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MAST CELL DEGRANULATING PEPTIDE FORMS VOLTAGE GATED AND CATION-SELECTIVE CHANNELS IN LIPID BILAYERS

Toru Ide, Takahisa Taguchi, Takuma Morita, Masayuki Sato, Kazuhiro Ikenaka*, Saburo Aimoto*, Tetsuro Kondo*, Hironobu Hojo*, Michiki Kasai, and Katsuhiko Mikoshiba*

Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

*Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan

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SUMMARY: Mast cell degranulating peptide, a toxin of bee venom, is a polypeptide composed of 22 amino acids. Exposure of the asolectin bilayer to the peptide results in the formation of channels that are more permeable to K^+ than Cl^-. These channels are activated when the voltage of the cis side, to which the peptide is added, is made positive.

Mast cell degranulating peptide (MCD) is a basic peptide composed of 22 amino acids which was isolated from bee venom (1). It was first noted for its mast cell degranulating activity (2), and it is also known to be neurotoxic (3) and, depending on the concentrations injected (4), induces convulsions and epileptogenic crisis. Recently, it has been shown that MCD induces long-term potentiation (LTP) of synaptic transmission in the CA-1 region of the hippocampus (5). High affinity binding sites for MCD have been identified in brain membranes (6).

Recently, we synthesized MCD and showed that the synthetic peptide was also capable of inducing LTP in the hippocampus (7). Therefore, it was concluded that the LTP-inducibility resided in MCD itself and not due to any contaminants that could not be removed during the purification process, such as phospholipases. During the course of studies aimed at understanding the mechanism of induction of LTP by MCD, we found that MCD interacts with the lipid bilayer to form cation-selective channels.

Abbreviations: MCD, Mast cell degranulating peptide; LTP, long-term potentiation.

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MATERIALS AND METHODS

Materials: Phosphatidylcholine (asolectin) was purchased from Sigma Chemical Co., USA. All other chemicals were commercial products of analytical grade.
Peptide Synthesis: The peptide was synthesized as described (7).
Bilayers and Peptides Incorporation: Planar bilayers consisting of asolectin were prepared by the painting method of Mueller and Rudin (8). The MCD peptide was dissolved in water, and an aliquot was added to one side (cis side) of a preformed bilayer with stirring.

The recorded data were digitized by a 12-bit A/D converter at an appropriate sampling rate, and analysis was carried out by a micro computer.

RESULTS

Figure 1 is a record of the current fluctuation of an asolectin bilayer exposed to 2.5 nM MCD peptide. Current fluctuations of various amplitudes are observed in this record. Some of them are integral multiples of the smaller ones. In Fig. 1-A, for example, there are 38 pS, 115 pS, and 230 pS channels. The minimum size of the current fluctuation observed corresponds to 3.8 pS (data not shown). However, it was found that not all of the fluctuation levels present in this and other records are integral multiples of the lower levels.

