ABSENCE OF INTERCHAIN DISULEIDE BRIDGES IN RABBIT HAPTOGLOBIN MOLECULE

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SUMMARY: Purified rabbit serum haptoglobin was partially characterized, and it was found that the hemoglobin-binding property and subunit structure were similar to those of human type 1-1, swine, canine, and equine haptoglobins. However, rabbit haptoglobin was dissociated into subunits and few intermediates only in the presence of urea or sodium dodecyl sulfate without reduction. Thus the absence of interchain disulfide bonds in rabbit haptoglobin is unique among many animal haptoglobins.

Haptoglobin (Hp) is a glycoprotein present in the plasma of human and most animals. Its unique property is to bind Hb stoichiometrically in vivo and in vitro. Human Hp has been well characterized biochemically and genetically, although the exact physiological function has not been understood well (1).

Most animal Hps tested so far including human Hp have been known to be composed of two kinds of polypeptide chains, α- and β-chains, held together by disulfide bonds (2-5). Hence, cleavage of interchain disulfide bonds is an essential step to dissociate Hp molecule into subunits. However, rabbit Hp was dissociated into subunits without disrupting the disulfide bridges.

MATERIALS AND METHODS

Swine and human Hps were prepared previously in this laboratory (4). Rabbit Hp was purified by DEAE-cellulose chromatography and gel filtration as described previously (4)

Abbreviations: Hp, haptoglobin; Hb, hemoglobin; SDS, sodium dodecyl sulfate.

from pooled serum. Each rabbit was injected with 1 ml of turpentine oil to raise Hp level two days before bleeding.

Separation of subunits of rabbit Hp by gel filtration after reduction and alkylation was performed as described previously for human, canine and swine Hps (3,4).

Dissociation of Hp was tested also under nonreducing condition by subjecting rabbit Hp dissolved in urea-saturated 0.1 M borate buffer (pH 8.9) to acid urea-starch-gel electrophoresis, or by subjecting rabbit Hp incubated at 37°C for 2 hours in 0.01 M phosphate buffer (pH 6.8) containing 0.2 %, 1 % SDS or 1 % SDS plus 8 M urea to SDS polyacrylamide gel electrophoresis.

For isolation of the dissociated products under nonreducing condition, 35 mg of rabbit Hp was dissolved in 2.5 ml of 0.25 M borate buffer (pH 8.9) containing 8 M urea, and it was applied on a Sephadex G-200 column (2.5 x 70 cm) which was equilibrated with 6 M urea-1 M propionic acid. Elution was carried out with 6 M urea-1 M propionic acid at a flow rate of 3 ml/hour, and 3 ml-aliquots were collected.

Urea-starch-gel electrophoresis was performed as described by Smithies et al. (6). SDS polyacrylamide gel electrophoresis was carried out by the method of Swank and Munkres (7) with modification incorporating procedure of Weber and Osborn (8) using slab gel tray instead of disc column.

RESULTS

The purified rabbit Hp migrated as a single band in alkaline starch gel electrophoresis, and the mobility was retarded by the formation of Hp-Hb complex with human Hb. Rabbit Hp dissolved in a small amount of borate buffer containing 8 M urea showed two bands in urea-starch-gel, and the both bands were further

