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A dipolar amino acid substitution induces voltage-dependent transitions between two stable conductance states in gramicidin channels

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INTRODUCTION

The linear gramicidins are a class of prototypical channel formers that are used to elucidate general aspects of ion channel function (for a review see Andersen et al., 1991). Gramicidin channels are formyl-NH-terminal-to-formyl-NH-terminal dimers of $\beta^{6.3}$-helices, as originally suggested by Urry (1971). The channels are stabilized by 6 intermolecular C = O · · · HN hydrogen bonds, and the channel appearances are usually denoted by stable (on the ms to s timescale) rectangular current transitions with a single predominant conducting state (e.g., Sawyer et al., 1989). (Gramicidin channels exhibit brief "flickers" to a low-conductance state with an average duration of ~1 μs (Heinemann and Sigworth [1991]; these events will not concern us here.) Gramicidin channels are generally presumed to be voltage independent, but we have recently found that some types of asymmetrical (heterodimeric) gramicidin channels exhibit voltage-dependent transitions between (at least) two conductance states.

These channels were discovered in an attempt to discern why heterodimers formed by [F_6Val^1]gramicidin A ([F_6Val^1]gA, in which Val^1 is replaced by 4,4,4,4′,4′,4′-hexafluorovaline), and [Val^1]gA are energetically destabilized relative to the symmetrical parent channels (Russell et al., 1986; Durkin et al., 1990). From the heterodimer appearance rate it could be concluded that [F_6Val^1]gA forms $\beta^{6.3}$-helical channels. Once formed however, these hybrid channels have very short durations, which could suggest that there were a "strain" at the join between the two chemically dissimilar $\beta^{6.3}$-helices (Durkin et al., 1990).

To examine this question further, we studied the behavior of heterodimers formed between [F_6Val^1]gA and [Gly^1]gA, in which Val^1 (with its β branch) had been replaced by a Gly. This substitution should introduce an additional flexibility of the peptide backbone, which might relieve any strain at the join. Heterodimers did indeed form between these two analogues, but they were still destabilized relative to the symmetrical channel types. More importantly, however, these hybrid channels had a fundamentally different appearance than all other heterodimers between two 15-residue analogues: they showed transitions between two "stable" conductance levels. This behavior was examined in detail in relatively solvent-depleted diphytanoylphosphatidylcholine (DPhPC)/*n*-hexadecane bilayers, in which the average channel durations were ~100-fold longer than in DPhPC/*n*-decane membranes.

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RESULTS AND DISCUSSION

Fig. 1 shows the basic channel behavior. The top two current traces show the two symmetrical and the two asymmetrical channel types. The two different heterodimers correspond to the two orientations of the [F_6Val^1]/[Gly^1]gA channels: in the high-conductance heterodimers, the [F_6Val^1]gA monomer is toward the positive solution; in the low-conductance heterodimers, the [Gly^1]gA half of the channel is at that position. The lower two traces show the two hybrid channel types in greater detail. For either channel type, channel formation is visible as a discrete current change, and there are rapid transitions between two conducting states: a high-conductance state (H) and a low-conductance state (L). Both conductance states are rectifying, but in opposite directions.

For either heterodimer orientation, the relative time the channels were in the H and L states were determined from current amplitude histograms (Fig. 2). The fraction of time spent in the H state (f_h) varied as a function of potential: when the [F_6Val^1]gA half is positive, most of the time is spent in the L state ($f_h \approx 0.1$); when the [Gly^1]gA half is positive, slightly less than half the time is spent in the H state ($f_h \approx 0.4$).

F_6Val possesses a dipole moment (μ) of ~1.6 Debye (cf. Russell et al., 1986). *A priori* one would thus expect that the transitions between H and L could result from a

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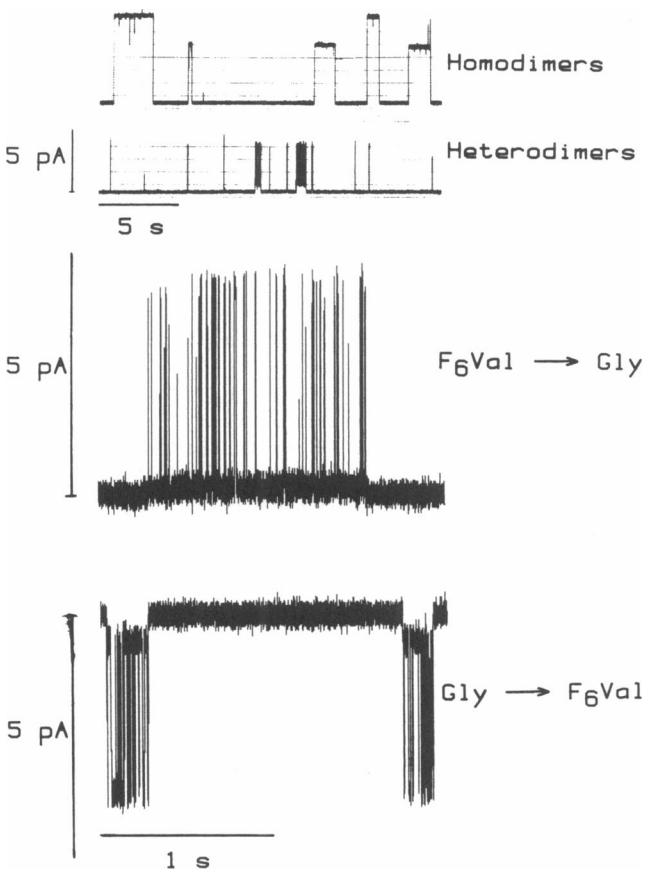

