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Crystallographic Studies on Acyl Ureas, a New Class of Inhibitors of Glycogen Phosphorylase

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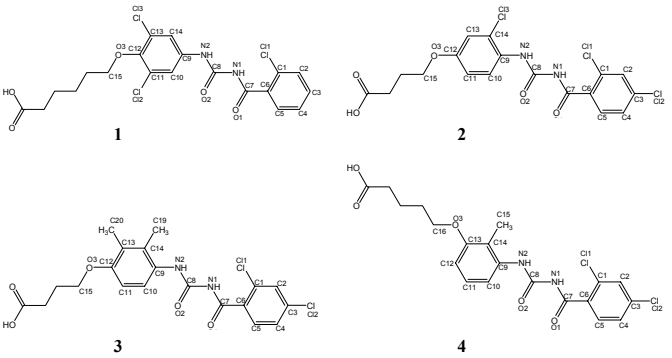
Acyl ureas were discovered as a novel class of inhibitors for glycogen phosphorylase, a molecular target to control hyperglycemia in type 2 diabetics [1]. This series is exemplified by 6-{2,6-Dichloro-4-[3-(2-chloro-benzoyl)-ureido]-phenoxy}-hexanoic acid (1), which inhibits human liver glycogen phosphorylase with an IC_{50} of 2.0 μM. Here we report on four crystal structures of acyl urea derivatives (1-4) in complex with rabbit muscle glycogen phosphorylase b to elucidate the mechanism of inhibition of these inhibitors. The structures were determined and refined to 2.26 Å resolution and demonstrate, that the inhibitors bind at the allosteric activator site, where the physiological activator AMP binds. Acyl ureas induce conformational changes in the vicinity of the allosteric site. The induced conformational changes are characteristic of the T' state conformation, and the key rearrangement is probably the backbone displacement of the loop 193-196 that allows for van der Waals interactions with the ligands similar to those observed with W1807 [2]. Our findings suggest that acyl ureas inhibit glycogen phosphorlyase by direct inhibition of AMP binding and by indirect inhibition of substrate binding through stabilization of the T' state. The structural results with the acyl ureas can be further exploited by means of chemical modification to produce new potential antidiabetic agents.

Scheme I. Chemical structures of the acyl ureas compounds **1-4**



1 **2** **3** **4**

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Keywords: type 2 diabetes, glycogen phosphorylase, X-ray crystallography

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X-ray Structures of Methylamine Dehydrogenase Reaction Intermediates

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Methylamine dehydrogenase (MADH) is a soluble periplasmic $\alpha_2\beta_2$ heterotetrameric enzyme, present in many methylotropic bacteria, that catalyzes the oxidation of methylamine to formaldehyde and ammonia. It is upregulated by the presence of substrate, and enables bacterial growth on methylamine as the sole carbon, nitrogen and energy source. The redox center is trytophan tryptophylquinone (TTQ) which is composed of two Trp residues that are posttranslational modified by the addition of two oxygens to form an O-quinone and a covalent cross-link. Amicyanin (a type I blue copper protein) is the redox partner of MADH and it is also induced in the presence of methylamine. The reaction during turnover gives distinct spectral features in the visible region, which define specific electronic states of the cofactor.

The use of single crystal kinetics, microspectrophotometry and X-ray crystallography of the holo- (with Cu) and apo- (without Cu) complexes of MADH with amicyanin allows the trapping of different catalytic intermediates in the crystal, and the determination of their x-ray structures. In this presentation the structural features of O-quinone, N-quinol and N-semiquinone catalytic intermediates will be discussed in terms of the current model for catalysis and electron transfer.

Keywords: quinoprotein, redox enzyme, single crystal microspectrophotometry

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Crystallographic Analysis of Maltohexaose-producing Amylase

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Maltohexaose-producing amylase (G6-amylase) from an alkalophilic *Bacillus* sp.707 mainly produces maltohexaose (G6) from starch and related α-1,4-glucans. To elucidate the reaction mechanism of G6-amylase, a crystal structure of its complex with G6 was determined at 1.9 Å resolution. The G6-amylase was crystallized by the hanging drop vapor diffusion method using with the reservoir solution containing 50% (v/v) 2-methylpentane-2,4-diol, 100 mM Tris-HCl (pH 8.5) and 200 mM ammonium phosphate and then the obtained crystals were soaked for about 1 hour in the crystallization solution containing 75mM G6. The crystallographic *R* value (R_{free} value) was 0.155 (0.184). The crystal structure revealed that the G6 occupies subsites –7 to –2 like an enzyme-product complex and its structure of the catalytic active site is very similar to that of the pseudo-maltononaose (pG9) complex and not its native structure. As same as in the pG9 complex structure determined previously, an indolyl ring of Trp140 stacks to the glucosyl residues at subsites –6 and –5. Almost α-amylases finally degrade G6 to glucose and/or maltose. This is achived by G6 binding to subsites –1 and +1 at least. However, G6-amylase would little hydrolyze G6 by non-productive binding to G6. Therefore, Trp140 may play an important role on G6 production and hydrolysis preventation to G6.

Keywords: amylases, carbohydrate degradation, crystallographic analysis