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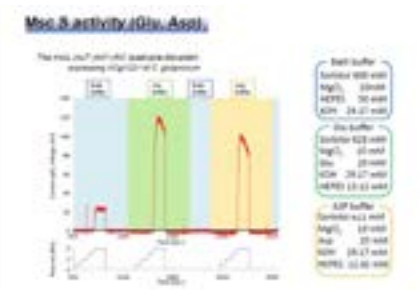
Analysis of microbial ion transport system by using electrophysiological method

The patch-clamp method is used to measure ion flux across biological membranes in the form of an electric current, using a small glass microelectrode. It has an advantage of high time resolution, and is highly quantitative in nature. Furthermore, this method allows for the quantitative changes in membrane tension, which is difficult to achieve with other methods, and for the manipulation of the microenvironment including membrane potential and substrate concentrations inside and outside of membrane. Hence, the patch-clamp method is suitable for measuring ion transport across membranes. The patch-clamp method is a well-known biophysical method. However, application of the patch-clamp method in microorganisms is very limited. The present study aimed to develop a unique system to perform the patch-clamp method on microorganisms, which allows for both whole-cell mode and whole-provacuole mode analyses and for analyzing membrane transport activity using membrane generated in vivo. Application of provacuoles with reversed membranes allows for analysis of cellular export (transport from the interior of a cell to the exterior) as an import (transport from the exterior of a provacuole to the interior), which is strikingly advantageous. *Corynebacterium glutamicum* is widely used for the industrial production of L-glutamate; however, its underlying mechanism is unclear. Using our unique microbial patch-clamp system, we report that the *NCgl1221* gene, critically involved in glutamate overproduction in *C. glutamicum*, encodes a mechanosensitive channel and glutamate is effluxed across the membrane through the *NCgl1221* channel via passive diffusion. Herein, the author shall discuss other characteristics of this channel, which facilitate glutamate efflux. In addition, the characteristics of the proton pumping activity of terminal oxidase of *Escherichia coli,* obtained using the present unique microbial patch-clamp system will also be discussed. The author believes that their unique microbial patch-clamp system will greatly contribute to the analysis of bacterial transmembrane channels and transporters and yield novel findings.



Recent Publications

1. Komine-Abe A, Nagano-Shoji M, Kubo S, Kawasaki H, Yoshida M, et al. (2017) Effect of lysine succinylation on the regulation of 2-oxoglutarate dehydrogenase inhibitor, OdhI, involved in glutamate production in *Corynebacterium glutamicum*. Biosci. Biotechnol. Biochem. 81:2130–2138.
2. Tomita A, Zhang M, Jin F, Zhuang W, Takeda H, et al. (2017) ATP-dependent modulation of MgtE in Mg2+ homeostasis. Nat. Commun. 8:148–158.
3. Mizuno Y, Nagano-Shoji M, Kubo S, Kawamura Y, Kawasaki H, et al. (2016) Altered acetylation and succinylation profiles in

Corynebacterium glutamicum in response to conditions inducing glutamate overproduction. Microbiologyopen 5:152–173.

4. Nakayama Y, Becker M, Ebrahimian H, Konishi T, Kawasaki H, et al. (2016) The impact of the C-terminal domain on the gating properties of MscCG from *Corynebacterium glutamicum*. Biochim. Biophys. Acta,-Biomembranes 1858:130–138.
5. Yamashita C, Hashimoto K, Kumagai K, Maeda T, Takada A, et al. (2013) L-Glutamate secretion by the N-terminal domain of the *Corynebacterium glutamicum NCgl1221* mechanosensitive channel. Biosci. Biotechnol. Biochem. 77:1008–1013.

Biography

Dr. Hisashi Kawasaki has a unique patch-clamp system for microbial cells, which enables the analysis of ion-transport across microbial cytoplasmic membrane. He and his collaborator Dr. Isamu Yabe have been improving the system, originally developed by Dr. Yabe (JBC 1998), for over 20 years now. This unique system would allow the electrophysiological analysis of not only microbial transmembrane channels and transporters, but also the respiratory and photosynthetic systems that are composed of several channels and transporters (related to microbial function). Hence, this system would help in novel findings and comprehensive understanding of ion-transport network.

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