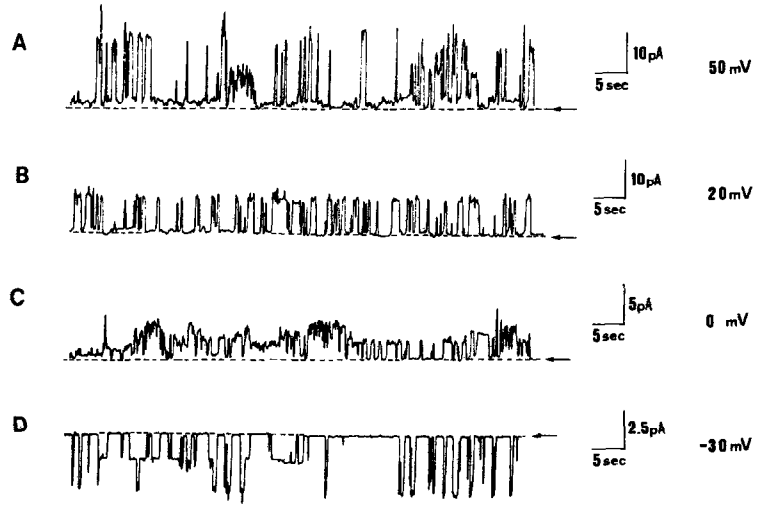

Fig.1. Current records of MCD peptide channels. About 10 minutes after the addition of 2.5 nM of peptide to the cis solution, current responses were recorded at the indicated holding potentials. The cis solution was composed of 0.3 M KCl, 15 mM Hepes/Tris, pH 7.2; and the trans was composed of 0.1 M KCl, 15 mM Hepes/Tris, pH 7.2. The baselines are indicated by dotted lines. In this experiment, we observed current fluctuations that corresponded to single-channel conductances of 38 pS, 60 pS, 115 pS, 170 pS, 230 pS, and 380 pS.

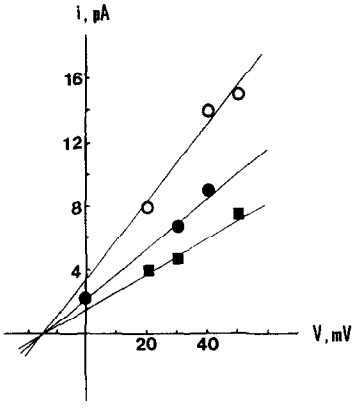

Fig.2. Current-voltage characteristics for MCD peptide channels in asymmetrical solutions. Three different current fluctuations were obtained at different voltages under the same conditions as in Fig. 1. The reversal potential was estimated to be about -15 mV based on linear extrapolation of the data for every fluctuation level. P_K/P_{Cl} was obtained using the Goldman equation and was found to be about 4. The slope conductances were 115 pS(■), 170 pS(●), and 230 pS(○), respectively.

We have found that the channel formed by MCD is selective for K^+ over Cl^-. The permeability ratio, P_K/P_{Cl}, was 4~6; it was calculated from the reversal potential obtained in this and other records according to the Goldman equation (Fig.2). We also found that Na^+ permeates through MCD peptide channels (data not shown). Current fluctuation that corresponded to a single-channel conductance of about 7 pS was observed in NaCl solution (cis: 0.3 M NaCl, 15 mM Hepes/Tris, pH 7.2, trans: 0.1 M NaCl, 15 mM Hepes/Tris, pH 7.2). This channel was selective for Na^+ over Cl^- ($P_{Na}/P_{Cl} \sim 8$). Although we observed larger current fluctuations in the NaCl solution, their marked instability prevented us from determining single-channel conductances or ion selectivities.

The MCD peptide channels we observed are voltage dependent. Their open probability increases with the increase of applied voltage as shown in Fig. 3. In this record, the open probability of the channel is 0.75 at 50 mV, decreased to 0.45 at 20 mV, and the channel closed within the time course of the record when the applied voltage was less than 10 mV. However, it was found that some other channels open even at negative voltage (see Fig. 1-D).

Furthermore, opening of the channel is dependent on the concentration of MCD peptide. Raising the peptide concentration increased the probability of

