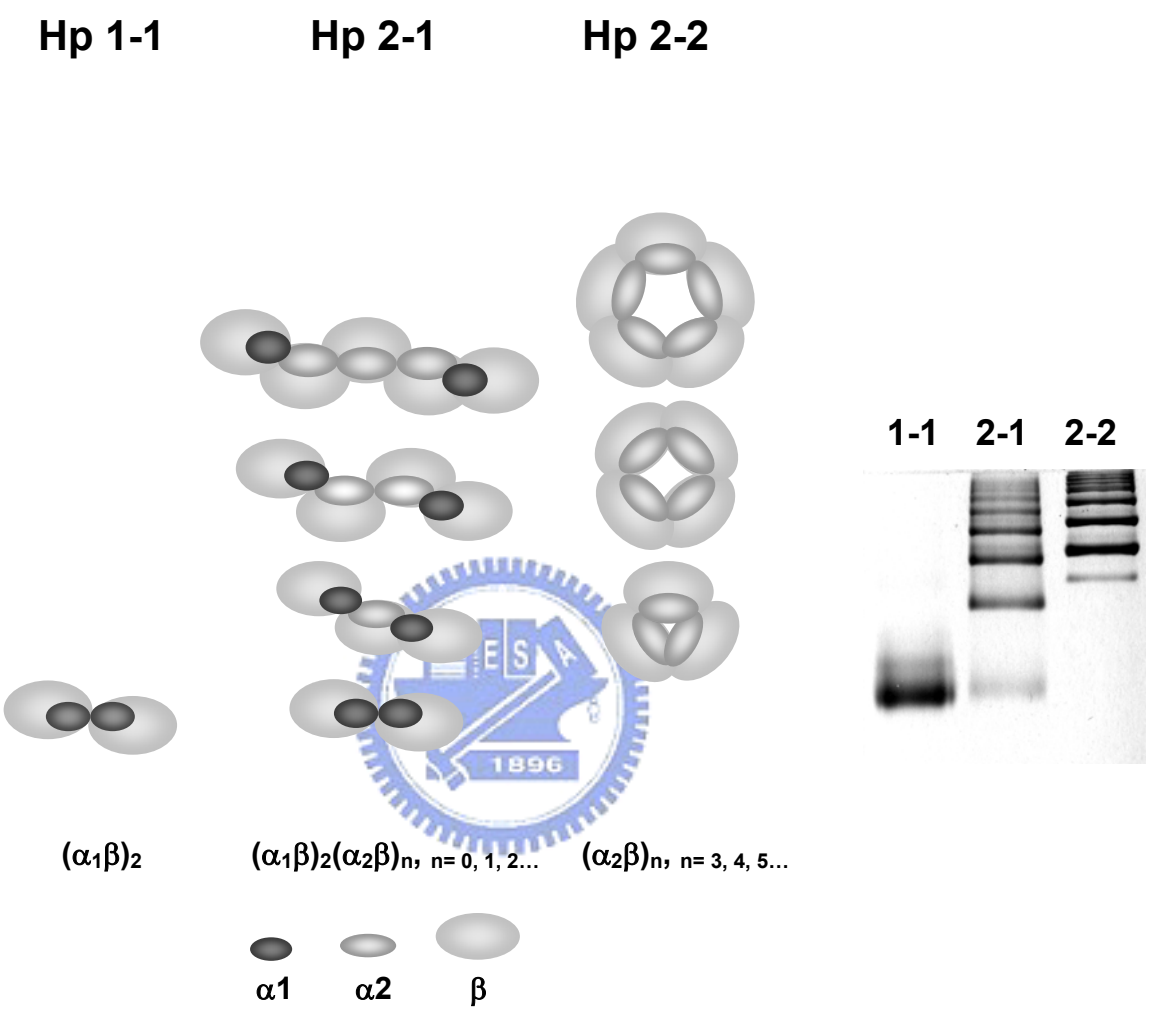

Fig. 1. Schematic drawing of molecular arrangement in Hp phenotypes. Hp 1-1 only has basic structure, the dimeric $(\alpha 1\text{-}\beta)_2$. while Hp 2-1 is comprised of many structures, a dimer $(\alpha 1\text{-}\beta)_2$, a trimer $(\alpha\text{-}\beta)_3$, and other linear polymers. Here, α represents both α1 and α2 chains. Hp 2-2 is comprised of a trimer $(\alpha 2\text{-}\beta)_3$ and other cyclic polymers. Each α1, α2, or β is 83, 142, or 245 amino acids in length, respectively. α2 is similar to α1, differing only by an additional insertion of a repeat identical to 3/4 of α1. Due to the extra Cys-74 in α2, Hp 2-1 and 2-2 form complicated polymers. *Right panel,* electrophoretic pattern of each purified type on a 7% native-PAGE.

