peak wavelength of gold, and processed with HKL2000. Phasing, model building and refinement were conducted using PHENIX, REFMAC5, COOT and other programs of CCP4. Electron densities for the catalytic domain of the enzyme were clearly obtained, but those for the linker and PHB-binding domains were not, in spite that there is remaining space for these domains to be packed in the crystal lattice.

The overall structure of the catalytic domain represented an α/β hydrolase fold which is often observed for the large superfamily of esterases including lipases and carboxypeptidases. A cervice was formed on the surface of the protein, at the bottom of which are located catalytic triad residues Ser-166, Asp-241 and His-300. The structure was compared with that of a fungal single-domain depolymerase with circular permuted polypeptide connectivity [1]. These two enzymes differ in the product composition. The main product is dimer of *R*-3-hydroxybutyrate for the bacterial enzyme, whereas it is monomer for the fungal enzyme. Structures of loop regions around the active site were different between the two enzymes. This may differentiate the mode of interactions with monomer units of the substrate polymer for these enzymes, which may explain the difference in the product composition.

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Intermediates along the NAD$^+$ cyclisation reaction pathway of ADP-ribosyl cyclase

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Calcium is a ubiquitous, versatile intracellular signal regulating numerous cellular processes [1]. The versatility of Ca^{2+} signalling emerges from the use of an extensive repertoire of signalling components known as a Ca^{2+} signalling toolkit that can be assembled in combinations to create signals with different spatial and temporal profiles. Mobilisation of intracellular Ca^{2+} stores into the cytoplasm is mediated through three structurally divergent messengers, one of them being cyclic ADP-ribose (cADPR) [2].

cADPR acts on the ryanodine receptor to elicit Ca^{2+} release. it is ubiquitous across species and is an endogenous modulator of Ca^{2+}-induced Ca^{2+} release thereby regulating a wide range of physiological processes [3]. cADPR is a cyclic nucleotide converted from NAD$^+$ by multifunctional enzymes of the ADP-ribosyl cyclase family [4]. *Aplysia* ADP-ribosyl cyclase (*Aplysia* cyclase) was the first enzyme identified to catalyse the cyclisation of NAD$^+$ to cADPR, with the release of nicotinamide . *Aplysia* cyclase is a 30kDa protein initially isolated from *Aplysia* ovotestis [5]. It is also found to be present in the neurons of the *Aplysia* buccal ganglion, where production of cADPR can enhance the evoked synaptic transmission [6]. Recently, it has been shown that *Aplysia* cyclase translocates from the cytosol into the nucleus upon depolarisation of *Aplysia* neurons, providing a mechanism for selective and specific activation of the nuclear Ca^{2+} store in neurons offering versatility for the neurons to respond to a wide range of stimuli [7].

Cyclisation of NAD$^+$ into cADPR involves a two step reaction, the elimination of the nicotinamide ring and the cyclisation of the intermediate resulting in the covalent attachment of the adenine ring to the anomeric carbon of the terminal ribose. Cyclisation of NAD$^+$ results in the linkage of the adenine and terminal ribose moieties established via the N1-position of the adenine ring, while cyclisation of NAD$^+$ analogues resulted in the covalent attachment to the terminal ribose via the N7 position of the purine ring of these analogues [8].

In this study, we have determined the structures of wildtype *Aplysia* cyclase complexed with its substrates, NAD$^+$ and NGD$^+$, and its products cADPR and cGDPR. In addition, we were also able to capture the reaction intermediates of the cyclisation reaction either by controlling the soaking time of the substrate or with the use of substrate analogues. Taken together, we are able to obtain snapshots of the cyclisation process of the dinucleotide resulting in either N1 or N7 linkage of the purine ring to the terminal ribose.

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Sulfolobus solfataricus adenine phosphoribosyltransferase

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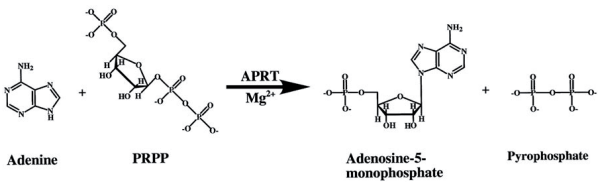
Phosphoribosyltransferases (PRTases) are a group of enzymes that catalyze the formation of nucleotide 5´-monophosphates as essential precursors in the synthesis DNA or RNA. **PRTases** all use a common substrate, 5-phosphoribosyl-α-1-pyrophosphate (PRPP), and in the presence of Mg^{2+} they transfer nucleobases to C1 of the ribose 5´-phosphate moiety of PRPP to form nucleotide 5´-monophosphates under the release of pyrophosphate ($P_2O_7^{4-}$). PRTases are involved in both the *de novo* biosynthesis of nucleotides, e.g. orotate PRTase (OPRTase) and in the salvage pathways, e.g. uracil PRTase (UPRTase), adenine PRTase (APRTase) and hypoxantine-guanine-xanthine PRTases (HGXPRTases; often with mixed specificity). PRTases share a common domain fold (type 1) which defines the specificity for PRPP and a variable domain for recognition of the various nucleobases.



Adenine PRTase structures are known for eukaryotes and bacteria, and details of their active sites have been mapped out. **APRTase** from the **thermophile archaean *Sulfolobus solfataricus*** is an enzyme with unexpected properties: The substrate binding order is reversed with adenine binding first followed by PRPP. It has a double pH optimum and is potently inhibited by AMP and ADP. Phosphate PO_4^{3-} seems to facilitate adenine binding.

Sequentially, ***Ss*APRTase** does not resemble other APRTases but merely HGXPRTases from eukaryotes, bacteria and archaea. The closest sequence homologues in PDB (1nul [1] & 1vdm [2]) used for molecular replacement share about 31 % sequence identity for a 143/210 residue stretch of the sequence. The remaining sequence does