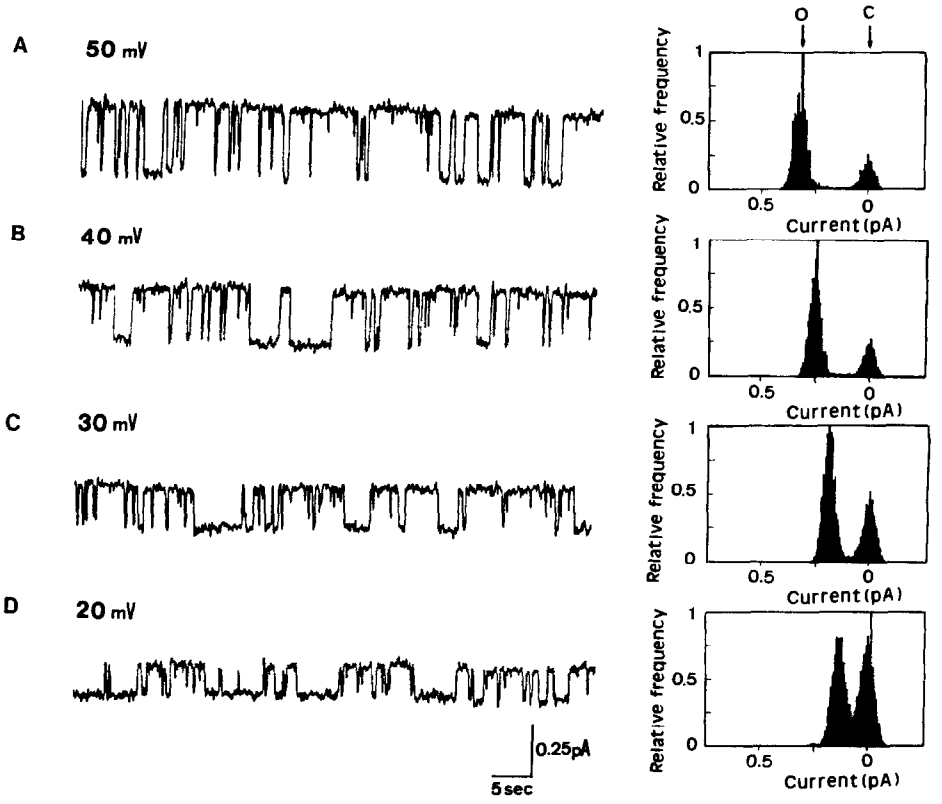

Fig.3. Voltage dependence of MCD peptide channel. In each figure, the left panel is the current recording at the indicated potentials in a symmetrical solution of 0.3 M KCl, 15 mM Hepes/Tris, pH 7.2. The right panel is the amplitude histogram corresponding to the data partially shown in the trace. Arrows indicate levels of open (O) and closed (C) states. Open probability is about 0.75 at 50 mV and about 0.45 at 20 mV.

the open state. As shown in Fig. 4, at the concentration of 1 μM, the channel is in the open state nearly all the time (cf. Fig. 1). It was also found that the latency, time required for forming an ion pathway after the addition of peptide, increases with an increase of peptide concentration.

DISCUSSION

The data described in this paper establish a new fact that MCD peptide interacts with an asolectin bilayer to form channels with various conductance levels. About 10 minutes after the addition of 2.5 nM peptide to the cis solution, current flickering of various sizes begins to appear. The flickering continues for several minutes until the size of the amplitude fluctuation gradually becomes constant. This suggests that the peptide

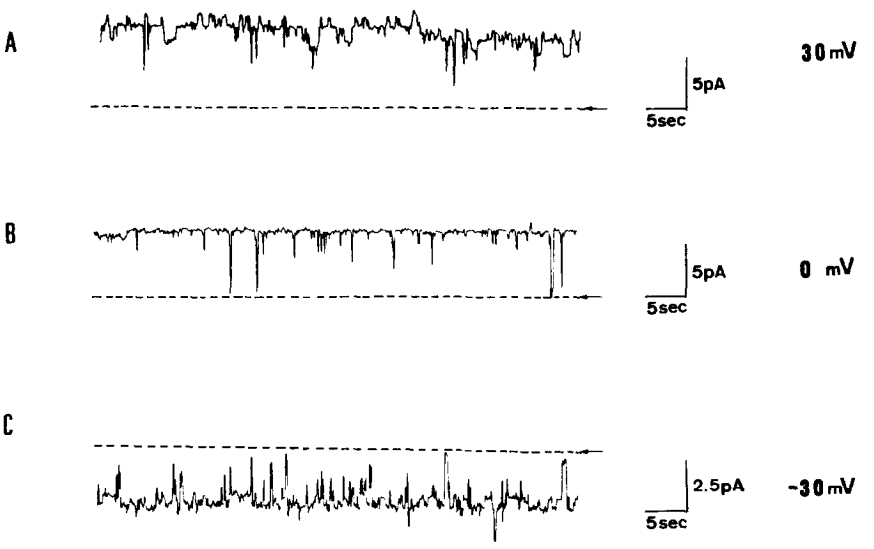

Fig.4. Current records at a high concentration of peptide. The current responses were recorded at the indicated voltage after the addition of 1μM of MCD peptide to the cis chamber. The cis solution was composed of 0.3 M KCl, 15 mM Hepes/Tris, pH 7.2; and the trans was composed of 0.1 M KCl, 15 mM Hepes/Tris, pH 7.2. The arrows denote the baseline current.