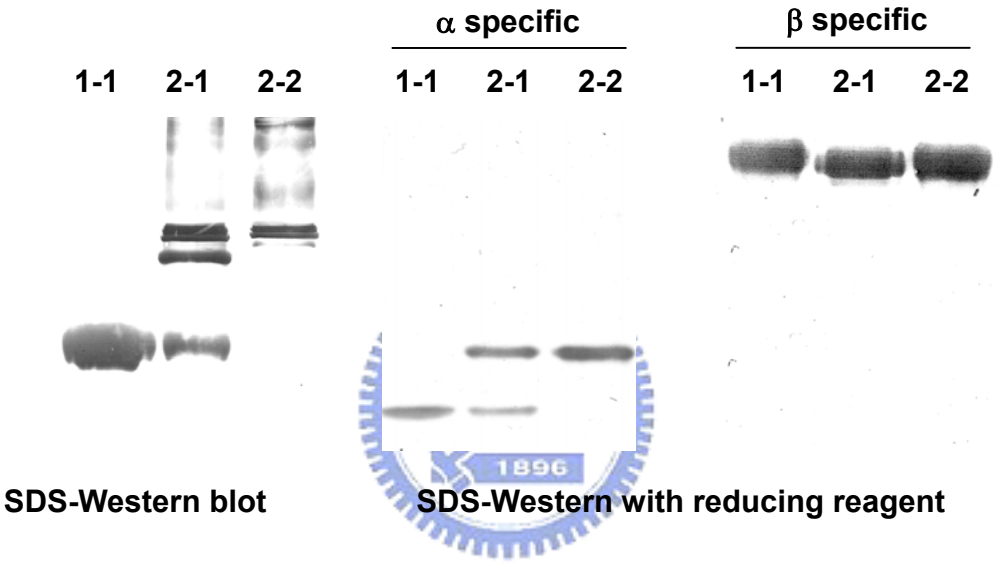

Fig. 2. Determination of the chain specificity of mAb. About 5 μg of purified Hp 1-1, 2-1, or 2-2 were loaded onto a 8% SDS-PAGE (*left*) or 15% SDS-PAGE in the presence of β-mercaptoethanol (*middle and right*), followed by a Western blot. Those mAb that did not show immunoreactivity following the β-mercaptoethanol treatment were defined as conformation-dependent mAb (not show).

