

Modification of Membrane and Organelle Proteins by Short-chain Poly-(R)-3-hydroxybutyrates

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When studying proteins, particularly proteins of membranes and/or organelles, it is important to determine whether the proteins are modified by covalent attachment of short-chain polymers (<15 residues) of R-3-hydroxybutyrate (cPHB) (PHBylation) [1]. cPHB are linear polymers in which hydrophobic methyl groups alternate with hydrophilic ester groups (Figure 1).

cPHB-modified proteins are found in all organisms – prokaryotes and eukaryotes. The presence of cPHB can be determined by analysis of the protein, isolated from cells or tissues, via a chemical assay [2], an antibody assay [3] or mass spectroscopy [4]. If cPHB is found, all further studies of the folding and function(s) of the protein should also be conducted with protein isolated from cells or tissues and not with protein obtained by overexpression, since post-translational modifications by cPHB may be absent or incomplete in the overexpressed protein.

It is also important that PHBylation renders a protein sensitive to temperature. Unlike polypeptide backbones, which are not very responsive to temperature, polyester backbones are highly flexible at physiological temperatures, but become increasingly more rigid as temperatures are lowered to room temperatures or below. This rigidity will influence the folding of the protein into its mature structure, and will thereby also influence protein function(s). Accordingly, the folding and the function(s) of cPHB-modified proteins should always be investigated at or above physiological temperatures.

An example of the influence of cPHB on protein folding and protein structure is the outer membrane protein A (OmpA) of *Escherichia coli*. OmpA is a major integral protein of the outer membrane, with ~10⁸ copies per cell [5]. It has multiple structural and physiological functions, including maintaining cell shape and stability, preserving the structural integrity of the outer membrane, increasing resistance to antimicrobial peptides, serving as an adhesin, an evasin, a colicin, a participant in biofilm formation, a therapeutic target for antimicrobials, an agent in the diagnosis of antimicrobial resistance, a bacteriophage receptor, and a mediator in F-dependent conjugation [5-11]. The latter two functions suggest that the pores formed by OmpA in the outer membrane are large enough to allow the passage of ss-DNA.

The 325 residue mature OmpA protein consists of two domains - a 171 residue N-terminal domain and a 154 residue C-terminal domain. Studies by Xian et al. [12] have shown that serine residues 163 and 167 of the N-terminal domain are modified by cPHB in the cytoplasm. Further studies by Negoda et al. [13] indicate that these modifications by cPHB are essential to the ability of the N-terminal domain to form a

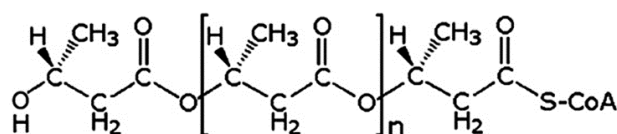
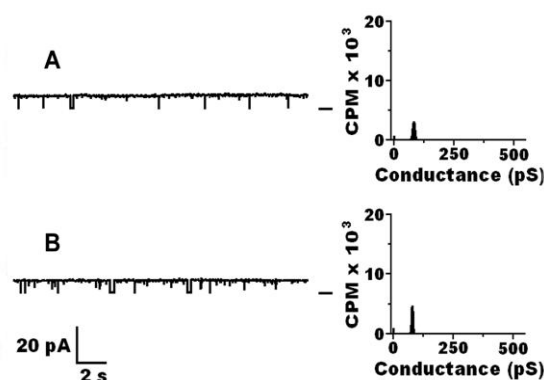


Figure 1:

OmpA from Inclusion Bodies



OmpA from Outer Membranes

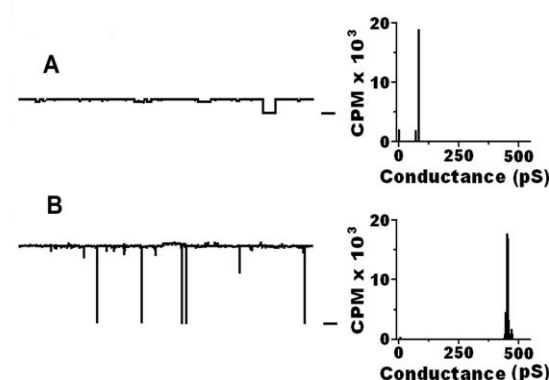


Figure 2: Representative single-channel current traces of OmpA in planar lipid bilayers of diphyanoyl phosphatidyl choline (DPHPC) between aqueous solutions of 20 mM Hepes (pH 7.4) and 1M KCl. The closed state is indicated by the bar at the right of each trace. The clamping potential was +100 mV with respect to ground (trans). The corresponding histograms, from 1 min of continuous recording, show the distribution of conductance magnitudes. CPM, counts per minute. A. Traces at 22°C. B. Traces at 22°C after incubation at ≥ 37°C [9].

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Received July 01, 2015; Accepted July 14, 2015; Published July 21, 2015

Citation: Reusch RN (2015) Modification of Membrane and Organelle Proteins by Short-chain Poly-(R)-3-hydroxybutyrates. J Microb Biochem Technol 7:4 226-227. doi:10.4172/1948-5948.1000211

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narrow pore. Modifications of the C-terminal domain by cPHB occur in the periplasm [14], which contains the enzyme cPHB synthase, ydcS [15]. Accordingly, OmpA obtained from cytoplasmic inclusion bodies contains cPHB only in its N-terminal domain whereas OmpA obtained from outer membranes contains cPHB in both N-terminal and C-terminal domains.

Planar lipid bilayer studies show that OmpA from inclusion bodies forms a two-domain narrow-pore structure (60 ± 30 pS) at room temperatures, which remains a two-domain narrow-pore structure when warmed to or above physiological temperatures for extended periods, whereas OmpA isolated from the outer membranes of *E. coli* forms a two-domain narrow-pore structure (60 ± 30 pS) at room temperatures, which undergoes a rearrangement into a single-domain large pore structure (450 ± 70 pS) when warmed above room temperatures [16,17] (Figure 2). The transition of a single molecule of OmpA from a narrow to a large pore in a bilayer of diphytanoylphosphatidylcholine (DPhPC) requires ~7 h at 26°C, ~30 min at 37°C, but only ~13 min at 42°C. The modification of the C-terminal of OmpA by cPHB in the periplasm is ostensibly essential to the formation of the mature large-pore structure. We may conclude that proteins obtained by overexpression into cytoplasmic inclusion bodies may not contain all the modifications that are essential to the formation of their mature structures, and thus their ability to perform their physiological functions.

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