# High-Resolution Crystal Structure of Intermediate Liganded State of $(\alpha^{Fe(II)})_2(\beta^{Mg(II)})_2$ Hybrid Hemoglobin

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## Introduction

Several metal substituted hybrid hemoglobins (Hbs), $(\alpha^{Fe(II)})_2(\beta^{M})_2$ and $(\alpha^{M})_2(\beta^{Fe(II)})_2$, where M denotes an ion other than the ferrous ion including the ferric ion and the porphyrin without metal ion, have been prepared to study the relation between the structure and the function of the Hb (Venkatesh et al., 1998). It has been found that the ligand affinities of ferrous subunits are changed in the full extent from R to T stare depending on the kind of metal ions in the partner subunits (Nagai et al., 1997; Unzai et al., 1996). Thus metal substituted hybrid Hbs have afforded important ways to study the interaction between the electronic state of the central metal ion in the heme and the protein moiety.

In the case of magnesium (Mg) portoporphyrin the crystal structures of Hb with all 4 Fe substituted by Mg (Kulia et al., 1991), and deoxy and CO-liganded $(\alpha^{Mg})_2(\beta^{Fe(II)})_2$ all belong to the T-structure (Park et al., 1996). Therefore Mg protoporphyrin has been thought to stabilize the T quaternary structure.

we report the crystallization, preliminary crystallographic characterization, of deoxy and CO-liganed forms of $(\alpha^{Fe(II)})_2(\beta^{Mg})_2$.

## Experimental and Results

HbA and its isolated chains were prepared in carbon monoxide forms as described. The preparation of $(\alpha^{Fe(II)})_2(\beta^{Mg})_2$ was carried out as follows. Apo β-chain (500mg) was dissolved in 300 ml of 20 mM-borate/NaOH buffer (pH=12). The spectrophotometric titration of apo β-globin with Mg-ppIX at 419 nm gave a well-definded inflection point from which a molarstoichiometry of 1:1 was estimated. The solution of apo β-chain was mixed with 1.2-fold excess of Mg-ppIX. And then concentrated by ultrafiltration and passed through a Sephadex G25. An equimolar amount of α-(Fe-CO)-chain was added to the β-(Mg)chains and was gently stirred at 4°C. The $(\alpha^{Fe(II)})_2(\beta^{Mg})_2$ solution was passed though a Sephadex G25, DE23, CM23-cellulose column.

Crystallization of $(\alpha^{Fe(II)})_2(\beta^{Mg})_2$ was carried out in ammonium sulfate buffer (pH6.5) according the method described by Perutz (1968) for human deoxy HbA, except that 50mM-sodium dethionite ($Na_2S_2O_4$) was used as a reducing agent in place of ferrous citrate. The best crystals grew from solution whose final ammonium sulfate buffer concentration was 2.5-2.4M. In order to obtain crystals of $(\alpha^{Fe\text{-}CO})_2(\beta^{Mg})_2$ the crystal in the capillaries was flushed with carbon monoxide under nitrogen-filled glove box. These crystals were found to be isomorphous with native deoxy HbA crystals, which belong to space group $P2_1$, a=63.15 Å, b=83.59 Å, c=53.80 Å, β=99.34°.

X-ray deflection data were collected on BL/44B2 using a Raxis-IV, with a crystal to film distance of 250 mm and a wavelength of 0.7Å. The intensity data up to 2.0 Å resolution were processed with program DENZO and were merge and scaled by using programs in the CCP4 program suite. And fine structural analysis in going on.

## References

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