NMR Study of CO Binding to the α and β chains of Hemoglobin Kansas

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We have measured the proton nmr spectra at 270 MHz of Hb-Kansas (β120 Asn → Thr) with IHP at different degrees of CO binding. The spectra showed the disappearance of paramagnetically shifted resonances assigned to deoxygenated α and β subunits simultaneous with the appearance of ring current shifted resonances assigned to CO complexes of the α and β subunits. Hb Kansas with IHP has previously been shown to be in the low affinity deoxy quaternary state T even when fully ligated with CO. As such it allows one to interpret the nmr results in terms of the affinity differences between the α and β subunits for CO without the complications of cooperative binding. The measurements indicate that the CO affinities of the α and β subunits are identical to within 20 % of experimental accuracy in this system where there is no switch of the quaternary state. In a hemoglobin kept in its low affinity T state the functional inequivalence of the α and β chains in the tetramer is negligible small compared to the functional difference of the two quaternary states.

The spectra of partially saturated hb Kansas show some difference from those of fully saturated and fully deoxygenated species in spite of the absence of quaternary change. Those differences clearly demonstrate in the heme environment the presence of subunit interaction due to the ligation at neighbouring subunits within the T state. However, since the cooperativity in the CO binding is essentially absent and since the α and β affinities are essentially equivalent, the subunit interactions observed in the nmr spectra do not bear significant energy. The residual cooperativity (n = 1.1) might be due to these, but the interaction energy can only be 0.4 Kcal/mole or less. In contrast the interaction energy in fully cooperative hemoglobin which involves a quaternary switch is about 3 Kcal/mole. The present nmr spectral changes in the intermediates illustrate that spectroscopic changes can be associated with the intermediate degrees of ligation without representing states of intermediate ligand affinities.

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