

## THE FATE OF VITAMIN B12

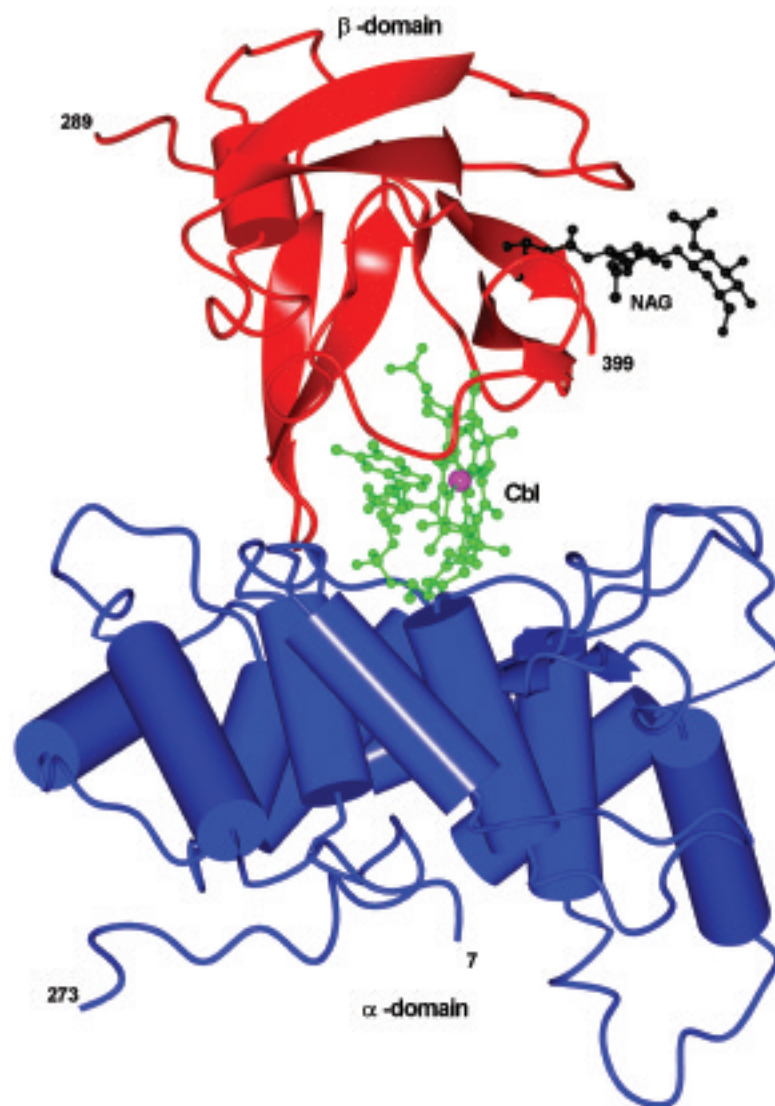


Fig. 1. Worms/tubes diagram of the IF-Cbl complex with  $\alpha$  and  $\beta$  domains shown in blue and red, respectively. The cobalamin and sugar (N-acetylglucosamine; NAG) molecules are shown in ball and stick and in green and black colors, respectively. The cobalt atom is shown as a pink sphere.

**V**itamin B12 (also known as cobalamin, or Cbl) is an essential vitamin for all mammals. But, unlike other water-soluble vitamins, cobalamin is stored in body tissues rather than being dispersed in urine. This can mask the effects of vitamin B12 deficiency in the diet for many years. Understanding the molecular processes that are involved in the delivery of Cbl to tissues in order to understand vitamin B12 deficiency clearly, and hopefully provide a target for new curative pharmaceuticals, was the goal of researchers using the NE-CAT 8-BM-B beamline at the APS. Their study provides important structural information that will facilitate those efforts.

Cobalamin delivery from food to tissues involves three successive transport proteins and their cellular receptors. The process begins when the food enters the digestive tract, where cobalamin is bound by a glycoprotein called haptocorrin (HC). After proteolysis of HC-Cbl complex in the small intestine, cobalamin is picked up by another glycoprotein called intrinsic factor (IF) that mediates the translocation of cobalamin into cells lining the ileum, the final section of the small intestine. Once IF has released cobalamin inside these cells, it is picked up by a protein called transcobalamin II (TC) that manages the final delivery into the circulation. The researchers using the NE-CAT facility at the APS determined the structure of the intrinsic factor-cobalamin complex at 2.6-Å resolution. The work reveals detailed information about the cobalamin binding site in IF. The identification of several important water molecules indicates their roles in Cbl binding, as well as the receptor binding site interactions at the N-terminal end of the protein. Comparison of the IF structure to that of TC reveals significant differences between them, though they perform a similar function. Determination of the IF structure enabled the team to investigate why rat IF and human IF are not interchangeable despite similarity at the amino acid sequence level. Recently, IF has generated interest as a possible drug discovery target that would allow uptake of small molecules linked either to Cbl or to a small peptide that would bind to the IF receptor.

The three-dimensional structure of IF is organized into two domains, an  $\alpha$ -domain that is arranged as an  $\alpha_6/\alpha_6$  helical barrel, and a  $\beta$ -domain that is made up of mostly  $\beta$ -sheet structures. The cobalamin binding site lies between these two domains (Fig. 1). The crystal structure

also reveals a major sugar binding site at Asparagine 395, which can accommodate at least two sugar molecules that may be important for IF function (Fig. 1).

Comparison of the overall structure of IF to the structure of TC revealed differences in the length and orientation of various helices in the  $\alpha$ -domain. Another important difference is that the  $\beta$ -side of the corrin ring of IF is empty even without any ordered water molecules with respect to TC. In IF the binding site and channel formation between the  $\alpha$  and  $\beta$  domains are dominated by negatively charged residues that may play a role in the binding of Cbl at low pH. In addition, the binding site for cobalamin in IF is broad and open on both sides compared to that for TC; this may have important implications for binding and dissociation of the cobalamin to these two transport proteins.

This study also investigated the observation that rat IF cannot substitute for human IF in binding to its cellular receptor, though they share 80% sequence identity. The predicted receptor binding site of 37 amino acids varies at only six of these locations between the two proteins. In this study, using a homology model, substitution of these six amino acids from the rat sequence into the human IF did not change the predicted structure of the binding region at all but did change the charged nature of the site. This change in charge environment may interfere with proper binding of the rat IF to the human IF receptor and thus inhibit its ability to leave the digestive tract with its cargo. — *Sandy Field*

**See:** F.S. Mathews<sup>1</sup>, M.M. Gordon<sup>2</sup>, Z. Chen<sup>1</sup>, K.R. Rajashankar<sup>3</sup>, S.E. Ealick<sup>3,4</sup>, D.H. Alpers<sup>2</sup>, and N. Sukumar<sup>3\*</sup>, "Crystal structure of human intrinsic factor: Cobalamin complex at 2.6-Å resolution," *Proc. Natl. Acad. Sci. USA* **104**(44) 17311 (October 30, 2007). DOI: 10.1073/pnas.0703228104

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