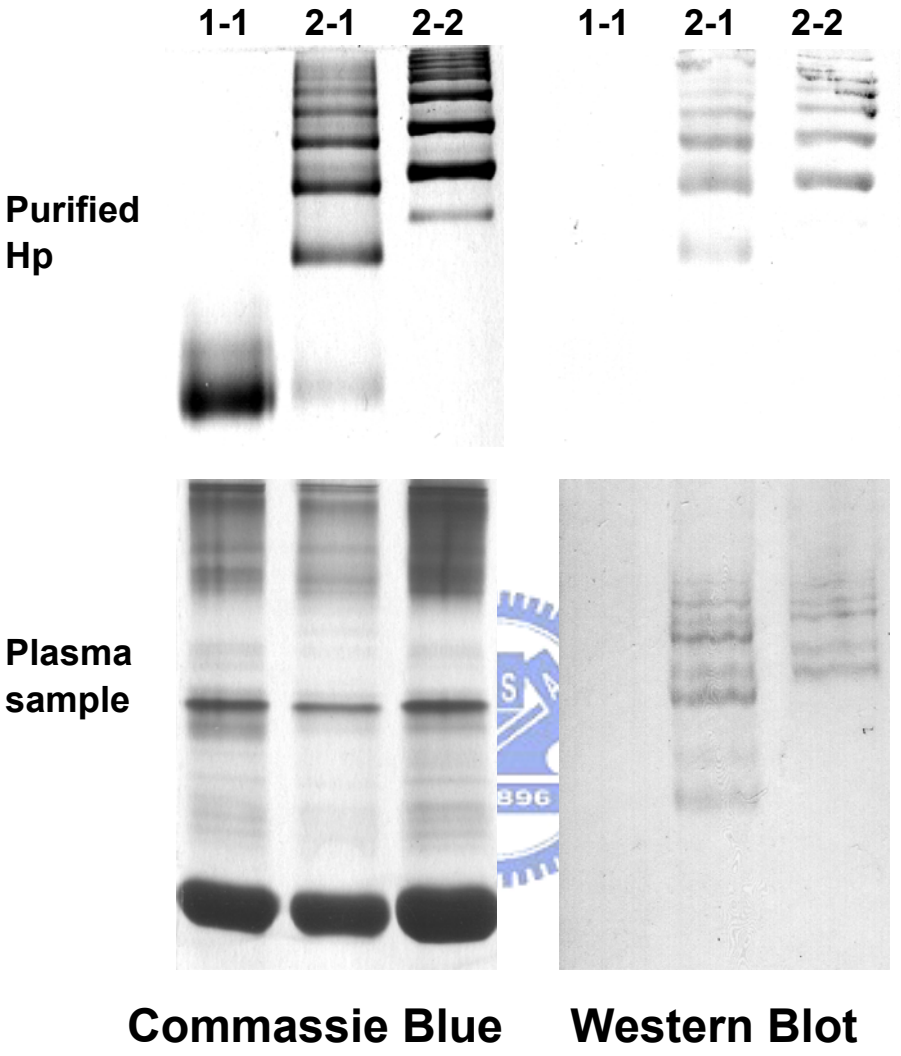

Fig. 3. Specificity of conformation-dependent mAb 3B7. About 5 μg of purified Hp 1-1, 2-1, and 2-2 (*top*) or 10 μl of human plasma (*bottom*) were loaded for 7% native-PAGE, followed by Commassie Blue staining (*left*) and Western blot (*right*) as indicated. This mAb was essentially specific to Hp 2-1 and 2-2, but not to 1-1.

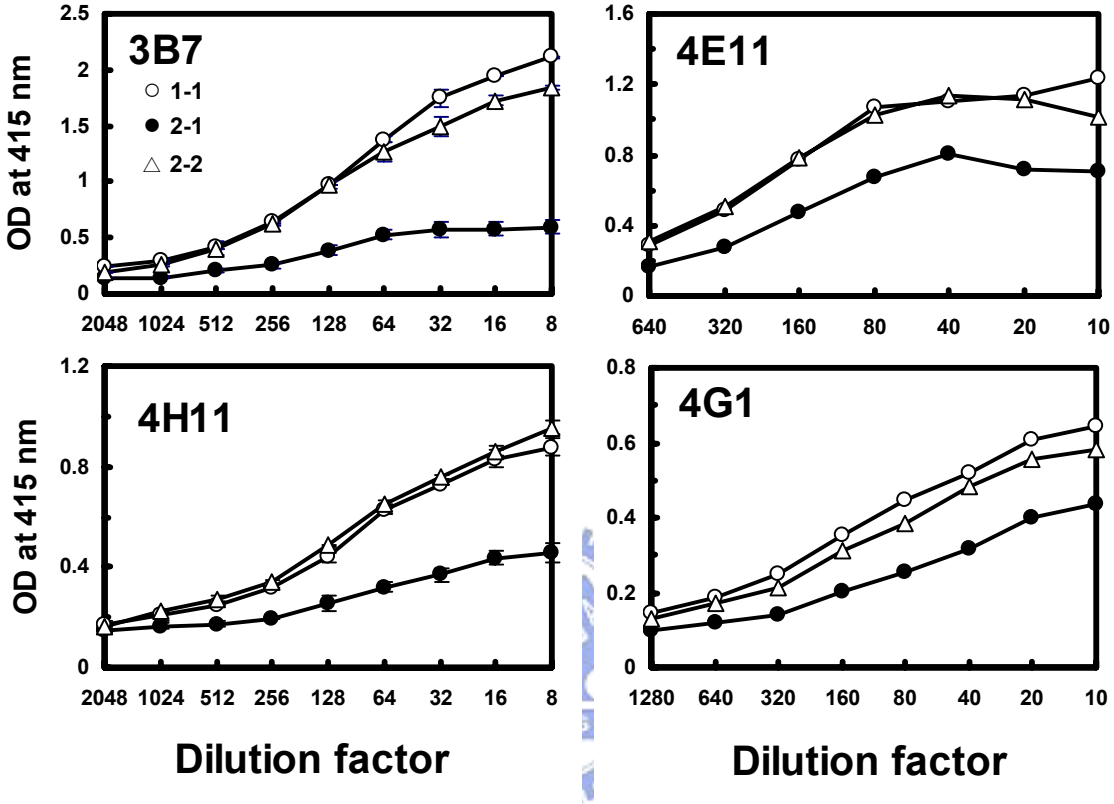

Fig. 4. Typical example for conformation-dependent mAb binding to Hp phenotypes using an ELISA. Equal amounts of Hp 1-1, 2-1 or 2-2 (200 ng) were coated onto each microtiter well, followed by the addition of mAb in serial dilutions. HRP-conjugated goat anti-mouse IgG was used for the final development of chromogenicity.

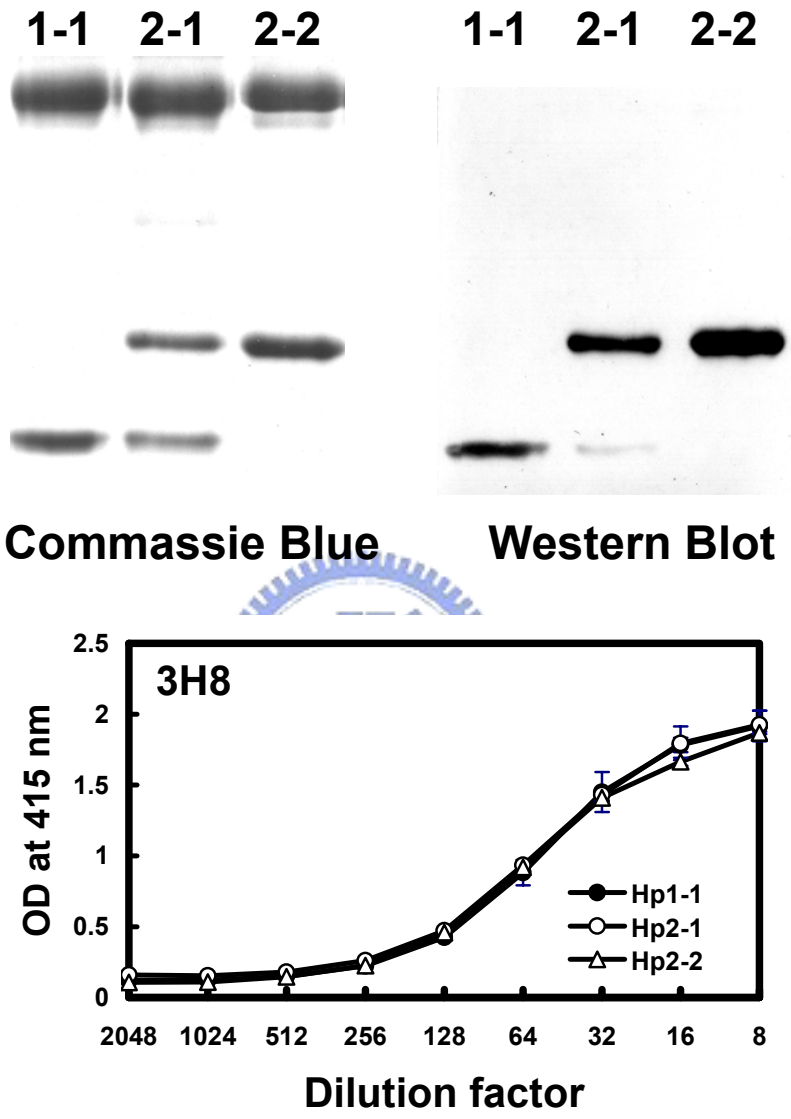

Fig. 5. Specificity and characterization of α-chain specific mAb (3H8). The isolated Hp 1-1, 2-1, or 2-2 (5 μg) were loaded onto a 15% SDS-PAGE gel in the presence of β-mercaptoethanol. The gel was stained with Commassie Blue (*top left*) or transferred to a nitrocellulose membrane for Western blot (*top right*). Direct-ELISA binding assay (*bottom panel*) was conducted using immobilized Hp 1-1, 2-1, or 2-2 as described in Fig. 4. The mAb reacted almost identically with each Hp phenotype. The assay was conducted smilar to that shown in Fig. 4.

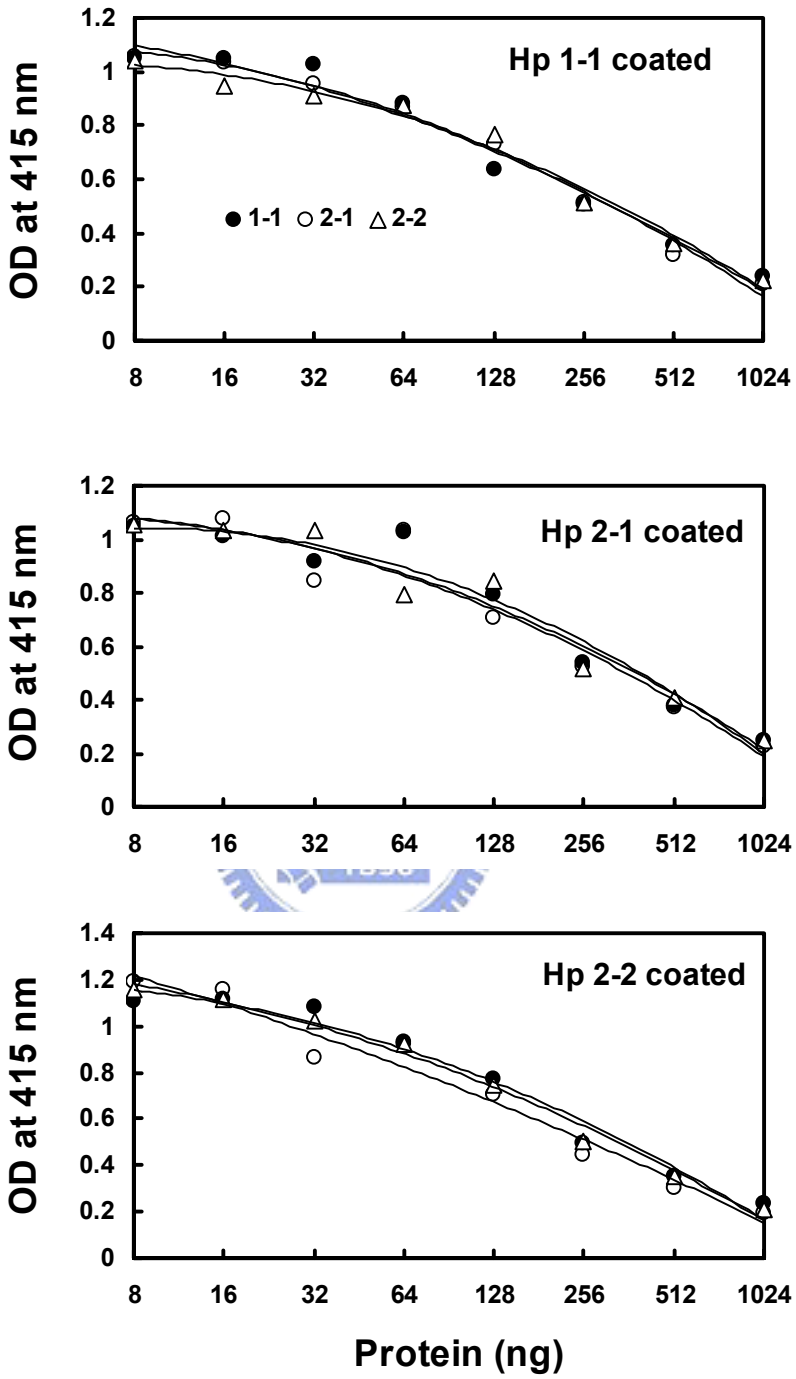

Fig. 6. Competitive ELISA among Hp 1-1, 2-1, and 2-2 using α-chain specific mAb 3H8. Standard displacement curves reveal the immunoreactivity was almost indistinguishable among Hp 1-1, 2-1, and 2-2 (x-axis) using immobilized each Hp type as indicated. Due to the standard procedures for immunoassays (expressed in mass concentrations) and the variable sizes of each phenotype, the molar concentrations are not equalized.

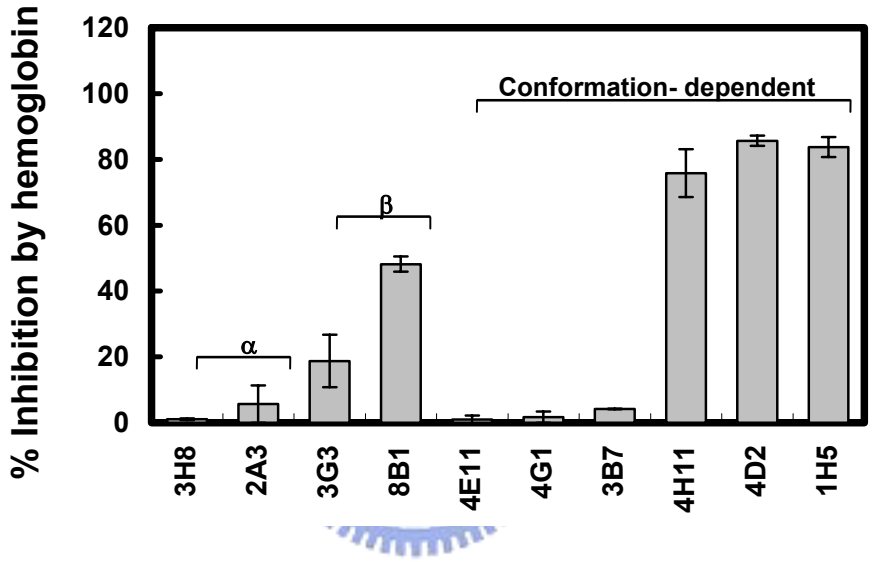

Fig. 7. Typical examples for mAb which immunoreactivity was inhibited by the presence of hemoglobin. ELISA binding for mAb and Hp 2-1 was conducted with or without the addition of hemoglobin. The percentage of inhibition for immunoreactivity was evaluated as 100[(A-B)/A]. Where, A = Absorbance at 415 nm of maximal binding – NSB; B = Absorbance at 415 nm in the presence of hemoglobin – NSB. The detailed assay procedures are given in Experimental Procedures.

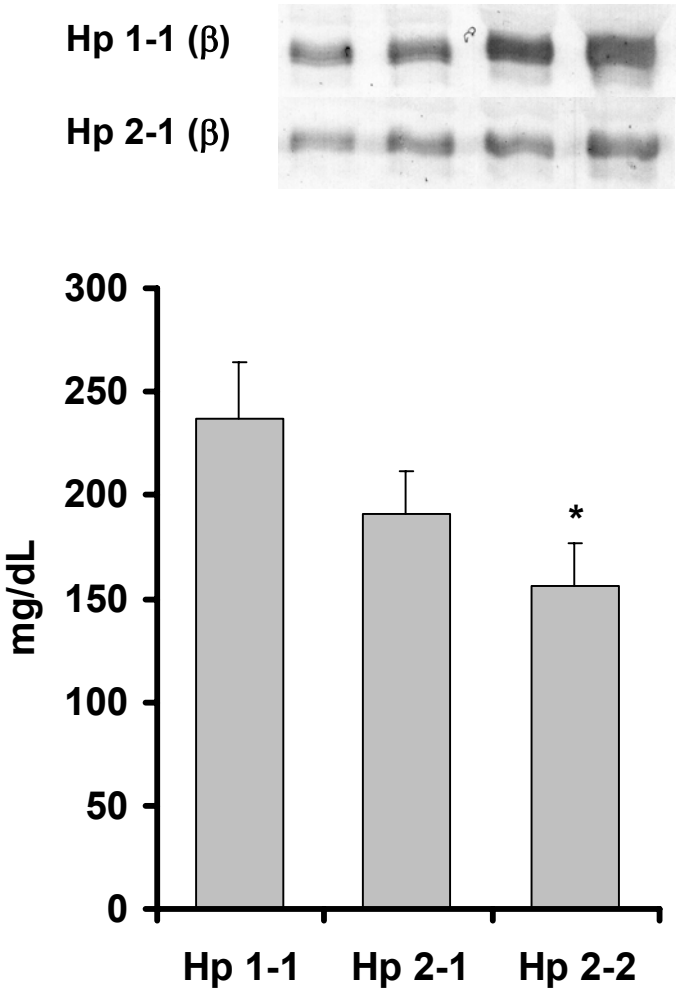

Fig. 8. Determination of plasma Hp concentrations in human subjects of various phenotypes using α-chain mAb (3H8). Hp levels in each group were extrapolated using the standard displacement curves similar to Fig. 6. Where each bar represents the Hp concentrations (mean ± SEM) in Hp 1-1 (236 ± 27.6 mg/dl; n = 11), 2-1 (191 ± 20.5 mg/dl; n = 13), or 2-2 subjects (155 ± 20.7 mg/dl; n = 13). Each individual sample was determined in triplicates. *Compared to Hp 1-1 ($p < 0.05$). *Top panel*: the pooled plasma (10μl) of Hp 1-1 (n = 11) and 2-2 (n = 13) were run on a 15% SDS-PAGE, followed by a Western blot using a β-chain mAb (8B1). The pooled plasma were diluted as 1:80, 1:40, 1:20 and 1:10, respectively (*lanes* from *left* to *right*)

