Determinants Involved in Kv1 Potassium Channel Folding in the Endoplasmic Reticulum, Glycosylation in the Golgi, and Cell Surface Expression*

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Kv1.1 and Kv1.4 potassium channels are expressed as mature glycosylated proteins in brain, whereas they exhibited striking differences in degree of trans-Golgi glycosylation conversion and high cell surface expression when they were transiently expressed as homomers in cell lines. Kv1.4 exhibited a 70% trans-Golgi glycosylation conversion, whereas Kv1.1 showed none, and Kv1.4 exhibited a ~20-fold higher cell surface expression level as compared with Kv1.1. Chimeras between Kv1.4 and Kv1.1 and site-directed mutants were constructed to identify amino acid determinants that affected these processes. Truncating the cytoplasmic C terminus of Kv1.4 inhibited its trans-Golgi glycosylation and high cell surface expression (as shown by Li, D., Takimoto, K., and Levitan, E. S. (2000) J. Biol. Chem. 275, 11597–11602), whereas truncating this region on Kv1.1 did not affect either of these events, indicating that its C terminus is not a negative determinant for these processes. Exchanging the C terminus between these constructs showed that there are other regions of the protein that exert a positive or negative effect on these processes. Chimeric constructs between Kv1.4 and Kv1.1 identified their outer pore regions as major positive and negative determinants, respectively, for both trans-Golgi glycosylation and cell surface expression. Site-directed mutagenesis identified a number of amino acids in the pore region that are involved in these processes. These data suggest that there are multiple positive and negative determinants on both Kv1.4 and Kv1.1 that affect channel folding, trans-Golgi glycosylation conversion, and cell surface expression.

Potassium (K⁺) channels are expressed by most cells and play important roles in cell physiology, including setting the resting membrane potential and repolarizing/modulating action potential waveforms in excitable tissues (1–3). Molecular cloning methods have identified a large, diverse family of voltage-gated α-K⁺ channels that includes the four subfamilies Shaker (Kv1), Shab (Kv2), Shaw (Kv3), and Shal (Kv4) (4, 5). Functional channels appear to be composed of noncovalently associated homo- and/or heteromeric tetramers of subunits within a subfamily, and numerous amino acid determinants involved in channel operation have been identified (4, 5).

Kv1.1 and Kv1.4 (6) are α-subunits of the brain dendrotoxin-binding protein (7), are heavily glycosylated proteins (7–10) that form heteromers (7, 11, 12), and are associated with cytoplasmic Kvβ-subunits (12, 13). These glycoproteins contain 20–25 kDa of carbohydrate per denatured monomer that was PNGase F-sensitive but Endo H glycosidase-insensitive, and they were also sialidase-sensitive (10). Endo H cleaves only high mannosetype immature N-linkages found on ER³ glycoproteins and some plasma membrane proteins, whereas PNGase F cleaves all N-linkages (high mannosetype, hybrid-, and complex-type linkages) (14). These findings imply that these glycoproteins were efficiently processed and sialilated in the trans-Golgi in native brain tissue. Glycosylation is a common posttranslational modification of membrane proteins and is thought to aid in their proper folding/oligomerization and, in some cases, targeting and function. Studies have suggested that glycosylation modified the expression or function of some channels (15–22), whereas in other cases, no change was reported (23, 24).

The amino acid determinants responsible for the efficient oligomerization, glycosylation, processing, and targeting of K⁺ channels to the cell surface are not well understood (25, 26). Tetramers appear to oligomerize in the ER, and motifs involved in this include the amino acid regions of the distal N terminus and the S1 and the S2 membrane-spanning domains (25, 26). A number of studies using cell lines transfected with Kv1 subfamily cDNAs have noted some differences in the extent of glycosylation of the expressed homomeric channel proteins versus those in native brain tissue, where the channels are all expressed as mature glycoproteins (7, 10, 15, 23, 28–30). Other work indicates that glycosylation and/or cell surface expression of some Kvα-subunits may be governed by a cytoplasmic C terminus VXXSL motif (28), that various Kvα-subunits may be differentially glycosylated and expressed on the cell surface (10, 30), and that cytoplasmic Kvβ-subunits promote the processing of some Kvα-subunits (31). The above-mentioned findings imply that for both efficient trans-Golgi glycosylation and high surface expression of heteromeric Kv1 subfamily channel members, there must be a favorable heteromeric subunit composition and/or stoichiometry. A recent report has identified regions on Kir-type K⁺ channels that appear to be possible ER export signals (32). Ion channel trafficking defects associated with human disorders have been summarized and point to the importance of correct protein folding, oligomerization, and targeting to the cell surface for normal function (33).

