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The mechanism of the formation reaction of biotinyl-CoA from biotin, ATP and CoA in BirA

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Biotin protein ligase, BirA, catalyzes the formation of biotinyl-CoA from biotin, ATP and CoA in the presence of Mg^{2+} ion (Biotin + ATP + CoA → biotinyl-CoA + AMP + PPi). This reaction corresponds to aminoacylation reaction on aminoacyl-tRNA synthetases (aaRSs) which facilitate formation reaction of aminoacyl-tRNA in the presence of Mg^{2+} ion (amino acid + ATP + tRNA → aminoacyl-tRNA + AMP + PPi). BirA and class II aaRSs have antiparallel β-sheet in common as ATP-binding catalytic site. The characteristics of the reaction by BirA are;

1. The biotinyl-CoA synthetase activity of BirA is optimal at a Mg^{2+}:ATP ratio of 1.0 [1]. 2. On BirA homolog from pea leaves, in the presence of ADP and ATP[S], the activity is 65 % of that in the presence of ATP, while the aminoacylation reaction on aaRSs was not observed in ADP and ATP[S] [2]. 3. The fact that the formation of biotinyl-NHOH from biotin + ATP + hydroxylamine is accelerated by three-fold in the presence of CoA [1], indicates that almost biotinyl-NHOH is formed via the reaction path between biotinyl-CoA and hydroxylamine. The whole synthesized biotinyl-AMP bound BirA reacts with hydroxylamine but 60 % of biotinyl-AMP is converted to biotinyl-CoA but 40% does not reacts with CoA [1]. This fact reveals that the biotinyl-AMP has active or inactive forms on BirA. This corresponds to the facts that 25% of the synthesized Ile-AMP on IleRS and 16% of the synthesized Val-AMP on ValRS does not react with the cognate tRNA, respectively [3, 4]. We determined the crystal structure of the complex of *T. thermophilus* BirA (Tt BirA) and biotin with R_{factor} = 24 % (R_{free} =29 %) at 2.35 Å resolution. Compared with reported structures of complexes of *P. holikoshii* BirA (Ph BirA), we constructed the model of ATP and CoA bound BirA on the basis of the position of biotin and propose the reaction mechanism that O of the carboxyl group of biotin attacks Pα of ATP and S of CoA attacks C of the carboxyl group.

The ring of biotin bound on β-sheet of Tt BirA occupies the same position as that in Ph BirA but fixed rotational isomers around C_{10}-C_{11} of the carboxyl group ($C_{11}O_1O_2H$) exist depending on crystallization conditions. The isomerization between these two distinct forms is blocked out. When O1 of the carboxyl group in biotin changes from sp^2 of double bond C_{11}=O_1 to sp^3 hybrid orbital, O_1 can form a bond with Pα of ATP through formation of the trigonal bipyramid coordination around Pα and successively, the Pα-O bond is transferred to O-Pβ bond to form PPi in S_N2 reaction. Since this reaction path occurs equally in the reverse direction, the attack by S of the end of CoA on C of the carboxyl group of biotin proceeds onward to form biotinyl-CoA, AMP and PPi. The fact that PPi releases as MgPPi, corresponds to the optimal ratio of Mg^{2+}:ATP = 1.0. The biotinyl-AMP is not formed from biotin and ADP.

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X-ray snapshots of HIV-1 protease catalysis and substrate recognition

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The Human Immunodeficiency Virus type-1 protease (HIV-1PR) is a virally encoded aspartyl protease that cleaves a series of nine peptide bonds in the viral *gag* and *gag-pol* polyproteins essential for the maturation of the virus. Crystal structures of active enzyme natural substrate(s) complexes will help in the development of new drugs. Earlier attempts to prepare such complexes had failed [1]. Employing the soaking method several crystalline complexes of HIV-1PR with substrate peptides are prepared containing both non-proline (type-II) and proline (type-I) cleavage sites.

In the crystal structure of the complex involving a type-II subst rate(AETF*YVDGAA,*cleavage site), the substrate was captured at two different stages of cleavage process by varying the pH of soaking solution. We have found that at pH 2.5 the substrate is *in situ* transformed into a tetrahedral intermediate (TI) [2] and cleaved into two product peptides at pH 6.2 [3]. We report here two structures at higher pHs: at pH 8 the substrate is trapped in the active site of the enzyme as an uncleaved peptide, refined to 1.89Å having R=16% and R-free=19% and apo-HIV-1 PR at pH 7.5 refined to 1.72Å having R=19% and R-free=23%. In uncleaved peptide complex structure, there is a hydrogen bond (HB) between the oxygen of scissile carbonyl and the outer oxygen of one of the catalytic aspartates, which is necessarily the first step in peptide bond hydrolysis. This HB becomes stronger, transforming to a short ionic HB in the TI-stage. These type-II substrate complexes enabled us to visualize the mechanism of peptide bond cleavage at atomic level.

In the apo HIV-1 PR structure at pH 7.5 there are two water molecules, hydrogen bonded to each other and the catalytic aspartates. The interaspartate HB is not a low barrier hydrogen bond contrary to the assumption in kinetic iso-mechanism [4]. Comparing apo with the uncleaved complex structure, it is seen that the near-attack geometry of scissile carbonyl cannot occur in presence of two water molecules, resulting in loss of activity at pH 8 and enabling entrapment of the uncleaved substrate.

The first glimpse of a substrate bound to HIV-1PR is obtained. A novel molecular mechanism for the loss of HIV-1PR activity at high pHs is elucidated based on the reported X-ray structures. Structures of type-II complexes indicate that the product release pathways are different, in the type-I substrate the Q-product moves out first whereas in type-II substrate the P-product (NH_2-AETF-CO_2H) moves out first. These are the first successful study on a complex involving an active protease and true substrate molecule providing atomic-level descriptions of substrate recognition and processing by HIV-1 PR.

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