# Structural Insights into Biopolymer PHA Granular Phasins: Comparison Analysis and Function Validation

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## 1 Introduction

Polyhydroxyalkanoates (PHA) are biodegradable polyesters synthesized and accumulated in many bacterial species as their intracellular carbon and energy storage compound under nutrient-limited growth conditions. The biopolyesters PHA attracted more and more attentions because of their excellent characteristics of biodegradability, biocompatibility, and bio-piezoelectricity; especially their immense potential as bio-plastics substitute to petroleum-derived thermoplastics (petrochemical plastics for "white pollution", environmentally unfriendly) and as scaffold or implant biomaterials in tissue engineering. PHA are a natural part of the renewable carbon cycle and a sustainable source of bio-plastics in the future.

The type of PHA-producing bacterium and growth conditions determined the chemical composition of PHA monomers. Typically poly(3-hydroxybutyrate) (PHB, C4 monomer, short chain length PHA) was homopolymer from *Wautersia eutropha* strain H16 and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx, C4 and C6 monomers, copolymer of short chain-medium chain length PHA) was heteropolymer from *Aeromonas hydrophila* strain 4AK4. Native PHA were accumulated as intracellular granules (inclusion bodies) *in vivo* and PHA granules were found to be enveloped by various granule-associated proteins. Non-enzymatic structural proteins associated to PHA granules were referred to PHA granular phasins, which occurred in any PHA accumulating bacterium. In bacterial PHA producers the absence of phasin reduced PHA content and granule number. Phasins have a granule stabilizing function, or may have several functions such as inhibiting individual granules from coalescing and agglutinating with other granules [1]. It was also speculated that phasins might have a protective function to reduce the passive attachment of cytosolic proteins [2]. In conclusion, it played a key role in PHA granule initial formation and polymer accumulation/stabilization *in vivo*. However, no crystal structure of PHA granular protein has been determined and little structural knowledge has been known about phasins. Here we focused on the comparison of two phasins, $PhaP_{We}$ from *Wautersia eutropha* and $PhaP_{Ah}$ from *Aeromonas hydrophila*, in order to elucidate their structure characteristics and function validation.

## 2 Methods

According to the computation results of the two phasins and their physical and chemical parameters from primary structure analysis tool **ProtParam** (EXPASY)**,** $PhaP_{We}$ and $PhaP_{Ah}$ were classified as stable protein. Molecular construction for high level expression were performed

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according to standard procedure. The genes encoding $PhaP_{We}$ (192 amino acid residue,20 kDa) and $PhaP_{Ah}$ (116 amino acid residue,13 kDa) were, respectively, PCR amplified and cloned into prokaryotic expression vector pET28a (+) or pGEX6p-1, and transformed into host cell *E.coli* BL21 (DE3) by electroporation method.

The expressed GST-tagged $PhaP_{We}$ and his-tagged $PhaP_{Ah}$ protein were purified by affinity chromatography. Protein homogeneity of $PhaP_{We}$ and $PhaP_{Ah}$ were identified by gel filtration chromatography. Secondary structure estimation of pure phasins was performed by circular dichroism chiropticalspectrometer (Jasco) to measure their 2D conformation. Dynamic light scattering technology was employed to evaluate protein crystallizability by DLS (DynaPro) for monodispersity of the phasins. Finally, crystallization trials were performed for good-shaped crystal and optimization experiments. Large single crystal with high quality for X-ray diffraction analysis were investigated in this study.

## 3 Results and discussion

Comparison between $PhaP_{Ah}$ from *A. hydrophila* 4AK4 and $PhaP_{We}$ from *Wautersia eutropha* (formerly *Ralstonia eutropha*) were studied regarding their primary amino acid sequence, secondary structure conformation from CD spectra, protein crystallizability from DLS data, and crystallization behavior.

Analysis of the primary structure of $PhaP_{Ah}$ and $PhaP_{We}$ showed that the respective disparity on molecular weights and amino acid sequences. PHB granular phasin from *W. eutropha* H16 was 20 kDa and PHBHHx granular phasins from *A. hydrophila* 4AK4 was 13 kDa. And the scores for positive and identity between $PhaP_{Ah}$ and $PhaP_{We}$ were, respectively, 15.1% and 6.3%. Obviously no protein homology was found and no significant similarity between them. Phasins had been proposed to be amphiphilic molecules that separate the hydrophobic core of the PHA inclusions from the hydrophilic cytoplasm [3]. The region of $PhaP_{Ah}$ protein amino acid sequence in *A. hydrophila* 4AK4 from Gln-63 to Gln-72 is predominantly hydrophobic. Hydrophilic domains of phasin might form the cytoplasmic face of the protein or possibly interact with other granule -bound proteins. CD results of $PhaP_{Ah}$ and $PhaP_{We}$ spectra showed β-sheet were main second structure for two phasins. Soβ-barrel structure may be suitable for PHA granular phasins. Futhermore, the crystal forms between two phasins were very similar in same crystallization conditions. Determination of the two phasin protein crystal structures is ongoing. Same function for PHA formation or similar effect on PHA biosynthesis of $PhaP_{Ah}$ and $PhaP_{We}$ with different primary structure , which indicated convergent evolution of the phasin homologes.

## References

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