Given the above-mentioned findings, the aims of this report were to use Kv1.4 and Kv1.1 chimeras and site-directed mu-

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transGolgi glycosylation conversion, and high cell surface expression.

**EXPERIMENTAL PROCEDURES**

**Cell Lines, cDNAs, and Transfections—**Chinese hamster ovary (CHO) pro cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's modified Eagle's medium, or in α minimum Eagle's medium, supplemented with 0.35 mM proline, with 10% fetal bovine serum at 37°C under 5% CO2. Cath-a-differentiated (CAD) cells were obtained from Dr. J. Wang (34), and a subclone from these cells was used. CAD cells are a subclone of Cath-a-cells that were derived from mouse central nervous system catecholaminergic brain stem neurons by SV40 large T immortalization. CAD cells have lost the expression of large T antigen but remain immortalized and neuronal-like. Undifferentiated CAD cells were maintained in Dulbecco's modified Eagle's medium/F-12 with 10% fetal bovine serum as described above for CHO cells. Rat brain Kv1 cDNAs (6) were engineered using the polymerase chain reaction (PCR) to contain a 5' Kozak enhanced ribosomal binding sequence (CCACC) before the start methionine and no endogenous 5'-untranslated regions or 3'-untranslated regions. C terminus truncation mutants were constructed by PCR by engineering a stop codon and a unique restriction enzyme site. The PCR products were cloned into eukaryotic expression vector pcDNA3, and the integrity of constructs was confirmed by DNA sequencing. Transient transfections were fashioned from 8161 corning glass (Warner Instruments) and had tip diameters of 1.5 μm. After transfection, the electrical activity was sampled with the pclampex software from a transient capacitance current. Mean linear membrane leak current was subtracted from the obtained current (1) using Ohm's law (G = I/Vp – EK) and a predicted Nernst K+ equilibrium potential (EK) of –83 mV. A test voltage value was corrected for the change of V introduced by the residue uncompensated Rs (change of V = I Rs; I is the net voltage-gated current). An unpaired t test was used to assess statistical differences of a control value compared with another one. p < 0.05 was considered significant.

**RESULTS**

TransGolgi Glycosylation and Cell Surface Expression Differences—In rat brain, Kv1.4 and Kv1.1 are expressed as mature glycoproteins of 110 kDa (appears to be a doublet) and 89 kDa, respectively (Fig. 1B, lanes 1 and 6, respectively). Both p110 and p80 were Endo H-insensitive glycoproteins that contained 20–25 kDa of Endo F-sensitive carbohydrate (data not shown), which implied they were efficiently processed and glycosylated in the Golgi/transGolgi at their one extracellular N-glycosylation site (Fig. 2, A and C). cDNA analysis showed
with their cytoplasmic C termini exchanged (Fig. 2B, chimera 1 and 2) were constructed, and their cDNAs were transiently transfected into CHO cells.

Full truncation of the C terminus of Kv1.4 (see Ref. 28) inhibited its trans-Golgi glycosylation dramatically for the Kv1.4 T1 mutant, whereas Kv1.4 T2 and T3 mutants with more of the C terminus showed some trans-Golgi glycosylation conversion of ~40% versus ~70% for wt Kv1.4 (Fig. 1B, compare lanes 1 and 2 with lanes 3–5). C-terminal truncation also dramatically inhibited cell surface expression of the Kv1.4 T1 mutant as monitored by patch clamping (Fig. 5, A and B). Because Kv1.1 exhibited very inefficient trans-Golgi glycosylation, we truncated its C terminus to test whether it was a negative determinant for this. Kv1.1 truncation mutants T1, T2, and T3 still exhibited inefficient trans-Golgi glycosylation (Fig. 1B, compare lane 6 with lanes 7–10), and the Kv1.1 T1 mutant also showed low cell surface expression levels similar to those of wt Kv1.1 (Fig. 5, A and B). For the truncation mutants, the predominant lower band for Kv1.4 and the bands for Kv1.1 (Fig. 1B, lanes 3–5 and 8–10, respectively) were Endo H-sensitive, which indicated that they were immature glycoproteins that were not trans-Golgi glycosylated (data not shown).

