

at 2.4 Å and 2.25 Å, respectively. This is the first reported structure of a prokaryotic UCK. The overall structure is highly similar to that of human uridine cytidine kinase 2 (UCK2). Structural comparison of the nucleoside-binding site between ttCK and human UCK2 revealed that most amino acid residues around the base moieties are identical between them, except for Tyr59 and Tyr93 in ttCK, corresponding to Phe83 and His117 in human UCK2. These two residues are located near the N4 amino group and the N3 nitrogen atom of cytosine: these atoms differ between cytosine and uracil. Many UCK homologues have a Phe and His residue at the position equivalent to Tyr59 and Tyr93 in ttCK, respectively. As the next step, we prepared ttCK mutants with amino acid substitution at Tyr59 and Tyr93. The substrate specificity of Y59F was the same as that for wild type. In contrast, Y93H had activity for both uridine and cytidine. Whereas the replacement of Tyr93 with Phe or Leu, unaffected the substrate specificity of ttCK, the replacement with Gln, Asn and Glu endowed ttCK with phosphorylation activity toward uridine. These results indicate that a hydrophilic residue at position 93 permits UCK to accept uridine as substrate. A potential hydrogen donor at this position may enable UCK to interact a keto group of uridine specifically. In addition to ttCK, other UCK homologues of 26 species including pathogens have tyrosine at the position equivalent to Tyr93, which predicts that these are cytidine-specific UCKs. This study points to the critical need for experimental studies even of enzymes whose annotation has been accepted.

**Keywords:** nucleoside, kinase, mutation

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#### Structure-based catalytic optimization of a type III rubisco

Yuichi Nishitani,<sup>a</sup> Shosuke Yoshida,<sup>b</sup> Masahiro Fujihashi,<sup>a</sup> Kazuya Kitagawa,<sup>a</sup> Takashi Doi,<sup>a</sup> Haruyuki Atomi,<sup>b</sup> Tadayuki Imanaka,<sup>c</sup> Kunio Miki,<sup>a</sup> <sup>a</sup>*Department of Chemistry, Graduate School of Science, Kyoto University (Japan).* <sup>b</sup>*Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University (Japan).* <sup>c</sup>*Department of Biotechnology, College of Life Sciences, Ritsumeikan University (Japan).* E-mail: ynishi@kuchem.kyoto-u.ac.jp

The Calvin-Benson-Bassham cycle is responsible for carbon dioxide fixation in all plants, algae, and cyanobacteria. The enzyme that catalyzes the carbon dioxide-fixing reaction is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco from a hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tk*-Rubisco) belongs to the type III group, and it shows high activity at high temperatures. We have previously determined the crystal structure in the apo-form of this enzyme [1]. We have also found that replacement of the entire  $\alpha$ -helix 6 of *Tk*-Rubisco with the corresponding region of the spinach enzyme (SP6 mutant) results in an improvement of catalytic performance at mesophilic temperatures, both *in vivo* and *in vitro*, whereas the former and latter half replacements of the  $\alpha$ -helix 6 (SP4 and SP5 mutants) do not yield such improvement [2]. We report here the crystal structures of the wild-type *Tk*-Rubisco and the mutants SP4 and SP6, and discuss the relationships between their structures and enzymatic activities [3].

A comparison among these structures shows the movement and the increase of temperature factors of  $\alpha$ -helix 6 induced by four essential factors. We thus supposed that an increase in the flexibility of the  $\alpha$ -helix 6 and loop 6 regions was important to increase the catalytic activity of *Tk*-Rubisco at ambient temperatures. Based on this structural information, we constructed a new mutant, SP5-V330T, which was designed to have significantly greater flexibility in the above region, and it proved to exhibit the highest activity among all mutants examined to date. The thermostability of the SP5-V330T mutant was lower than that

of wild-type *Tk*-Rubisco, providing further support on the relationship between flexibility and activity at ambient temperatures.

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#### TTP binding in the active conformation of dCTP deaminase: dUTPase

Pernille Harris,<sup>a</sup> Monika Nøhr Løvgreen,<sup>a</sup> Martin Willemoës,<sup>b</sup> <sup>a</sup>*Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, (Denmark).* <sup>b</sup>*Department of Biology, University of Copenhagen, DK-2200 Copenhagen, (Denmark).* E-mail: ph@kemi.dtu.dk

The bifunctional dCTP deaminase:dUTPase is a homotrimeric enzyme, which is closely related to the monofunctional dCTPases and dUTPases. The enzyme catalyses the deamination and the phosphate hydrolysis of dCTP to dUMP and it is end-product inhibited by dTTP. Until now the structures of the wild type *Mycobacterium tuberculosis* bifunctional enzyme in its apo form (pdb-entry 2qlp) and with TTP (pdb-entry 2qxx) bound have been solved [1] and the *Methanocaldococcus jannaschii* bifunctional enzyme in the apo form (pdb-entries 1ogh, 2hxb, 1pkh, [2,3]) is also known. Furthermore, two variants of the *M. jannaschii* enzyme have been crystallized: the E145Q variant with diphosphate and magnesium (pdb-entry 3GF0) and the E145A variant of the *M. jannaschii* enzyme with  $\alpha,\beta$ -imido dUTP and magnesium (pdb-entry 2hxd) [4]. It has been concluded that the bifunctional dCTP deaminase:dUTPase exists in two mutually exclusive conformations. An active form, which resembles the apo-form and the structure where  $\alpha,\beta$ -imido dUTP binds and an inactive form that binds dTTP. To investigate the inhibition by dTTP we have made several mutant enzymes, one of which is able to bind dTTP in the active conformation. Furthermore, the wild type enzyme and this mutant enzyme have been enzymatically characterized and interestingly, the mutant enzyme, which binds dTTP in the active conformation, is still inhibited by dTTP.

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#### Structural studies of the aminotransferases LivB and NeoB

M. Schöpfel,<sup>a</sup> U. Bräuer,<sup>a</sup> C. Parthier,<sup>a</sup> D. Clausnitzer,<sup>b</sup> T. Beck,<sup>c</sup> U.F. Wehmeier,<sup>d</sup> and M.T. Stubbs,<sup>a</sup> <sup>a</sup>*Institute of Biochemistry and Biotechnology, Martin-Luther-University Halle-Wittenberg.* <sup>b</sup>*Institute of Chemical Microbiology, University of Wuppertal.* <sup>c</sup>*Department of Structural Chemistry, Georg-August University Göttingen.* <sup>d</sup>*Institute of Sport Medicine, University of Wuppertal. (Germany).* E-mail: