International Conference & Exihibition on
Analytical and Bioanalytical Techniques 2010
ANANBIOANAL - 2010
Pharmaceutical R & D Summit
doi:10.4172/2155-9872.1000082

Quaternary Structure Analysis of Human Hemoglobin by a Near-UV CD Spectroscopy

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Human adult hemoglobin (HbA) has four subunits, namely, two-α subunits and two β-subunits. X-ray crystallographic analysis have indicated that there are two distinct quaternary structures, namely, the deoxy state, represented by the tense (T), low-affinity structure, and oxy state, represented by the relaxed (R), high-affinity structure. A characteristic spectral change of HbA in the near-UV CD occurs: from a small positive band in the oxy-R form to a negative CD band with a distinct peak at 287nm in the deoxy-T form. This negative CD band of deoxyHbA known as T-state marker has been supposed to derive from the changes of Tyr and Trp residues at the α1β2 subunit interface. To identify the aromatic residue responsible for the CD band, we have synthesized five recombinant Hbs in *E. coli* in which non aromatic residue is substituted for Tyr or Trp residue; rHb (α14Trp→Leu), rHb (β15Trp→Leu), rHb (β37Trp→His), rHb (α42Tyr→Ser), and rHb (β145Tyr→Thr). We examined the near-UV CD spectra of these rHbs and a natural mutant, Hb Rouen (α140Tyr→His). The CD spectra of individual aromatic residue were extracted from the difference between Hb A and each mutant. We concluded that changes in CD bands arising froṃβ37Trp, α140Tyr, β145Tyr and α42Tyr residues contributed to the appearance of the negative CD bands at 287nm. To examine different signals of CD band among aromatic residues, the effects of environments on CD spectra were examined using model compounds of Tyr and Trp dissolved in various solvants.