We next tested the effects of exchanging the cytoplasmic C terminus of Kv1.4 and Kv1.1 on trans-Golgi glycosylation and cell surface expression. If the C terminus of Kv1.4 is required for both of these processes, then exchanging it with the C terminus of Kv1.1 is predicted to inhibit them. Chimera 1, which is Kv1.4 with the C terminus of Kv1.1, exhibited a significant trans-Golgi glycosylation conversion of 34% (Fig. 3A, lane 1), but it was less than the trans-Golgi glycosylation conversion of ~70% for wt Kv1.4. The intermediate band in lane 1 of Fig. 3A was not trans-Golgi glycosylated because it was sensitive to Endo H digestion (Fig. 3A, lane 2), and this band was responsible for the lower trans-Golgi glycosylation conversion. It appears that this band had an additional post-translational modification(s) that may be due to phosphorylation of the C terminus of Kv1.1 at S446 in chimera 1 (36). In contrast, there was a dramatic reduction in cell surface expression of chimera 1, and it was expressed at low levels like wt Kv1.1 using patch clamping (Fig. 5, A–C) and biotinylation of cell surface glycoproteins (Fig. 5, C and D). The biotinylation method gave a lower expression level than patch clamping for this chimera (Fig. 5C), which suggests that the Kv1.1 C terminus on Kv1.4 resulted in current enhancement. Also note that Kv1.4 and C1 cell surface biotinylated proteins in the immunoblot in Fig. 5D are enriched in trans-Golgi glycosylated proteins when compared with other whole cell membrane immunoblots (see the other figures). Will the Kv1.4 C terminus transplanted to Kv1.1 stimulate efficient trans-Golgi glycosylation and high cell surface expression? It appeared that it did not. Chimera 2, which is Kv1.1 with the C terminus of Kv1.4, displayed no trans-Golgi glycosylation (Fig. 3A, lane 4) as well as low cell surface expression levels like wt Kv1.1 as monitored by patch clamping (Fig. 5, A–C) and biotinylation methods (Fig. 5, C and D). These results suggest that trans-Golgi glycosylation and cell surface expression for Kv1.4 are differentially affected by transplanting the Kv1.1 C terminus on it. Although chimera 1 exhibited inhibition of both trans-Golgi glycosylation and cell surface expression versus wt Kv1.4, there was a much more dramatic effect on the inhibition of cell surface expression. In addition, the C terminus of Kv1.4 transplanted to Kv1.1 did not stimulate its efficient trans-Golgi glycosylation or its high cell surface expression.

Effects of Exchanging N-terminal Regions or Pore Regions on Trans-Golgi Glycosylation and Cell Surface Expression—The
results above suggest that there are other dominant-negative and dominant-positive determinants in the N-terminal region (the N terminus through the S6 region, see Fig. 2) of these channels involved in these processes, and a number of chimeras between Kv1.4 and Kv1.1 were constructed to map them.

We first transplanted the extracellular S1-S2 loop of Kv1.1 to Kv1.4, which contains the only extracellular N-linked site that is glycosylated, to test whether efficient trans-Golgi glycosylation was affected by the different amino acids in this region (Fig. 2, B and C). No evidence was found that the extracellular S1-S2 loop of Kv1.4 was required for high trans-Golgi glycosylation or high cell surface expression because the loop of Kv1.1 on Kv1.4 chimera 3 was efficiently glycosylated (Fig. 3, B, lane 2), and the chimera exhibited similar surface expression levels as wt Kv1.4 (Fig. 5, B, lane 2).

Progressively replacing the N-terminal region of Kv1.4 with Kv1.1 (Fig. 2, B, chimeras 4–6) inhibited trans-Golgi glycosylation somewhat versus wt Kv1.4 (Fig. 3, B, lane 3–5), but the conversion was still much greater than Kv1.1; cell surface expression for chimeras 4 and 5 showed little change, whereas there was some inhibition for chimera 6 (Fig. 5, B).

