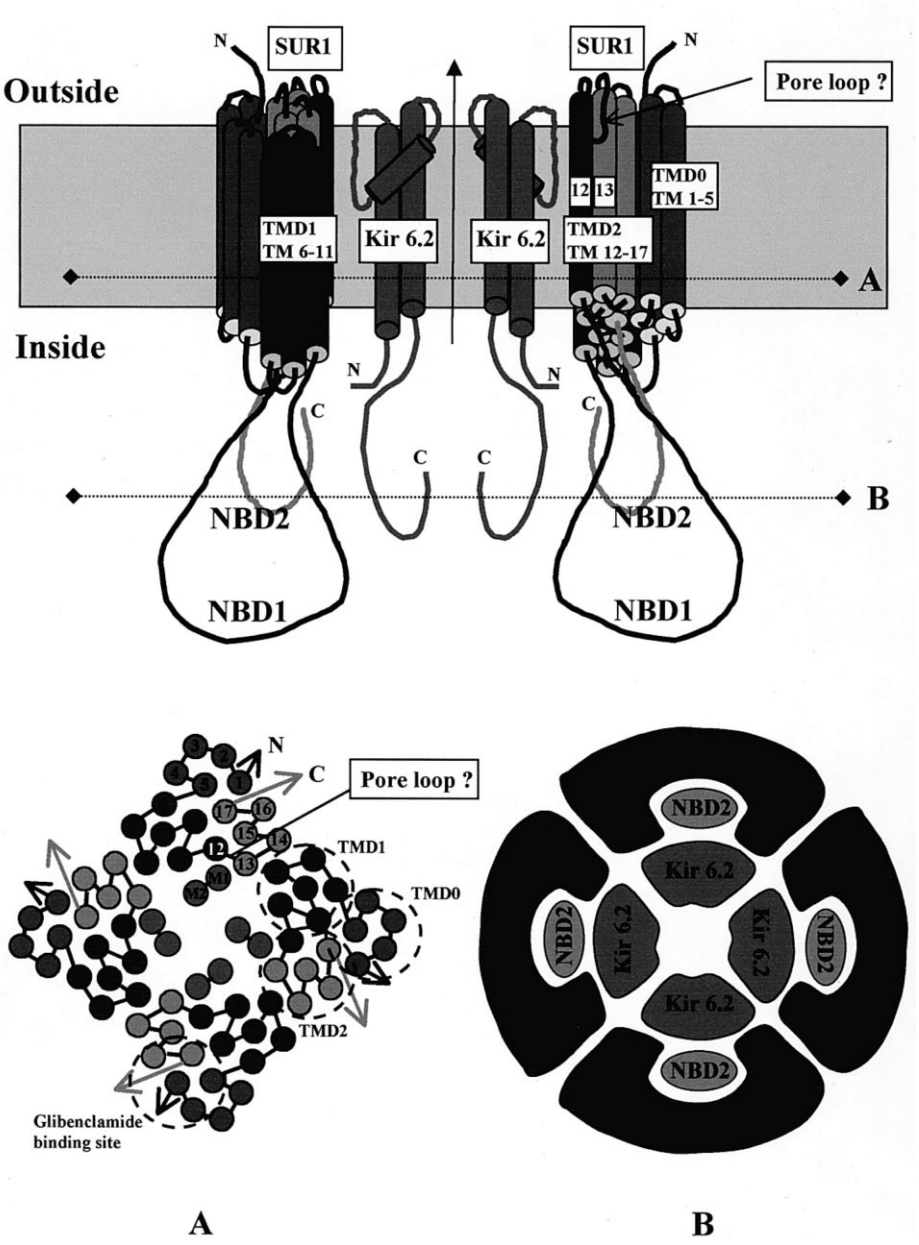

Fig. 5. Model of K_{ATP} channel. The upper panel shows the suggested transmembrane topology of the β-cell K_{ATP} channel. The lower panel provides cross-sectional views within the membrane (A) and within the cytosol (B). The key features of the model are: (i) within each SUR1–Kir6.2 pair: interactions between NBD1 and NBD2, between NBD2 and Kir6.2, between SUR1 TM12 and TM13, between SUR1 TM12 and Kir6.2, and between the N- and C-terminal cytosolic regions of Kir6.2; (ii) between neighbouring SUR1–Kir6.2 pairs: interactions between NBD1. The model also shows the presumed internal location of Kir6.2 to form the permeation pathway, and incorporates the suggestion that the sequence of SUR1 between TM12 and TM13 may be a pore-like region.

