Structural dynamics of potassium ion channels revealed by side-chain methyl ^{13}C-^{1}H multiple quantum relaxation analyses

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Electrophysiological and biophysical studies of potassium ion (K^+) channels have revealed that K^+ channels are not static and exchanging between functionally different states on the timescale of microsecond to second, and interconversion between these states play critical roles in their biological functions. Therefore, to fully understand the structural mechanism of K^+ channels, it is important to characterize the chemical exchange processes of K^+ channels. Developments in methyl-transverse relaxation optimized spectroscopy (methyl-TROSY), which observes the slowly relaxing ^{13}C-^{1}H multiple quantum (MQ) coherences of side-chain methyl groups, have enabled the studies of large systems such as K^+ channels, however, the analyses of microsecond to millisecond chemical exchange processes based on the methyl-TROSY principle are still challenging, because the interpretation of the chemical exchange contributions to the MQ relaxation profiles is complicated, as significant chemical shift differences occur in both ^{1}H and ^{13}C nuclei.

To overcome these difficulties, we established a new methyl-based NMR method, which enables quantitative evaluations of chemical exchange contributions in MQ relaxation rates, the methyl heteronuclear double resonance (methyl-HDR) method. The methyl-HDR method exploits the fact that the exchange contributions in differential MQ relaxation rates ($\Delta R_{MQ,ex}$) can be selectively quenched by applying heteronuclear double resonance (HDR) pulses sufficiently faster in field strength than the chemical exchange processes. Thus, we can obtain the $\Delta R_{MQ,ex}$ rates as the difference of the ΔR_{MQ} rates measured in the presence and absence of the HDR pulses. We applied the method to an inwardly-rectifying potassium channel, which has an apparent molecular weight of over 200 K as a functional tetrameric form in detergent micelles, and successfully identified the regions that exist in a chemical exchange process on millisecond time scale. Further applications of the method to reveal ligand-dependent activation mechanism of K^+ channels will be discussed in the presentation.

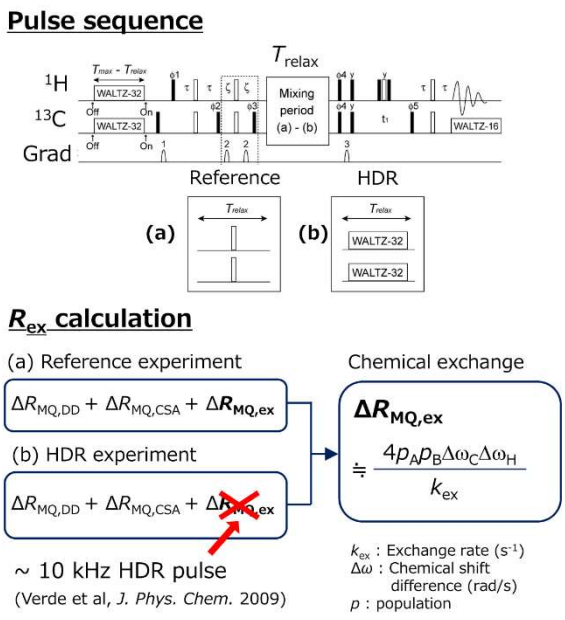

Figure. Pulse sequence and schematic explanation of the methyl-HDR method

References

Yuki Toyama, Masanori Osawa, Mariko Yokogawa, Ichio Shimada *Journal of the American Chemical Society* (2016) 138(7), 2302-2311.

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