We next constructed chimeras between Kv1.4 and Kv1.1 in their pore region (Fig. 2, B, chimeras 7–16). In the S5-pore-S6 region of Kv1.4 and Kv1.1, there are only nine amino acid differences, five in the outer pore region a, three in the outer pore region b, and one in the deep pore region c (Fig. 2, D). Chimera 7, which is Kv1.4 with both the outer pore regions a and b of Kv1.1, exhibited little or no trans-Golgi glycosylation (Fig. 3, C, lane 2; Fig. 5, D, lane 3) and low cell surface expression levels similar to those of wt Kv1.1 using both patch clamping (Fig. 5, B and C) and biotinylation methods (Fig. 5, C and D). Chimera 16, which is Kv1.1 with regions a, b, and c of Kv1.4, exhibited a dramatically efficient trans-Golgi glycosylation (Fig. 3, C, lane 12) and a high cell surface expression that was similar to that of wt Kv1.4 by both patch clamping (Fig. 5, B).
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and C) and biotinylation methods (Fig. 5, C and D). Also note that C16 cell surface biotinylated proteins in the immunoblot in Fig. 5D are enriched in trans-Golgi glycosylated proteins when compared with other whole cell membrane immunoblots (compare with Fig. 3C, lane 12).

Having identified the pore region as containing positive and negative determinants for Kv1.4 and Kv1.1, respectively, for both trans-Golgi glycosylation and cell surface expression, we next sought to identify which of these subregions were involved. The outer pore region a or b was exchanged between Kv1.4 and Kv1.1 (Fig. 2B, chimera 8–12). For Kv1.4, replacing its a or b region with those of Kv1.1 (chimeras 8 and 9, respectively) inhibited both trans-Golgi glycosylation (Fig. 3C, lanes 3 and 4) and cell surface expression (Fig. 5B) versus wt Kv1.4, but the inhibition in both cases was less than that recorded for chimera 7, which has both of these regions of Kv1.1. For Kv1.1, replacing its a and b regions with those of Kv1.4 (chimera 10) did not stimulate trans-Golgi glycosylation (Fig. 3C, lane 6), and no detectable currents were recorded from this chimera (Fig. 5B). Kv1.1 with the a or b region of Kv1.4 (chimeras 11 and 12, respectively) did not rescue trans-Golgi glycosylation (Fig. 3C, lanes 7 and 8) or high cell surface expression for chimera 11 (although it was expressed slightly better than wt Kv1.1), whereas no detectable currents were recorded from chimera 12 (Fig. 5B). Kv1.1 with the a and c regions of Kv1.4 (chimera 13) exhibited trans-Golgi glycosylation (Fig. 3C, lane 9) and cell surface expression (Fig. 5B) that was greater than those of Kv1.1 but less than those of Kv1.4. Kv1.1 with the c region of Kv1.4 (chimera 14; S to T mutation) showed trans-Golgi glycosylation (Fig. 3C, lane 10) that was greater than that of Kv1.1 (with filter/film overexposure) but much less than that of Kv1.4, whereas cell surface expression levels (Fig. 5B) were greater than those of Kv1.1 and only somewhat less than those of Kv1.4. Kv1.1 with the b and c regions of Kv1.4 (chimera 15) exhibited efficient trans-Golgi glycosylation, although somewhat less than that of Kv1.4 (Fig. 3C, lane 11), and high cell surface expression (Fig. 5B) that was similar to that of Kv1.4 but less than that of chimera 16. All lower bands in Fig. 3, B and C, were core N-glycosylated in the ER because they were sensitive to treatment with Endo H glycosidase (data not shown).

These results suggest that the pore region of Kv1.4 and Kv1.1 is a major positive and negative determinant, respectively, for both trans-Golgi glycosylation and cell surface expression. The Kv1.4 pore region exerted a dominant-positive effect when transplanted to Kv1.1 (chimera 16) and completely rescued efficient trans-Golgi glycosylation and high cell surface expression that was similar to Kv1.4. Different combinations of Kv1.4 pore regions transplanted to Kv1.1 had a trend toward stimulating high cell surface expression as follows: a, b, c > c a, b, c > c a, c; and this sequence was slightly different from the trend toward promoting efficient trans-Golgi glycosylation (a, b, c > b, c > a, c). This implies that the outer pore regions a or b and the c region of Kv1.4 are required for efficient trans-Golgi glycosylation on the Kv1.1 pore chimera, and the c region promotes surface expression. Although the Kv1.4 pore exerted a dominant-positive effect on Kv1.1 for both processes, it only promoted high trans-Golgi glycosylation on chimera 1 (Kv1.4 with the C terminus of Kv1.1) and did not stimulate high cell surface expression. The Kv1.1 pore regions a and b had a dominant-negative effect on both of these processes when transplanted to Kv1.4 (chimera 7), and either Kv1.1 pore region a or b alone (chimeras 8 and 9, respectively) inhibited these processes, but to a lesser extent than they did when together.

