Direct Observation of Photolysis-Induced Structural Changes in Hemoglobin

Hemoglobin (Hb) is a tetrameric protein that consists of two α and two β subunits represented by $\alpha_2\beta_2$ and transports oxygen from lungs to tissues for use in respiration. The α and β subunits are structurally and evolutionarily related to each other, and each subunit has an oxygen binding site which is called heme. Thus, Hb can bind a total of four oxygen molecules. It is well known that Hb is not only a simple oxygen tank, but also functions as a sophisticated oxygen delivery system to provide the proper amount of oxygen to tissues under a wide variety of circumstances. This feature is closely related to a cooperative interaction between oxygen binding sites; that is, the binding of oxygen at one subunit increases the affinity for additional oxygen at another subunit. In this context, Hb has played a central role in exploring the mechanism of the cooperative interaction of proteins in general. It is well established by X-ray crystallographic studies that Hb has two end-structures which are called relaxed (or R) and tense (or T-) states, which correspond to high and low oxygen affinities, respectively [1]. However, intermediate structures between the two end-structures, which must be related to a key structure to understand the cooperative regulation mechanism of Hb, have not been directly observed yet. In particular, the essential part of the mechanism is the structural restraints in the T-state Hb on ligand binding, because the oxygen affinity of the R-state is close to that of the isolated α and β subunits, while the oxygen affinity of the T-state is lower by two orders of magnitude than those of the R-state and isolated subunits. We noted that the structural restrains in the T-state Hb can be elucidated at the atomic level by determining the structure of the photoproduct of T-state HbCO by X-ray crystallography, and here we present an X-ray crystallographic study of CO complexes of the T- and R-states of Hb at cryogenic temperatures in both resting and photolysed states [2].

The experiments were carried out at the RIKEN Structural Biology beamline **BL44B2**. We clearly observed the photodissociation of CO within a single crystal of T- and R-state Hbs, and directly monitored subsequent tertiary structural changes of the α and β subunits in terms of electron density movements. We used iron-nickel hybrid Hb as T-state liganded Hb, in which iron atoms either in the α or β subunit are replaced by nickel atoms. Nickel binds neither oxygen nor CO and mimics deoxy heme, thus the quaternary structure remains in the T-state even two CO molecules bind to either α or β subunit. The 2Fo-Fc map of the α subunit of α-iron β-nickel hemoglobin in the T-state is shown in Fig. 1. A photolyzed CO molecule is clearly observed immediately above the

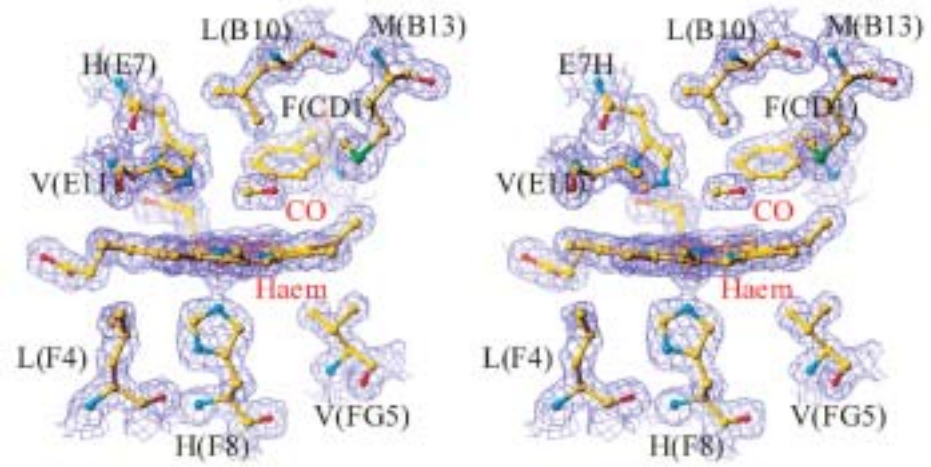

Fig. 1. Stereoview of the electron density map ($2F_o$-F_c map) of the active-site structure of the photolysed CO complex of T-state hybrid Hbs contoured at 1.3 σ. The α_1 heme region in photolysed iron-nickel hybrid HbCO at 25 K is shown [2].

