Gene products from Bacillus megaterium involved in the metabolism of polyhydroxyalkanoic acid (PHA) and the biogenesis of PHA inclusion -bodies

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Abstract

Polyhydroxyalkanoates (PHAs) comprise a family of macromolecules produced by many bacteria as a carbon and energy reserve and are perceived to have commercial potential as biodegradable thermoplastics. To investigate the biogenesis of PHA inclusion-bodies and the functions of inclusion-body proteins from *Bacillus megaterium* strain 11561, we identified and cloned a 7.9 kb DNA fragment harboring five genes, *phaP, -Q, -R, -B*, and *-C* specifying proteins having known or putative functions in PHA metabolism and/or inclusion-body biogenesis. Sequence similarities to known *pha* genes identified *phaB* and *-C* as specifying acetoacetyl-CoA reductase and PHA synthase, respectively. Putative proteins encoded by *phaP, -Q*, and *-R* were not ascribed functions due to lack of significant similarities to known proteins. Both the functionality of the *pha* gene cluster with respect to PHA accumulation and the transcriptional organization of the genes were determined. Subsequent studies were carried out to further investigate functions of *phaP, -Q*, and *-R*.^ PhaP was established as a major PHA inclusion-body associated protein and was shown to localize to inclusion-bodies in living cells. Further, we demonstrated a phasin-like role for this protein due to its affect on the formation of PHA inclusion-bodies. In addition, our data is consistent with PhaP functioning as a storage protein, implying that the role of PHA inclusion-bodies may be that of a reserve of amino acids in addition to reduced carbon. Regulation of *phaP* was influenced by PhaQ We showed that PhaQ is a transcriptional repressor of *phaP*. ^ Moreover, we demonstrated the binding of PhaQ to inclusion-bodies, suggesting that its mode of regulation may involve its localization. Similarly, we showed that PhaR is bound to PHA inclusion-bodies. Our data demonstrated the

requirement of *phaR* for PHA accumulation *in vivo* and that both PhaC and PhaR were necessary for PHA synthase activity *in vitro*. Evidence suggests that PHA synthase from strain 11561 can exist in an active or inactive state and that this state is either directly or indirectly influenced by PhaR. A working model is proposed to describe the roles of PhaP, -Q and -R in the metabolism of PHA and biogenesis of PHA inclusion-bodies. ^

Subject Area

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