glibenclamide binding sites was obtained when NSUR2+CSUR2 were co-expressed with Kir6.2 – from 863 000 to 428 000 glibenclamide binding sites per cell (Table 1, lines 4 and 5); or when NSUR3+CSUR3 were co-expressed with Kir6.2 – from 270 000 to 170 000 sites per cell (Table 1, lines 6 and 7). However, an *increased* number of glibenclamide binding sites – from 108 000 to 211 000 sites per cell – was obtained after co-expression of NSUR1+CSUR1 with Kir6.2 (Table 1, lines 1 and 2). This interaction is specific to Kir6.2 because when we used Kir4.1 instead of Kir6.2 no increase of glibenclamide binding sites was observed (Table 1, line 3). In view of the previously proposed instability of TM12 in NSUR1 these data suggest that Kir6.2 interacts with TM12 to facilitate assembly of NH_2- and COOH-parts of SUR1. Additional confirmation of this proposal was obtained after investigation of NSUR1+CSUR3 expression; only when co-expressed with Kir6.2 was a significant number of glibenclamide binding sites observed – 77 000 (Table 1, lines 12 and 13).

3.6. A model for the β-cell K_{ATP} channel

There is growing evidence on the regions of K_{ATP} channel subunits which may interact to form the active channel. Since the fusion protein SUR1–Kir6.2 leads to active K_{ATP} channels [12,13,34] it seemed likely that in the native protein there is close juxtaposition of the C-terminus of SUR1 and the N-

terminus of Kir6.2. Using an in vitro protein–protein interaction assay it has been demonstrated that the two intracellular domains of Kir6.2 do mutually interact [35]. A highly conserved region within the N-terminus is responsible for this interaction and a mutation within this region (G40D) which disrupts the interaction severely interferes with the ability of Kir6.2 to form a functional K_{ATP} channel.

There is evidence for a direct interaction between Kir6.2 and SUR1. From a mixture of Kir6.2 and SUR1 in vitro translated proteins, and from COS cells transfected with both channel subunits, a Kir6.2-specific antibody co-immunoprecipitated Kir6.2 and SUR1 [19]. Kir6.2ΔC37 also co-immunoprecipitated with SUR1, suggesting that the distal carboxy-terminus of Kir6.2 is unnecessary for subunit association. However, a co-immunoprecipitation approach in HEK293 cells stably transfected with SUR1 and Kir6.2 suggested that a domain in the C-terminus of Kir6.2 (amino acids 208–279) was involved in biochemical interaction with SUR1 [20]. The domain, while necessary, was not sufficient, however, and full reconstitution of K_{ATP} channels required largely intact N- and C-termini. A requirement for a proximal C-terminal domain has also been observed for association of Kir6.2 and SUR2A [36]. However, a study using a trafficking-based assay for detection of interactions showed that either TM1 or the N-terminus of Kir6.2 was sufficient for conferring assembly with SUR1 [21]. This study also indicated that Kir6.2 interacts with transmembrane domains of SUR1. Consistent with these observations our data further suggest that TM12 of SUR1 interacts with Kir6.2.

We have previously provided evidence for an interaction between NBD1 and NBD2 [1]. NBD1 expressed in Sf9 cells as a green fluorescent protein (GFP) fusion protein was distributed throughout the cell. After co-expression of NBD1–GFP with the C-terminal half of SUR1, NBD1–GFP was localised near the plasma membrane. This effect disappeared when NBD2 was deleted from the C-terminal fragment indicating strong interaction between NBD1 and NBD2. Co-expression of NBD1–GFP with Kir6.2 did not localise NBD1–GFP to the plasma membrane suggesting a lack of strong interaction between Kir6.2 and NBD1. We have also previously shown that purified NBD1 alone shows a marked ability to form a tetramer [1], suggesting that self-interactions between NBD1 in different subunits contribute to channel assembly. The present study further supports such an essential role for NBD1.

Fig. 5 presents a model for K_{ATP} channel structure based upon the interactions suggested by these data.

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