Development of Red-Shifted Channelrhodopsin Variants Having Chemically Modified Retinylidene Chromophore

レチニリデン発色団の化学修飾による赤色光吸収チャネルロドプシンの開発

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Introduction

Channelrhodopsin (ChR) is a light-gated cation channel found in algae. It absorbs light and forms a hydrophilic channel in its structure, letting various cations pass across cell membrane. The generation of cation flow using light energy by ChR shows its capability for optogenetics application, a neuromodulation method to manipulate membrane potential in living organism by illumination. However, naturally existing ChR variants are generally optimized for blue-green light, which is highly scattered or absorbed by animal tissues. To overcome this, red-shift of ChR absorption spectra is demanded.

In this study, we propose a new and effective strategy to red-shift the ChR spectrum by replacing the chromophore, *all-trans* retinal (ATR1), to its chemically modified analogues. C=C elongated retinal analogues were introduced into widely studied ChR variant, C1C2. The spectrum of C1C2 was effectively red-shifted, and the channel opening (conducting) intermediate states could be observed in the photocycle of each C1C2 analogue.

Results and Discussion

Chemically modified analogues of ATR1 and ATR2 (3,4-didehydro-*all-trans*-retinal) were synthesized. These retinal analogues have elongated conjugated double bond system with inserted extra C=C at different positions on the polyene chain (C6−C7, C10−C11, and C14−C15, referred to as 6ex, 10ex, and 14ex, respectively). Totally 8 kinds of retinals and analogues were introduced into ChR C1C2.

The absorption spectrum of C1C2 was red-shifted by introducing elongated ATR1 and ATR2 analogues. The degrees of relative pigment yield and spectral red-shift were dependent to the location of C=C insertion. Among 8 kinds of retinals and analogues, ATR2-10ex most effectively red-shifted the C1C2 spectrum. It red-shifted the absorption maximum from 477 nm to 510 nm, and the long-wavelength boundary from

514 nm to 599 nm.

By testing the photocycle of analogue pigments, we demonstrated that C1C2 analogues could complete the photocycle, although they had elongated bulky retinal analogues as their chromophore. The channel opening state, P2 state, could be observed in all C1C2 analogues, indicating a putative channel activity. Further analysis of the photocycle kinetics using SVD analysis showed that the time constant of each intermediate state varied by the location of insertion of extra C=C on polyene chain. In short, 6ex analogues decelerated P3 formation, 14ex analogues accelerated P3 formation, and 10ex analogues extremely decelerated the whole photocycle reaction.

While almost all the combinations of C1C2 and retinal analogues efficiently formed pigments, some combination showed low pigment yield because of the extra steric effect. ATR1 analogues had low relative pigment yield, especially for ATR1-6ex, which formed almost no pigment. To release the steric hindrance caused by these bulky analogues, Phe265 in C1C2 chromophore binding pocket was mutated to Ala. The F265A mutation resulted in more effective pigments formation by ATR1 analogues, without significant influences to the spectral characteristics. Further analysis of photocycle kinetics of F265A analogues was also performed, showing the same photocycle patterns but longer channel open state comparing to their C1C2 counterparts.

Conclusion

Our results show that elongation of the conjugated double bond system of retinal is a promising strategy for improving the ability of ChR to absorb long-wavelength light. In C1C2, these retinal analogues gave maximally 33 nm red-shift of the absorption maximum, and 84 nm red-shift of the long-wavelength boundary. Although the bulky retinal analogues could decrease the ChR pigment yield, this could be compensated by mutagenesis for the residues in the chromophore binding pocket. Also, although the photocycle kinetics were altered, channel open intermediate states could be observed in all C1C2 analogues, indicating a putative channel activity.

Hereafter, we would expand our elongated retinal analogue system into other ChR variants. The tuning ability of spectra of these analogues could further red-shift the spectra of naturally red-shifted ChR variants, such as ReaChR and ChrimsonR, making them more efficiently utilize the light passing through the biological optical window.