Bacterial Clathrate-Binding Proteins Alter Gas Clathrate Morphology

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Gas clathrates are found on Earth along continental margins and in permafrost and are extreme habitats due to low water activity, high salinity, low temperatures, and high pressures. Survival strategies used by gas clathrate-inhabiting microbes could provide clues for searching for life beyond Earth, such as Mars, Titan, and Pluto. Our hypothesis is that bacteria living in gas clathrates employ a strategy similar to that of cold-water fish that express ice-binding proteins (IBPs) to inhibit the growth of ice crystals. Antarctic fish IBPs have been successfully tested for their efficacy to inhibit gas clathrate formation. To test our hypothesis, we engineered a temperature-controlled chamber to synthesize tetrahydrofuran (THF) clathrate in the presence of recombinantly-expressed bacterial proteins. We chose eight protein sequences that were predicted to be IBPs from metagenomes sequenced from methane clathrate sediments from coastal Oregon, USA and coastal Japan. All assayed proteins have been confirmed to be clathrate-binding proteins (CBPs). We assayed CBPs 2-6 by testing their effect on morphology of THF-clathrate crystal structure, visualization of clathrate binding via fluorescence of tagged enhanced green fluorescent protein (eGFP), and protein quantification. Winter-flounder antifreeze protein was used as a positive control, which formed numerous small, platy crystals. Phosphate-buffered saline solution, cytochrome c, and eGFP were used as negative controls, which formed single, native-like crystals. In the presence of CBPs, THF-clathrate crystals formed nonnative-like morphologies. CBPs 2 and 3 formed small, platy crystals, similar to the positive control, whereas CBPs 4-6 formed larger, flat crystals. eGFP-tagged CBPs showed clear evidence of clathrate binding compared to eGFP alone. CBPs were more enriched in the clathrate compared to the negative controls. Taken together, these data support our hypothesis that CBPs are employed by microorganisms in clathrate-rich zones for survival. Future studies include directly assaying CBPs for activity toward methane hydrate.

Keywords: hydrate, clathrate, microbe

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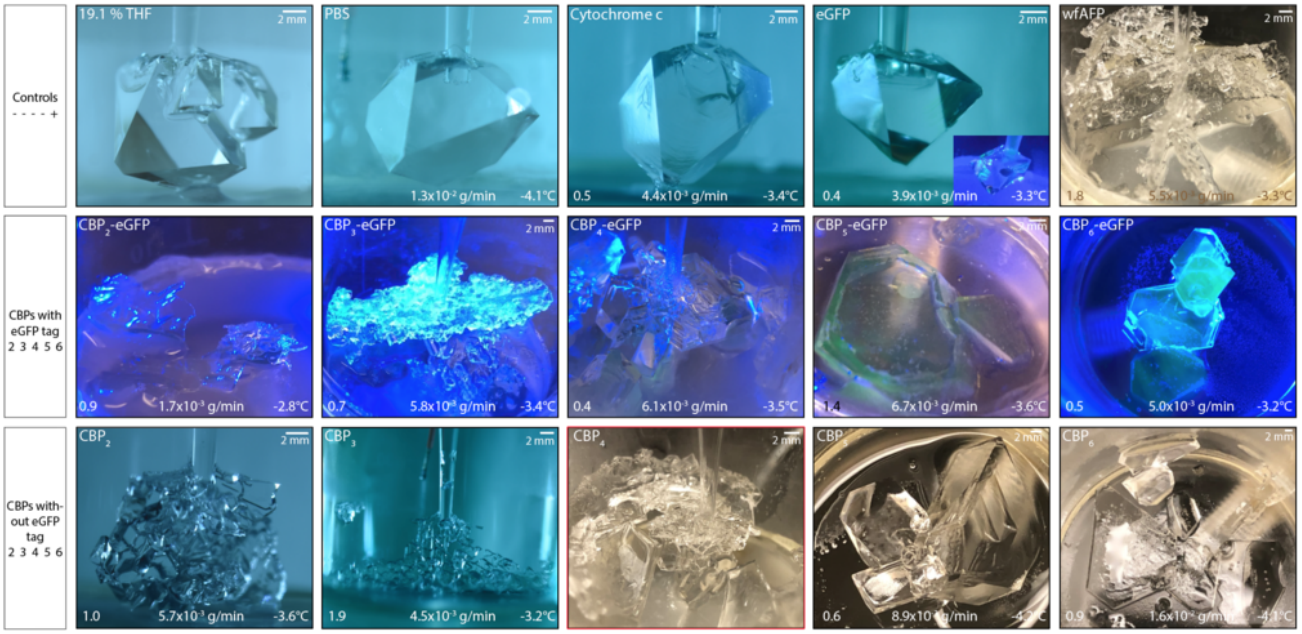

Figure 1. Representative images of each treatment. The top row includes the negative controls (19.1% THF with no additives, the phosphate buffered solution in which all proteins were suspended, cytochrome c, and enhanced green fluorescent protein) and the positive control (winter flounder antifreeze protein). The middle row contains CBPs 2-6 with eGFP tags. The bottom row contains CBPs 2-6 without eGFP tags. Each image is labeled with the treatment (top left), the scale (top right), the average protein concentration ratio in crystal : non-crystallized solution (bottom left), the crystal growth rate (bottom middle) and the nucleation temperature (bottom right).

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