Mapping hydrophobic residues of the interaction surface of charybdotoxin

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INTRODUCTION

Charybdotoxin (CTX) is a 37-residue peptide that inhibits in the nanomolar concentration range numerous K^+ channels by binding to a receptor located in the outer mouth of the channel and literally blocking K^+ conduction (1–3). CTX is a basic peptide originally isolated from the venom of the scorpion *Leiurus quinquestriatus* (4). Recently, Park et al. (5) produced fully active recombinant CTX using a synthetic gene coding for CTX fused onto an *E. coli* expression plasmid. Using this method we constructed point mutations of CTX by polymerase chain reaction (PCR) mutagenesis and assayed these on single high-conductance Ca^{2+}-activated K^+ channels reconstituted into planar lipid bilayer membranes. The binding of CTX in a simple bimolecular way to K^+-channels is seen at the single-channel level as the appearance of "long-lived" nonconducting intervals that are easily distinguished from "short-lived" channel closing events with average dwell times in the range of 1–5 ms (1) (see Fig. 1). We measured the on- and off-rates and calculated the dissociation constant (K_d) as described by Anderson et al. (6) in their model for CTX binding to K^+ channels. The structure of CTX has previously been determined by two-dimensional NMR (7). This work is therefore intended to be a step on the way to link the structure and the function of the charybdotoxin molecule.

RESULTS AND DISCUSSION

Though CTX is a highly charged molecule previous chemical modification work (4, 8) suggested that hydrophobic interaction may contribute to toxin-channel recognition. We tested this proposal by producing point mutations at several hydrophobic residues. All CTX variants examined here blocked the K^+-channels with an affinity equal to or lower than the wild-type (Fig. 1 and Table 1). The single exponential nature of the blocked dwell times indicates that in each case we are examining a single species of CTX (Fig. 2). Furthermore, the association rates are all within a factor of two to three of the wild-type value and the block times are voltage dependent. This strongly supports the assumption that the mutants are all similar to wild-type CTX in overall structure.

At position 29 even a very conservative mutation (Met to Leu) produces a drastic decrease in affinity. The tyrosine at position 36 represents a special case where it is possible to produce a series of mutations with different effects. Phe, which is the most conservative substitute, has no effect on the channel blocking; replacement of the large hydrophobic Tyr with the small polar Asn decreases the affinity 20-fold. Substituting by Ala, His or Pro totally abolishes blocking activity. The 7- and

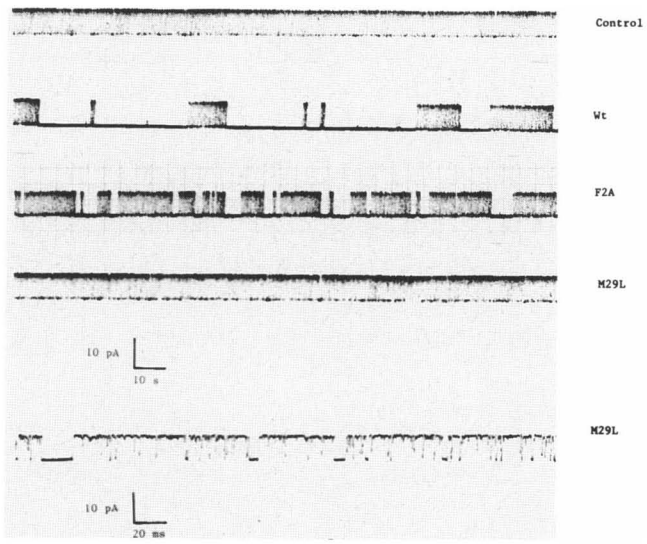

FIGURE 1 Block of Ca^{2+}-activated K^+ channels by recombinant wild type and point mutated CTX. A single channel was inserted into a planar bilayer and observed in symmetrical 150 mM KCl medium, at a holding potential of 30 mV inside (*cis*) positive. CTX was added to the outside (*trans*) chamber. (*First trace*) Control record, no CTX added. (*Second trace*) 50 nM wild type CTX. (*Third trace*) 100 nM Phe2 to Ala mutated CTX. (*Fourth trace*) 75 nM Met29 to Leu mutated CTX. Trace five is similar to trace four, but with an expanded time scale.

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TABLE 1 **The dissociation constant (K_d) of recombinant wild type CTX and different hydrophobic point mutations of CTX.**

Mutant	K_d (nM)	K_d(Mut)/K_d (Wt)
Wild type	15	—
pE1Q	110	7
F2A	160	11
V5E	12	1
V16E	25	2
L20N	14	1
M29L	2,300	150
Y36F	12	1
Y36N	160	11
Y36(A,H,P)	no blocks	—

The third column gives the K_d value of the mutant relative to the wild type. The conditions were as described in the legend to Fig. 1.

11-fold affinity changes at position 1 and 2 are caused by more radical changes (pGlu to Gln and Phe to Ala). At positions 5, 16, and 20 even very radical mutations (Val to Glu and Leu to Asn) produce only minor changes in affinity. These results are naturally rationalized in terms of the CTX solution structure. The toxin is of a lumpy, slightly oblate, ellipsoidal "moon-lander" shape with axes ~2 and 2.5 nm (7, 8). All strongly destabilizing mutations (pGlu1, Phe2, Met29, Tyr36) are residues forming a surface on the "lower" part of the molecule, whereas the functionally unimportant mutations (Val5, Val16, Leu20) are residues located on the opposite side of the molecule. Furthermore Met29 and Tyr36 are probably in intimate contact with the channel and therefore more important for contact than pGlu1 and Phe2. These results are consistent with the mutations reported by Park and Miller (unpublished results). They changed the eight charged residues of CTX, and the three mutations that lowered the blocking affinity of the molecule are all located in the same area found here for the hydrophobic residues.

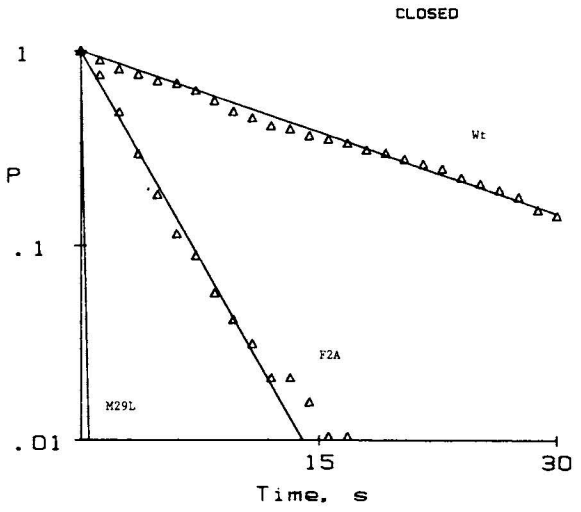

FIGURE 2 Exponential distribution of CTX blocked times. The cumulative distribution function $P(t)$ is defined as the probability that a given dwell time is greater than or equal to time t. A blocked state was defined as a nonconducting interval of duration >300 ms (for M29L > 50 ms). Under these conditions this is 50 (10)-fold longer than the mean closed time. Otherwise the conditions were as described in the legend to Fig. 1. The off rates are *Wt*: 0.067 s^{-1}, F2A: 0.33 s^{-1} and M29L: 9.1 s^{-1}.

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