FIGURE 1 Single-channel current traces obtained with [F_6Val^1]gA and [Gly^1]gA. The top two traces were filtered at 100 Hz. The upper trace shows the homodimers, the channels with the higher conductance being [Gly^1]gA channels. The lower trace shows the heterodimers. They are less stable and show "flickery" behavior. The lower two current traces (filtered at 1 kHz) show the two forms in which the heterodimers occur. They correspond to the two possible heterodimer orientations (shown in experiments where each analogue was added to only one side of a preformed bilayer). Experimental conditions: 1.0 M CsCl; 200 mV; the bilayers were formed from DPhPC + *n*-hexadecane monolayers at the tip of a silanized bilayer-punch pipet.

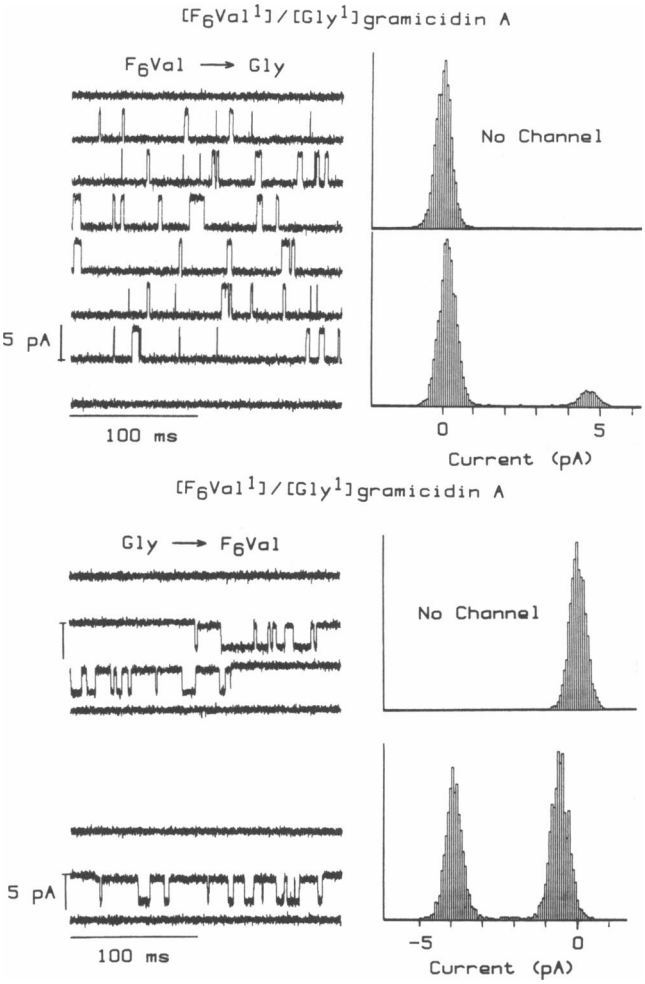

FIGURE 2 Results at higher time resolution. The top part of the figure shows results obtained in the F_6Val → Gly direction, the bottom part shows results obtained in the Gly → F_6Val direction. The current traces denote consecutive sweeps. The current histograms to the right show results obtained without (*top*) and with a channel (*bottom*). In the latter case, there are two peaks, corresponding to the H and L states. Even in the F_6Val → Gly direction, the L state has a finite conductance. The area under each peak is proportional to the time spent in each state. The average conductances and durations of H and L were: for F_6Val → Gly, 22.8 and 0.9 pS, 2.8 and 26 ms; for Gly → F_6Val, 19.7 and 2.9 pS, 5.3 and 12 ms. Experimental conditions as in Fig. 1; filtered at 3 kHz.

reorientation of the F_6Val side chain between two relatively stable rotameric states. In the simplest version of this model, the voltage dependence of f_h would result from the difference in electrostatic energy between the two rotamer states (dipole orientations). But side chain reorientation alone cannot account for the results: one can estimate the magnitude of this electrostatic energy difference (ΔE) from the f_h estimates at ±200 mV (taking the [F_6Val^1]gA side as the electrical reference),

$$f_h(+200)/f_h(-200) = \exp\{-2 \cdot \Delta E/RT\}. \quad (1)$$

$f_h(+200)/f_h(-200) \approx 4$; ΔE is thus estimated to be ~ -3.4 kJ/mol. This is sixfold larger than can be accounted for by the electrostatic field interacting with the F_6Val dipole (rotating it 180°): ~ 0.5 kJ/mol (~ 0.2 kT at 25°C) for a field of $8 \cdot 10^7$ V/m. Nevertheless, from the sign of ΔE, it appears that the H state is stabilized when the two CF_3 groups point away from the [F_6Val^1]gA monomer and toward the [Gly^1]gA monomer.

There is little doubt that the F_6Val side chain "triggers" the conductance changes; these H ↔ L transitions must, however, result from a larger conformational change because we cannot account for the necessary energy by invoking only the F_6Val side chain reorientation. It is in this respect important that the heterodimers have a finite conductance in the L state (Fig. 2) because this implies that the basic channel structure remains intact (the pore has not collapsed), which constrains the possible conformational rearrangements. (If the "missing" energy results from the reorientation of additional dipoles, the conformational change involves at least two peptide groups [$\mu \approx 3.6$ Debye] or 4 water molecules [$\mu \approx 1.8$].)

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