Active site Structure of Actinorhodin Polyketide (*act* III) Reductase

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Polyketides produced by bacteria and other organisms include antibiotics, anticancer and antifungal compounds. In Type II polyketide synthesis, a minimal system of three enzymes are sufficient to form a polyketide of the requisite chain length, one of which is acyl carrier protein (ACP) which mediates transport of pathway intermediates between the proteins. Addition of ketoreductase (KR) to this system results in the correctly cyclised and reduced product. We have determined the 2.5 Å crystal structure of the tetrameric polyketide Type II ketoreductase, a member of the SDR family, with its cofactor $NADP^+$. Of two subunits in the crystallographic asymmetric unit, one is 'closed' around the active site. Formate is observed in the other 'open' subunit, indicating possible locations for substrate binding. A model for the binding of ACP has been constructed, based on observed non-crystallographic contacts. Based on these observations, we hypothesize that approach and binding of ACP triggers a conformational change from the closed to the open, active, form of the enzyme that allows the polyketide chain to enter the active site and be reduced. The model suggests a mechanism for ACP recognition which is applicable more generally to a range of protein families with NAD(P) cofactors which rely on ACP to provide the substrate. Substrate analogue soaks are being carried out to elucidate the binding of the substrate in the active site.

Keywords: ketoreductase, polyketide synthesis, ACP