Effects of Point Mutations in the Pore Region of Kv1.4 on Trans-Golgi Glycosylation and Cell Surface Expression—A number of point mutations were then constructed in the outer pore regions of Kv1.4 to map the amino acid determinants involved in its efficient trans-Golgi glycosylation and high cell surface expression. All of the amino acids in the outer pore regions a and b of Kv1.4 were individually mutated to the corresponding ones in Kv1.1 (Fig. 2D). Cells transfected with these constructs were analyzed by immunoblotting, patch clamping, and biotinylation. For trans-Golgi glycosylation conversion, our results suggest that the mutants can be arranged into three groups: 1) mutations that did not alter conversion (T507E, T508S, and I535V, Fig. 4A, lanes 4, 6, and 9, respectively); 2) mutations that had an inhibitory effect, but some conversion could be detected with filter/film overexposure (D504E, P506A/T507E, Q511S, and V537I, Fig. 4A, lanes 2, 5, 7, and 10, respectively); and 3) mutations that had a dramatic inhibitory effect, and little or no conversion could be detected with filter/film overexposure (D506A and K533Y, Fig. 4A, lanes 3 and 8). For cell surface expression levels estimated by patch clamping (Fig. 5, E and F) and biotinylation (Fig. 5, F and G), the mutants did not always fall into the same groups as described above. For example, group 1 members had high cell surface expression similar to wt Kv1.4, but so did some members of group 2, e.g. D504E and V537I. Group 3 members (P506A and K533Y) exhibited low surface expression versus Kv1.4. Patch clamping and biotinylation gave similar estimates for cell surface expression for most of the mutants. A major exception appears to be the Q511S mutant, which exhib-
protein (p230 or GM130) were also used to help determine whether channels were localized at high or low levels to these internal organelles. Kv1.1 showed a reticulate-like signal that radiated out from the nucleus, suggestive of high retention in the ER, whereas Kv1.4 did not show this signal but rather exhibited a diffuse cell surface signal and a high signal at the cell’s perimenter. Similar results have been shown for Kv1.1 and Kv1.4 in transfected COS7 cells (35). Chimera 1, Kv1.4 with cytoplasmic Kv1.1 C terminus, exhibited a unique pattern of intracellular localization. Chimera 2, Kv1.1 with the cytoplasmic Kv1.4 C terminus, showed a pattern similar to Kv1.1 suggesting that it continued to exhibit high retention in the ER. Chimera 16, Kv1.1 with the pore of Kv1.4, showed a localization pattern similar to Kv1.4, whereas chimera 7, Kv1.4 with the outer pore regions of Kv1.1, exhibited a pattern similar to Kv1.1. Overall, these data suggest that our patch clamp and biotinylation data are a good reflection of cell surface expression levels and that mutations here that caused reduced voltage-gated currents are due to partial intracellular organelle retention and not high cell surface expression of dysfunctional channels.

**DISCUSSION**

Evidence has been presented in this report that Kv1.4 and Kv1.1 have multiple positive and negative determinants that affect channel folding and retention in the ER, trans-Golgi glycosylation conversion, and cell surface expression. It has been suggested that *Shaker*-like potassium channels are oligomerized in the ER by dimerization of monomers followed by dimerization of dimers to produce properly folded tetramers (26). It also appears that *Shaker*-like channels in the ER are functional because K+ channel activity has been recorded from planar lipid bilayers that had fused rough microsomes isolated from a cell-free protein processing system programmed with *Shaker* B1(6–46) cRNA (Ref. 17; see Ref. 25 for review). Thus, it appears that *Shaker*-like channels in the ER are only transported to the Golgi as properly oligomerized tetramers, and additional glyco-processing may occur in the Golgi (including trans-Golgi glycosylation).

