Residues in Cyclodextrin Glycosyl Transferase that Determine Product Specificity

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CONFORMATIONAL FEATURES OF CORNEAL PROTEOGLYCANS

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The molecular conformation, interaction and dynamics of the two structural protein components of the cornea, keratan sulfate proteoglycan (KSPG) and dermatan sulfate proteoglycan (DSPG), were monitored using fluorescence spectroscopy.

KSPG exhibits the red edge excitation effect and dipole relaxation indicating the Trp region to be in a polar and motionally restricted environment. It also possesses significant surface hydrophobicity and thus a dual character. The consequent ability of KSPG to interact with both collagen and model membranes renders it the role of a "filler" in the extracellular matrix.

The lone Trp residue in DSPG exhibits a doublet fluorescence emission, arising from the Trp being located in two different environments, as indicated by fluorescence quenching, ANS binding and denaturation studies. Such a situation could arise either due to differential glycosylation of the core proteins or by duplexation and aggregation of the glycosaminoglycan chains.

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RESIDUES IN CYCLODEXTRIN GLYCOSYL TRANSFERASE THAT DETERMINE PRODUCT SPECIFICITY

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Cyclodextrin Glycosyl Transferase (CGTase) produces cyclodextrins from starch. Normally, a mixture of α, β and γ cyclodextrins is produced by the CGTase from Bacillus Circulans 251. In an attempt to understand the factors determining the product ratio of CGTase, a combination of protein crystallography, steady state kinetics and site directed mutagenesis was used.

The structure of CGTase was studied in complex with natural substrates of diverse lengths. Amino acids that were important for interactions with intermediates were mutated and the new interactions with substrates redetermined by crystallography.

The crystallographic information, coupled to the changed kinetic behaviour of the mutants sheds a first light on the pathways followed during formation of α, β and γ cyclodextrin.

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THE 3D STRUCTURE OF R-PHYCOERYTHRIN FROM *POLYSIPHONIA URCEOLATA*

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Purpose: R-phycoerythrin (R-PE) is one of phycobiliproteins and is located at the tip of the rod-like phycobilisome in the light-harvesting system from the red alga *Polysiphonia Urceolata*. The subunit composition of the R-PE is $(\alpha_2\beta_2)_3\gamma$ and the sequence unknown, but from the results of the determined structure, we are sure that 164 and 177 residues in α and β respectively. Each (αβ) unit contains four phycoerthrobilins (PEB) and one phycourobilin (PUB). The γ subunit is 33 Kda which contains one PEB and three PUB.

Methods and Results: The crystal of R-PE belongs to space group R3 with the unit cell of a=b=189.8, c=60.1Å. There are two (αβ) units in an unsymmetric unit of the cell. The 3D structure of R-PE was solved by MIR method with four heavyatom derivatives, such as K_2AuCl_4, K_2PtCl_4, PCMS, PHMH. The final figure-of-merit is 0.835 at 3Å resolution. As the first stage the structure of R-PE was determined at 5Å resolution and the peptide traceing and preliminary position determination of Cα was proceeded. As the second stage the MIR map at 3Å resolution was caculuted and then the resolution was extended up to 2.8Å. The R-factor of the final model of the R-PE molecule with 132 water molecules is 18.0%.The four PEB chromophores α84, α14a, β84 and β155 in an (αβ) unit are each covelently bonund to a cycteine β52 by ring A. The PUB chromophore is bound to cycteine β52 by ring A and bound to β61 by ring D. The four (A,B,C and D) rings form a boat shaped structure. The γ subunit assumed in the central channel of the molecule disc $(\alpha_2\beta_2)_3$.