monomers in the lipid membrane associate randomly to form different kinds of oligomers, and they transform to more stable channels which conduct ions.

We have found that at higher peptide concentrations, formation of channels takes a longer time. This latency lasts an hour or more at 1 μM, whereas it takes about 10 minutes at 2.5 nM. We hypothesize that the peptide has a tendency to form a hydrophilic aggregate in aqueous solution, and it is difficult for the aggregate to incorporate into the bilayer.

The channel induced by MCD peptide is cation-selective, although the peptide has many positive charges. This may be due to the permeant anions bound to the positively charged side chains of the peptide. We have found that various sizes of single-channel conductances are induced by MCD peptide as shown in Fig. 1. Some of them are integral multiples of the smaller ones, but others are not. There are two ways of explaining these different sizes of single-channel conductances. One is that MCD molecules have a tendency to aggregate laterally, forming oligomers containing different numbers of monomers, like the channels of alamethicin and its analogues (9, 10). The other explanation is that like it has been proposed for the Cl^- channel (11),

the channels consist of different numbers of identical conducting units (protochannels or cochannels), each opening and closing simultaneously owing to cooperative interaction. The minimum size of the single-channel conductance that we observed is 3.8 pS, which may be the basic unit channel.

The channel formed by MCD is voltage dependent. Tosteson et al. (12) proposed that the voltage dependency of channels formed by peptides depends on the presence of positive charges on the side chains of some of the amino acid residues. MCD peptide has many positive charges, and this suggests that the voltage dependency of the channel induced by MCD peptide depends on the primary structure of the peptide.

MCD peptide has been suspected to bind to a K^+ channel and modulate the K^+ current. However, our finding that MCD itself can form cation selective channels in lipid bilayers adds a new perspective for understanding the mechanism of the pharmacological action of MCD.

REFERENCES

1. Adams, J. C. (1977) Neuroscience 2, 141-145.
2. Breithaupt, H., and Habermann, E. (1968) Naunyn-Schmiedeberg's Arch. Exp. Pathol. 261, 252-270.
3. Banks, B. E. C., German, A. J., and Habermann, E. (1978) J. Physiol. 284, 160p-161p.
4. Bidard, J.-N., Gandolfo, G., Mourre, C., Gottesmann, C., and Lazdunski, M. (1987) Brain Res. 418, 235-244.
5. Cherubini, E., Ben Ari, Y., Gho, M., Bidard, J.-N., and Lazdunski, M. (1987) Nature, 328, 70-73.
6. Taylor, J. W., Bidard, J.-N., and Lazdunski, M. (1984) J. Biol. Chem. 259, 13957-13967.
7. Ikenaka, K., Kondo, T., Kato, H., Ito, K., Aimoto, S., Hojo, H., and Mikoshiba, K., submitted for publication.
8. Muller, P., and Rudin, D. O. (1969) in Laboratory Technique in Membrane Biophysics (Passow, H. and Stampfli, R. eds) pp.141-156, Spring-Verlag, Berlin.
9. Baumann, G., and Mueller, P. (1974) J. Supramol. Struct. 2, 538-557.
10. Boheim, G. (1974) J. Membr. Biol. 19, 277-303.
11. Miller, C. (1982) Phil. Trans. R. Soc. Biol. Sci. 299, 401-411.
12. Tosteson, M., Auld, D., and Tosteson, D. (1989) Proc. Natl. Acad. Sci. USA 86, 707-710.