# Molecular Recognition of Biotin Derivatives

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by

Yu-Lin Jiang B.Sc. (Mas)

ADELAIDE UNIVERSITY
AUSTRALIA

The Department of Chemistry
The University of Adelaide
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# Abstract

In *Escherichia coli* , biotin dependent carboxylase catalyses the first step of the acetyl-CoA carboxylase reaction, which is the first committed step of fatty acid biosynthesis. In its functional cycle the biotin carboxyl carrier protein (BCCP) participates in three heterologous protein-protein interactions, depending on its state of post-translational modification. Athappilly and co-worker revealed the internal multiple biotin-mediated interactions in the crystal structure of the biotinylated BCCP of the acetyl CoA carboxylase (Athappilly, F. K.; Hendrickson, W. A. *Structure* **1995**, *3*, 1407). However, the results were clouded by the later finding of the absence of observable biotin-protein interactions in the dilute solution of the 1.3 subunit of transcarboxylase (Reddy, D. V.; Shenoy, B. C.; Carey, P. R.; Sonnichsen, F. D. *Biochemistry* **1997**, *36*, 14676). Biotin is attached via an amide linkage to a specific lysine residue of the biotin dependent enzymes by the intermediate BirA-biotinyl-5'-AMP. However, chemically, the interactions between biotin and the various proteins, between biotin and nucleic acids and between different biotin dependent enzymes are not well documented.

The intra-molecular hydrogen binding of biotin in the mixed organic solvent $CDCl_3$-DMSO-$d_6$ was initially revealed by multiple NMR techniques and provided a support for the proposed mechanism (Goodall, G. J.; Prager, R.; Wallace, J. C.; Keech, D. B. *FEBS Lett.* **1983**, *163*, 6) and preferential carboxylation at N-1' of biotin (Fry, D. C.; Fox, T.; Lane, M. D.; Dilavan, A. S. *Ann. N. Y. Acad. Sci.* **1985**, *447*, 140). The intra-molecular hydrogen bonding in biotin esters was investigated as well as the aminolysis of active biotin esters. For various biotin peptides, intra-molecular hydrogen bonding networks were observed, which resulted in the pro-chiral vicinal hydrogen differentiation of side chain methylenes and hydrophobic interactions between biotin and aromatic side chains. During the investigation of biotin nucleobases, Bt-Ade (**51**), Bt-Cyt (**52**), Bt-Thy (**53**), Bt-Ura (**54**) and Bt-Thioura (**55**), the nucleobases, cytosine, adenine, thymine and uracil form hydrogen bonding pairs with the NHb and carbonyl group of biotin, however, thiouracil preferentially formed a hydrogen bonding pair with the NHa and the carbonyl group of biotin.

A series of potential receptors for biotin derivatives based on the 2, 6-pyridinedicarboxamide motifs were investigated. The three-centred intra-molecular hydrogen bonds gave rise to a planar conformation, which also resulted in efficient intermolecular $\pi$–$\pi$ stacking interactions. Efficient bindings were observed between these receptors and biotin esters and between these receptors and biotin peptides.

Receptors were designed for the recognition of adenine through efficient hydrogen bonding and $\pi$–$\pi$ stacking interactions. Receptor **73** was used to bind DNA base pair doublet and triplet analogues by hydrogen bonding and $\pi$–$\pi$ stacking interactions. DNA base pair interactions and Watson-Crick and Hoogsteen hydrogen bonding, were also observed within a series of artificial base pair doublets and triplets.

The crystal structure of carboethoxyimidazolidinone, and structures on the conformation of biotin derivatives and various receptors provided support for a literature mechanism (Kluger, R.; Tsao, B. *J. Am. Chem. Soc.* **1993**, *115*, 2089) for decarboxylation of carboxybiotin. Tetrapeptide receptor **73** was used to investigate the binding of free biotin, and inorganic substrates such as hydrogen phosphate, bicarbonate, carbonate and organic acetate anion which are the basic materials used in the carboxylation of biotin. The binding interactions between various biotin peptides and bicarbonate were also investigated.

Based on the above information, models were proposed for the formation of biotin-5'-AMP and carboxyphosphate, the carboxylation of biotin by carboxyphosphate and the transdecarboxylation of carboxybiotin and carboxylation of malonyl with retention of configuration.