The Kv1.4 C Terminus Is Required for High Cell Surface Expression but Not Trans-Golgi Glycosylation on Kv1.4, but It Does Not Affect These Processes When Transplanted to Kv1.1—

Truncating the C terminus of Kv1.4 inhibited both efficient trans-Golgi glycosylation conversion and high cell surface expression as first reported by Li et al. (28). In addition, we found that truncating the C terminus of Kv1.1 did not affect either of the processes, which indicates that it does not play a role as a negative determinant on the wild type Kv1.1. Exchanging the C termini between Kv1.4 and Kv1.1 produced results that were informative. Chimera 1, Kv1.4 with the C terminus of Kv1.1, still exhibited a trans-Golgi glycosylation conversion of ~35% but showed low cell surface expression versus wt Kv1.4. Moreover, chimera 2, Kv1.1 with the C terminus of Kv1.4, did not exhibit either efficient trans-Golgi glycosylation or high cell surface expression. Chimera 1 and 2 results suggest that: 1) 35% efficient trans-Golgi glycosylation can be obtained without high cell surface expression, so that these two processes appear to be directed by different determinants on Kv1.4; 2) the Kv1.4 C terminus contains a determinant for high cell surface expression that is presumably the VXXSL motif as described by Li et al. (28), and the C terminus of Kv1.1 cannot substitute for it; and 3) the C terminus of Kv1.4 transplanted to Kv1.1 cannot rescue either efficient trans-Golgi glycosylation or high cell surface expression; it appears that the outer pore region of Kv1.1 contains dominant-negative determinants for these processes. What explains the finding that truncating the C terminus of Kv1.4 (the T1 mutant) blocked trans-Golgi glycosylation...
FIG. 5. Mapping the amino acid determinants of Kv1.4 and Kv1.1 that affect their cell surface expression. Whole cell patch clamping and cell surface biotinylation of glycoproteins were performed on CHO cells transfected with various Kv1 constructs to estimate cell surface expression levels as described under “Experimental Procedures.” For patch clamping, all data are from cells that were held at −80 mV and depolarized to a maximum activating voltage of 50 mV. For biotinylation, data were normalized to Kv1.4, which was taken as 100.0 as outlined under “Experimental Procedures,” and selective Gm/Cm data were also normalized to Kv1.4, which was taken as 100.0 for comparison of the two methods. A, representative whole cell current traces of selected Kv1.4 and Kv1.1 constructs from patch clamping. B, group patch clamping data for peak conductance/capacitance (Gm/Cm) expression levels of different Kv1.4 and Kv1.1 constructs. C, normalized group biotinylation data for cell surface protein expression levels of selected Kv1.1 and Kv1.4 constructs compared with selected normalized Gm/Cm data. D, representative immunoblot with Kv1.4 or Kv1.1 antibodies that was exposed to the same film for the same length of time from the biotinylation method of transfected cells. E, group patch clamping data for peak conductance/capacitance (Gm/Cm) expression levels of different Kv1.4 point mutants. F, normalized group biotinylation data for cell surface protein expression levels of Kv1.4 point mutants compared with selected normalized Gm/Cm data. G, representative immunoblot with Kv1.4 antibodies that was exposed to the same film for the same length of time from the biotinylation method of transfected cells. Middle and bottom panel immunoblots used antibodies to actin and GFP, and these panels show that the cell density and the transfection efficiency, respectively, were similar among the different transfected dishes. The n values in B and E are listed above the error bars, and the n values for biotinylation data in C and F = 3. The error bars in B and E and the ± values in C and F are S.E.
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but that transplanting the C terminus of Kv1.1 to Kv1.4 did not? The simplest explanation is that the majority of Kv1.4 truncation mutants were partially retained in the ER or shuttled off to the cytoplasm for degradation or to newly described cytoplasmic structures called aggresomes (39). This would result in both inefficient trans-Golgi glycosylation and low or no cell surface expression. In contrast, chimera 1, which is Kv1.4 with the C terminus of Kv1.1, was folded and oligomerized properly in the ER and transported to the Golgi, where it was somewhat efficiently glycosylated. However, once in the trans-Golgi or trans-Golgi network, it was inefficiently delivered to the cell surface because it did not have the C terminus of Kv1.4. Thus, it appears that the Kv1.4 C terminus contains determinants recognized by the trans-Golgi processing machinery involved in the high cell surface delivery of Kv1.4.

The Pore Region of Kv1.4 Has Positive Determinants for Trans-Golgi Glycosylation and Cell Surface Expression, Whereas the Pore Region of Kv1.1 Has Negative Determinants—Both outer pore regions \( a \) and \( b \) of Kv1.4 are required for Kv1.4’s efficient trans-Golgi glycosylation and high surface expression because exchanging these regions with Kv1.1 regions inhibited both processes (chimeras 7–9). To rescue Kv1.1 for both processes required a minimum of Kv1.4 pore regions \( b \) and \( c \), although its \( a \), \( b \), and \( c \) regions together gave the best rescue (chimeras 10–16). The outer pore regions \( a \) and \( b \) of Kv1 channels may be in close proximity to each other because these equivalent regions in the Kcsa \( K^+ \) channel (Fig. 2D) are in close juxtaposition in the three-dimensional crystal structure of the protein (40). The \( a \) region forms a protruding turret on the perimeter of the external face of each Kcsa subunit, whereas the \( b \) region is somewhat lower and is positioned inward from that position. If the \( a \) and \( b \) regions of Kv1 channels fold in a similar fashion, then our mapped determinant is composed of regions that are nonadjacent in the primary sequence but are situated close to one another in the folded three-dimensional state. Kv1.1 with pore regions \( b \) and \( c \) or \( a \), \( b \), and \( c \) of Kv1.4 (chimeras 15 and 16, respectively) exhibited both high trans-Golgi glycosylation and cell surface expression without having the identified high expression cytoplasmic C terminus VXXSL motif (28) of Kv1.4. Therefore, Kv1.4 and Kv1.1 have positive and negative determinants, respectively, for both of these processes, but Kv1.4 also requires its own cytoplasmic C terminus for the process of high cell surface expression.

Point mutations in both outer pore regions \( a \) and \( b \) of Kv1.4 to identical amino acids in Kv1.1 identified a number of determinants critical for Kv1.4’s proper folding in the ER, efficient trans-Golgi glycosylation, or high cell surface expression. All of the point mutations exhibited cell surface expression as assayed by patch clamping or surface biotinylation. Group 1 mutants T507E or T508S in region \( a \) and I535V in region \( b \) did not affect any of these processes and were indistinguishable from wt Kv1.4. Group 2 mutations such as D504E in region \( a \) and V536I in region \( b \) resulted in inefficient trans-Golgi glycosylation of channels but did not affect their high cell surface expression versus wt Kv1.4; these mutations appear to be determinants only for efficient glycosylation in the Golgi, and they do not affect the channel’s proper folding and rapid exit from the ER. Group 3 mutations P506A and K533Y in region \( a \) and \( b \), respectively, presumably caused partial retention in the ER of channels and reduced both trans-Golgi glycosylation and surface expression versus wt Kv1.4; these mutations inhibited proper folding and rapid exit from the ER, which is the probable cause of their reduced cell surface expression, but they also disrupted the determinant for efficient glycosylation in the Golgi.

Thus, it appears that wild type Kv1.4’s outer pore regions \( a \) and \( b \) promote its efficient folding and rapid exit from the ER as well as containing positive determinants for efficient trans-Golgi glycosylation, but its cytoplasmic C terminus is required for high cell surface expression. For wt Kv1.1, outer pore regions \( a \) and \( b \) inhibit efficient folding and rapid exit from the ER, which appears to be the cause of its low cell surface expression, and contain negative determinants for trans-Golgi glycosylation. However, a chimeric Kv1.1 with the pore of Kv1.4 was completely rescued and exhibited rapid exit from the ER, efficient trans-Golgi glycosylation, and high cell surface expression.

Although the trans-Golgi glycosylation of Kv1.1 in transiently transfected cells was extremely low, we have selected a stably transfected CHO cell clone expressing Kv1.1 that exhibited a higher conversion ratio (15), but this ratio was still much less than the conversion of Kv1.4. Furthermore, the Kv1.1 trans-Golgi glycosylation conversion in Kv1.1 cRNA-injected oocytes was also low, but a mature p80 band (similar to that found in brain) was enriched in plasma membranes (36). Apparently, some expression systems are capable of trans-Golgi glycosylation of exogenously expressed homomeric Kv1.1, but they do so inefficiently.

