

Solution properties of human plasma haptoglobin as
probed by monoclonal antibodies

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ABSTRACT

Human plasma haptoglobin (Hp) is classified as three distinct phenotypes (Hp 1-1, 2-1, and 2-2) due to the insertion of a 3/4 redundant sequence of α chain (residues 12-70 from 1-83) in Hp 2-1 and 2-2 individuals. As a result, the Hp structure in 2-1 and 2-2 individuals exhibits complicated linear and cyclic polymers, respectively. In this study, we prepared a battery of monoclonal antibodies (mAb) in which some of them were restrictedly conformational dependent. Two of them, mAb 3B7 and 4H11, recognized the Hp 2-1 and 2-2 but not 1-1. One unique α -chain specific mAb (3H8) directed to the sequence of 31-39 (or RYQCKNYYK) was established following the antigenic mapping using synthetic peptides. This 3H8 mAb recognized the region that repeated twice in the α chain of Hp 2-1 and 2-2, but failed to show increased immunoreactivity in Hp 2-1 and 2-2 molecules. However, markedly increased immunoreactivity was observed, while using recombinant α chains ($\alpha 1$ and $\alpha 2$) as antigen under reducing condition. These results suggest that the amino acid region of 31-39 was recognized in whole Hp; as such the epitope expression in Hp 2-1 and 2-2 was not fully exposed due to the formation of polymers. By chance, the expressed immunoreactivity for this α -chain mAb was equal in Hp three types in a competitive ELISA and therefore provided a unique opportunity in determining the plasma Hp levels. In conclusion, the present study provides a new insight in understanding the immunochemical diversity among the Hp phenotypes. The antigenic mapping further demonstrates the “masked” region involved in the molecules of Hp 2-1 and 2-2 and provides a valuable reference and strategy in producing a useful immunochemical region.