

# Cinko-bakteriochlorofilo A' sąveikos tyrimai *Chloroacidobacterium thermophilum* reakcijų centruose naudojamą EPR spektroskopiją

## Elucidating the Role of Zinc-Bacteriochlorophyll A' in the *Chloroacidobacterium thermophilum* Reaction Centers Using EPR Spectroscopy

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*Chloroacidobacterium (C.) thermophilum* is a microaerophilic, chlorophototrophic species in the phylum Acidobacteria that uses homodimeric type-1 reaction centers (RC) to convert light energy into chemical energy using (bacterio)chlorophyll ((B)Chl) cofactors. Pigment analyses show that these RCs contain BChl  $a_P$ , Chl  $a_{PD}$ , and  $Zn^{2+}$ -BChl  $a_P$  in the approximate ratio 7.1:5.4:1. However, the functional roles of these three different Chl species are not yet fully understood. It was recently demonstrated that Chl  $a_{PD}$  is the primary electron acceptor. Because  $Zn^{2+}$ -(B)Chl  $a_P$  is present at low abundance, it was suggested that the primary electron donor might be a dimer of  $Zn^{2+}$ -BChl  $a_P$  molecules. In this study, we utilize isotopic enrichment and electron paramagnetic resonance (EPR) high-resolution two-dimensional (2D)  $^{14}N$  and  $^{67}Zn$  hyperfine sublevel correlation (HYSCORE) spectroscopy to demonstrate that the primary donor cation,  $P_{840}^+$ , in the *C. thermophilum* RC is indeed a  $Zn^{2+}$ -BChl  $a_P$  dimer. Density functional theory (DFT) calculations and the measured electron-nuclear hyperfine parameters of  $P_{840}^+$  indicate that the electron spin density on  $P_{840}^+$  is distributed nearly symmetrically over two  $Zn^{2+}$ -(B)Chl  $a_P$  molecules as expected in a homodimeric RC. To our knowledge this is the only example of a photochemical RC in which the Chl molecules of the primary donor are metallated differently than those of the antenna.

The 2D  $^{67}Zn$  HYSCORE spectroscopy measurements provide direct evidence that the excitonically coupled BChl dimer in the primary donor cation,  $P_{840}^+$ , is comprised of  $Zn$ -BChl  $a_P$  molecules. Supporting this conclusion, (1) the maximal light induced bleaching at 840nm, (2) the relatively narrow line width of the continuous wave (CW) EPR signal of  $P_{840}^+$ , (3) the smaller  $^{14}N$  hyperfine coupling constants of the ring nitrogen atoms in comparison with monomeric  $Chl^+$  and (4) DFT calculations showing a

distributed electron density in a dimeric  $Zn^{2+}$ -BChl  $a_P$  cation model all indicate that the charge density should be highly delocalized over a strongly excitonically coupled BChl dimer in the primary donor cation,  $P_{840}^+$ , of *C. thermophilum*. This is the first example of a photochemical RC in which the (B)Chl molecules in the primary donor are metallated differently than the other (B)Chls in the RC and antenna. In other respects, these unusual *C. thermophilum* RCs are nevertheless similar to other homodimeric type-1 RCs, as the spin density in  $P_{840}^+$  is distributed symmetrically over the two Chls of the dimer. This work also demonstrates the utility of 2D  $^{67}Zn$  HYSCORE spectroscopy for the study of zinc sites in paramagnetic systems. By the direct detection of  $^{67}Zn$  hyperfine and quadrupolar couplings in a paramagnetic excited state of a  $^{67}Zn$  labelled reaction center, we anticipate that in the future 2D  $^{67}Zn$  HYSCORE spectroscopy will play an important role in the study of  $^{67}Zn$ -enriched metalloproteins. Furthermore, a combination of 2D  $^{67}Zn$  HYSCORE data and DFT calculations has the potential to be used as a tool to characterize  $Zn$ -containing paramagnetic systems with low  $^{67}Zn$  concentrations. Overall, this study is a noteworthy contribution toward probing the relationship between  $^{67}Zn$  hyperfine and quadrupolar interaction tensors and molecular structure in a paramagnetic system and provides excellent opportunities for the application of 2D  $^{67}Zn$  HYSCORE in the investigation of zinc binding sites in a wide variety of bioinorganic systems.

**Keywords:** *electron paramagnetic resonance, oxygen-evolving complex, photosystem-II, zinc-complexes.*

### References

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