Other Mutagenesis Studies on the Outer Pore Regions of \( K^+ \) Channels—Mutagenesis studies in Kv1 \( a \) and \( b \) outer pore regions, which have the greatest amino acid sequence differences in the pore in a subfamily, suggest that they are important in defining different properties among closely related channels such as external IC\(_{50}\) block for tetraethylammonium ion and various toxins, slow C-type inactivation, modulation by external pH, and, in some cases, modulation by external \( K^+ \) and single channel conductance (4, 5, 27). Our study also suggests that these regions are important determinants on channels involved in the effects outlined in this report. However, it is not apparent from inspection of equivalent \( a \) and \( b \) sequences in other Kv1 channels or in Kv2, Kv3, and Kv4 channels that there is a highly conserved motif in these regions. Kv1.5 exhibits its high cell surface expression that is only somewhat less than that of Kv1.4 (28), and its \( a \) region is distinct from Kv1.4. Shaker B also exhibits high surface expression and trans-Golgi glycosylation, although its \( a \) and \( b \) regions are as different from Kv1.4 as Kv1.1 sequences are. It may be that the secondary structures of the \( a \) and \( b \) regions are more important than their primary sequence in forming this combined region motif.

N-terminal Regions—Progressively replacing the N-terminal region of Kv1.4 with Kv1.1 regions (chimeras 4–6) inhibited efficient trans-Golgi glycosylation but did not affect high cell surface expression for chimeras 4 and 5. We propose that this finding is most likely due to these chimeras (N terminus and S1-S4 helices of Kv1.1 and the S5-pore-S6 region of Kv1.4) folding in such a manner as to change the conformation of the positive determinants in the outer pore Kv1.4 regions \( a \) and \( b \) required for efficient glycosylation. This is more likely than the possibility of negative determinants for glycosylation in the N-terminal region of Kv1.1 because chimera 16 (Kv1.1 with the 1.4 pore region), which identified the outer pore region as a major determinant for these processes, exhibited both efficient glycosylation conversion and high cell surface expression.

Possible Role of Determinants in Affecting Steady-state Levels of Kv1 Channels in the Plasma Membrane—It appears that there are multiple determinants on both Kv1.4 and Kv1.1 that are involved in inhibiting or stimulating trans-Golgi glycosylation conversion and cell surface expression of these very closely related channels. These determinants may positively or negatively affect the folding, oligomerization, and stability of a homomeric or heteromeric protein complex, and/or they may associate transiently with non-Kv1 proteins involved in these...
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processes in cellular organelles. Determinants on wt Kv1.1 or mutant constructs that cause partial ER retention may influence intrasubunit folding and/or intersubunit dimerization processes, and if inefficient oligomerization occurs, then chaperone-mediated retention may take place, or they may interact directly with the ER processing machinery through specific amino acids. Because Kv1 channels do not contain canonical ER retention or retrieval signals signals (e.g. RXXR(R), KDEL) it appears more likely that high ER retention of a given subunit used in this study is due to inefficient folding or insufficient stability causing chaperone-mediated partial retention. Furthermore, Kv1.4 does not contain the putative ER export signals that have been mapped on Kir-type K⁺ channels (32). In contrast, the positive determinants for trans-Golgi glycosylation on Kv1.4 outer pore regions may exert their influence by interacting directly with a molecule(s) in the processing machinery pathway that promotes glyco-processing or by not interacting with a molecule(s) in the pathway that inhibits glyco-processing. Negative determinants for trans-Golgi glycosylation in the outer pore region of Kv1.1 may act by either of these modes. These negative determinants may be involved in preventing a channel from coming in efficient contact with the Golgi glyco-processing enzymes, possibly due to the channel tetramer moving through the Golgi in a lipid subdomain that avoids them.

Kv1.1 is efficiently trans-Golgi glycosylated in native brain tissue. This finding and the work in this report suggest that Kv1.1 must be expressed as a heteromer in brain with other Kv1 and Kvβ-subunits in an unknown stoichiometry that leads to its efficient trans-Golgi glycosylation and cell surface expression. Kv1.1 may be preferentially retained in brain neurons in the ER due to its outer pore a and b regions, which appear to act like a “partial retention signal,” until other Kv1 subunits are coexpressed with it, which would give robust cell surface expression of the heteromer. Thus, Kv1 cell surface expression and steady-state levels can be influenced by the channel’s subunit composition. The determinants mapped in this study are important in understanding the amino acids involved in proper channel folding, efficient trans-Golgi glycosylation conversion, and high cell surface expression. Furthermore, we have suggested that Kv1 channels with truncated carbohydrate trees can affect their functional properties (15). Thus, the expression level of a channel in the plasma membrane and its functional properties may be affected by these determinants and contribute to the functional diversity of K⁺ channels in different tissue.

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