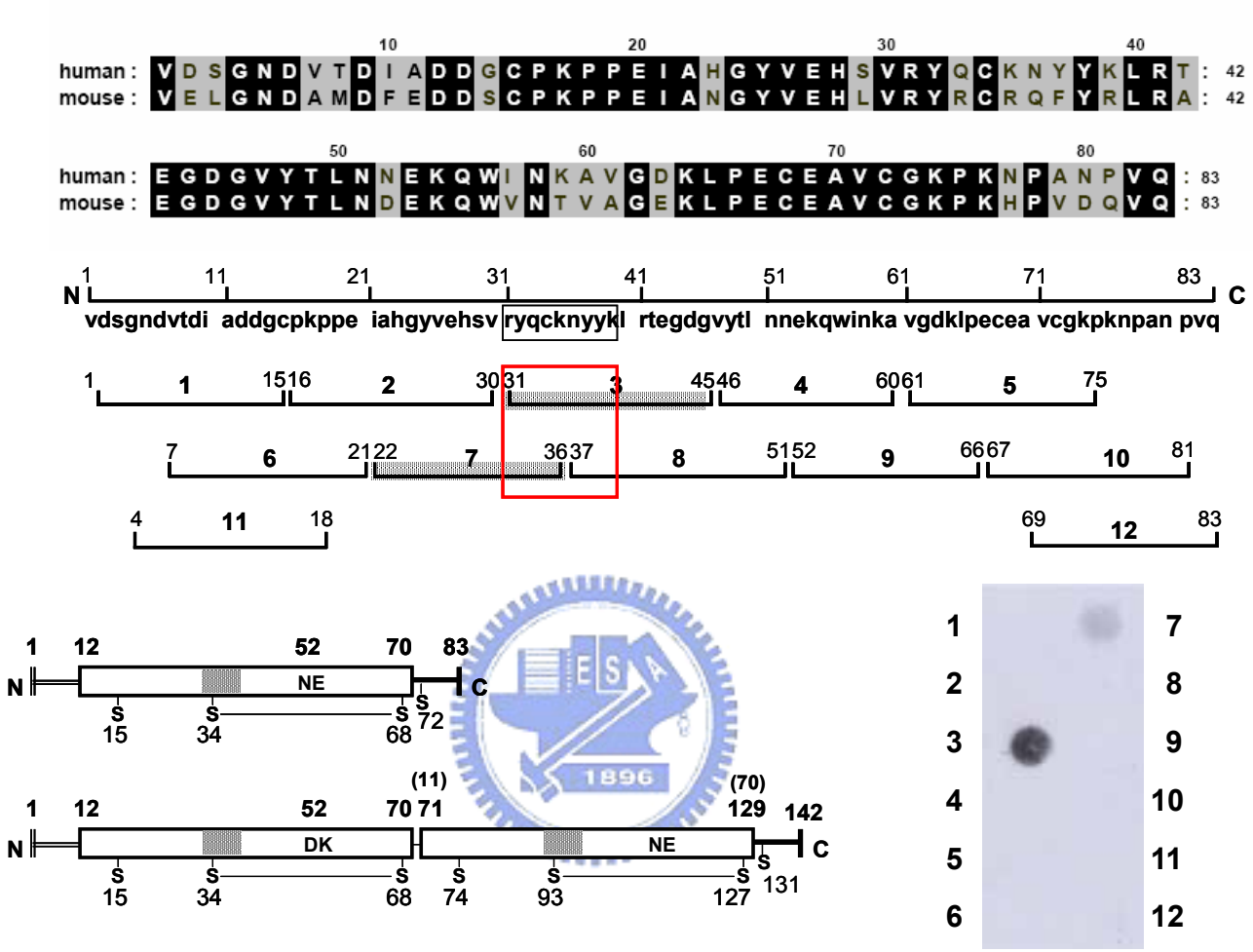

Fig. 9. Sequence homology between human and mouse Hp α chain and delineation of an epitope recognized by α-chain specific mAb (3H8). The variable regions between human and mouse Hp α chain are shaded in grey in the *top panel.* Twelve peptides (containing 15 amino acids of each) corresponding to human Hp α1 chain (residues 1-83) were directly synthesized *in situ* on nitrocellulose membrane (33). Binding of antibody (*bottom right*) was conducted using HRP-labeled secondary antibody with chemiluminescent agent as a developer. The specificity of mapping was demonstrated previously (33). The boxed region represents the proposed epitope (residues 31-39). The difference between the amino acid sequence of human α1 and α2 is shown in the *bottom left*. The 3/4 repeat (residues 12-70) of α1 appears in the α2 chain as residues 71-129. The open bar represents the duplicated region. The extra Cys74 in α2 allows the polymerization of Hp 2-1 or 2-2 into many different structures. Notably, the NH_2-terminus of α2 exhibits DK at residues 52 and 53.