heme iron in the heme pocket. Difference Fourier maps between T-state photoproducts and CO-bound structures gave a clear picture of the dynamic responses of the hemes and protein moieties after photolysis (Fig. 2). We found two important structural differences between the α(Fe) and β(Fe) subunits. First, the downward movement of an F-helix and the bent motion of a pyrrole ring are more marked in the α(Fe) subunit than in the β(Fe) subunit. Secondly, sliding motion of the heme is observed only in the β(Fe) subunit. These results reflect structural restraints retained in each subunit and clearly show that the structural basis of the low affinity of T-state Hb is completely different between the α and β subunits, even though these subunits have similar tertiary structures. This direct observation of photolysis-induced structural changes in Hb suggests that the reduced ligand affinity of T-state Hb is mainly contributed by the structures of relatively local and specific sites close to the heme moiety.

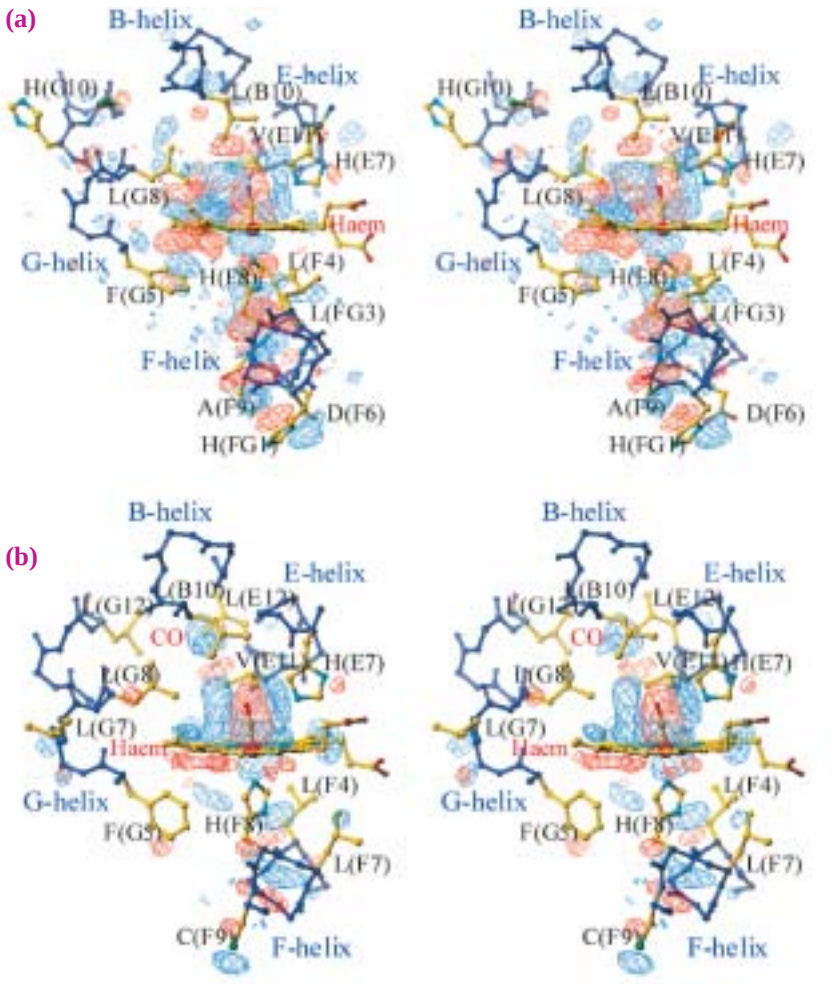

Fig. 2. Stereoview of the difference Fourier map of the active-site structure between the T-state protoproduct and CO-bound forms at ±3σ. **(a)** The α_1 heme region in iron-nickel hybrid HbCO at 25 K. **(b)** The β_2 heme region in iron-nickel hybrid HbCO at 25 K [2].

Shin-ichi Adachi[a,*], Sam-Yong Park[b] and Naoya Shibayama[c]

(a) SPring-8 / RIKEN
(b) Protein Design Laboratory, Yokohama City University
(c) Department of Physiology, Jichi Medical School

* *Present address* : Photon Factory, High Energy Accelerator Research Organization (KEK)

E-mail: shinichi.adachi@kek.jp

References

[1] M. F. Perutz: Nature **228** (1970) 726.
[2] S. Adachi, S.-Y. Park, J.R.H. Tame, Y. Shiro and N. Shibayama: Proc. Natl. Acad. Sci. USA **100** (2003) 7039.