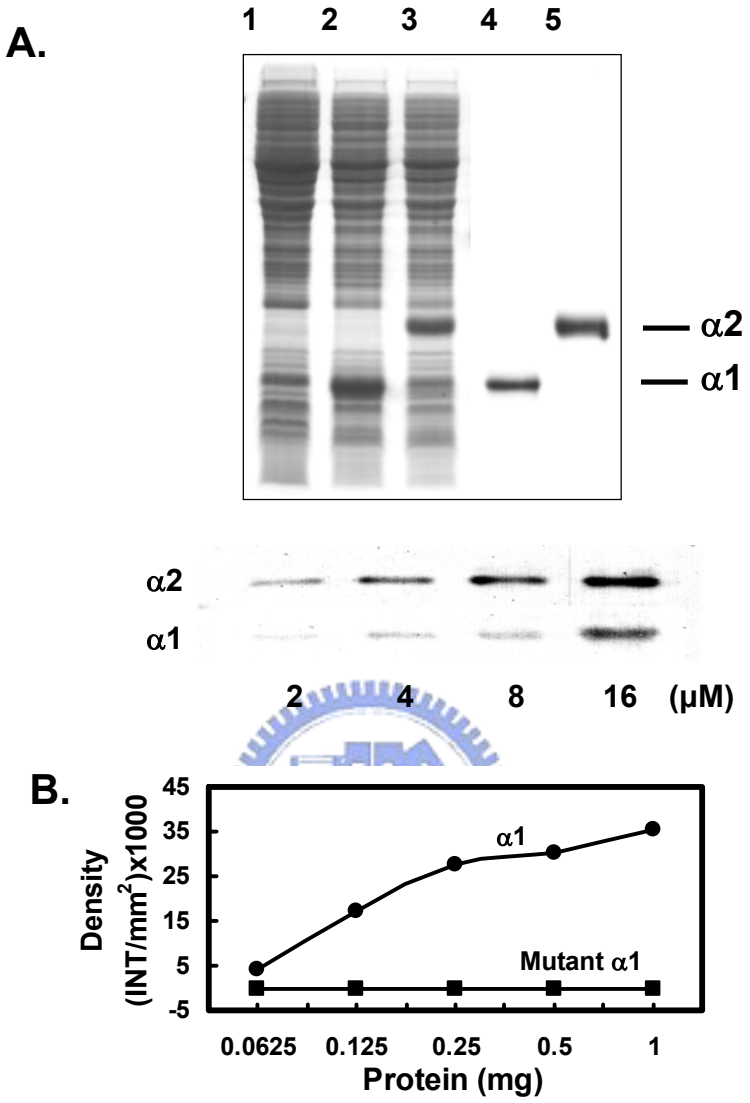

Fig. 10. ***A,*** **Expression and purification of recombinant Hp α1 and α2 subunits and their immunoreactivity to α-chain specific mAb 3H8.** Recombinant α subunits were expressed in *E. coli* using pQE30 vector with T5 promoter (*A*). *Lane 1*: whole cell lysate control of *E. coli* M15 [pREP4]; *Lanes 2 and 3*: whole cell lysate of *E. coli* M15 containing α1 and α2, respectively; *Lanes 4 and 5*: purified recombinant α1 and α2, respectively. All samples were run on a 15% SDS-PAGE in the presence of 1% β-mercaptoethanol. Western blot analysis of mAb 3H8 for recombinant α subunits at various concentrations is shown (*middle panel*). *B,* Effect of Tyr-32 substitution on immunoreactivity. Immunoreactivity of recombinant α1 or mutant-α1 (Y32F) at various concentrations was determined by dot blot using mAb 3H8. The reaction density level was measured by evaluating the intensity (*INT*) developed on the membrane and plotted using BioRad Quantity One software (BioRad, Hercules, CA, USA).

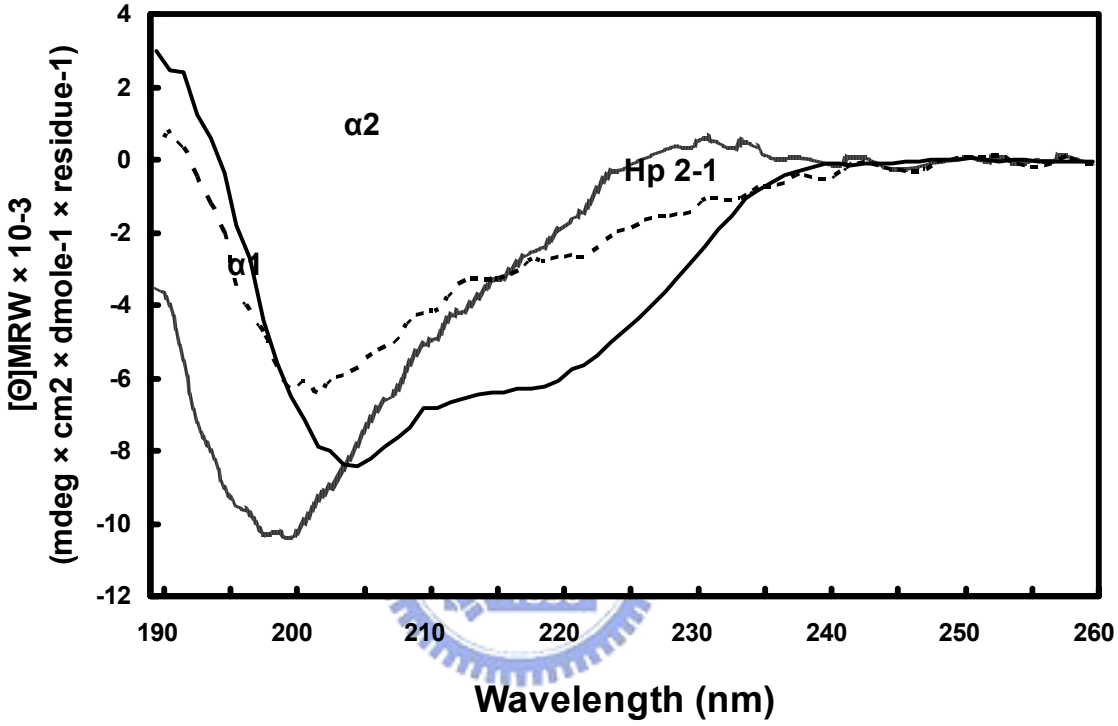

Fig. 11. Circular dichroic spectra of recombinant α1 and α2 chains and native Hp 2-1. Native Hp or recombinant subunit in 10 mM phosphate buffer, pH 7.4, (final concentration 0.2 mg/ml) was monitored by a circular dichroic spectrophotometer. Each spectrum represents a mean of 20 determinations. Essentially, recombinant α1 and α2 subunits are typically disordered in structure.