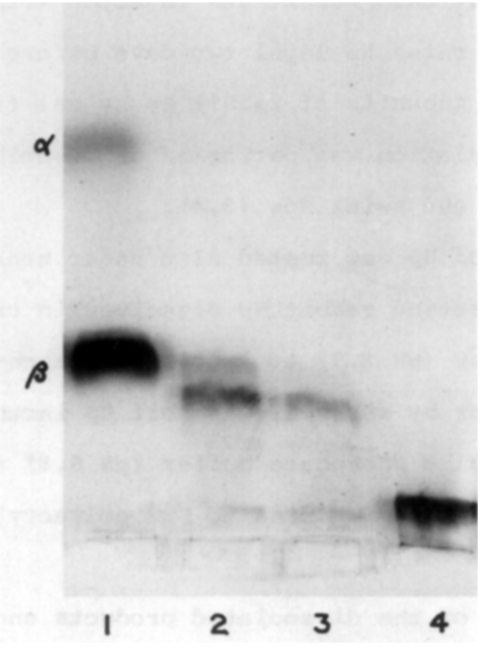

Fig. 1. SDS-polyacrylamide slab gel electrophoretic pattern of rabbit Hp under reducing and nonreducing conditions.
Before electrophoresis, rabbit Hp was preincubated at 37°C for 2 hours in 0.01 M phosphate buffer of pH 6.8 containing 1 % SDS-8 M urea-1 % β-mercaptoethanol (slot 1), 1 % SDS-8 M urea (slot 2), or 0.2 % SDS only (slot 3). Preincubated pig Hp with 1 % SDS-8 M urea at the same condition (slot 4) was run as control. Gel electrophoresis was carried out in 0.1 % SDS-10 % acrylamide-0.01 M phosohate buffer of pH 6.8 for 15 hours. Coomassie brilliant blue stained.
Rabbit Hp incubated with β-mercaptoethanol was completely dissociated into its subunits (slot 1), but it was also dissociated into few bands only in the presence of detergent (slot 2 & 3), while pig Hp in the same condition was not dissociated (slot 4).

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split into α- and β-chains by reduction. Similarly rabbit Hp incubated in 0.2 %, 1 % SDS or 1 % SDS plus urea at 37°C for 2 hours gave several bands in SDS polyacrylamide gel electrophoresis. Swine Hp did not show such bands after the same treatment (Fig. 1).

Hence, rabbit Hp dissolved in urea-containing borate buffer was applied on a Sephadex G-200 column. The gel filtration profile was shown in Fig. 2a. The eluate under the four peaks

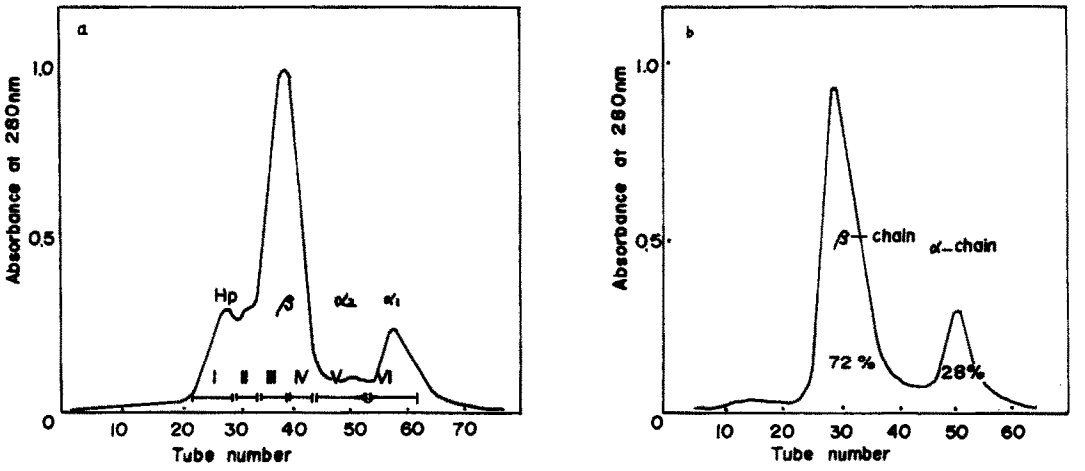

Fig. 2a. The gel filtration profile of nonreduced rabbit Hp on a Sephadex G-200 column (2.5 x 70 cm). Thirty-five mg of purified rabbit Hp was dissolved in 2.5 ml of borate buffer of pH 8.9 containing 8 M urea, and was applied on the column. Elution was done with 6 M urea-1 M propionic acid mixture at a flow rate of 3 ml/hour.

Fig. 2b. The gel filtration profile of reduced and alkylated rabbit Hp on a Sephadex G-100 column (3 x 61 cm). Thirty-five mg of rabbit Hp was reduced with β-mercaptoethanol in the presence of 8 M urea and alkylated with iodoacetamide. Elution was performed with 4 M urea-1 M propionic acid at a flow rate of 3 ml/hour.

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was pooled into six fractions, I to VI, and each fraction was characterized by urea-starch-gel or SDS polyacrylamide gel electrophoresis before and after reduction with β-mercaptoethanol. Fraction I was intact molecule, while fraction II was dissociated products with smaller molecular size than intact Hp molecule. Fraction III and IV were β-chains, but fraction III was contaminated with components of fraction II. Fraction VI was α-chain monomer, while fraction V was α-chain dimer (Fig. 3a).

The control experiments with completely reduced and alkylated rabbit Hp were shown in Fig. 2b and 3b, respectively.

DISCUSSION

The molecular weight of rabbit Hp was reported to be 70,000

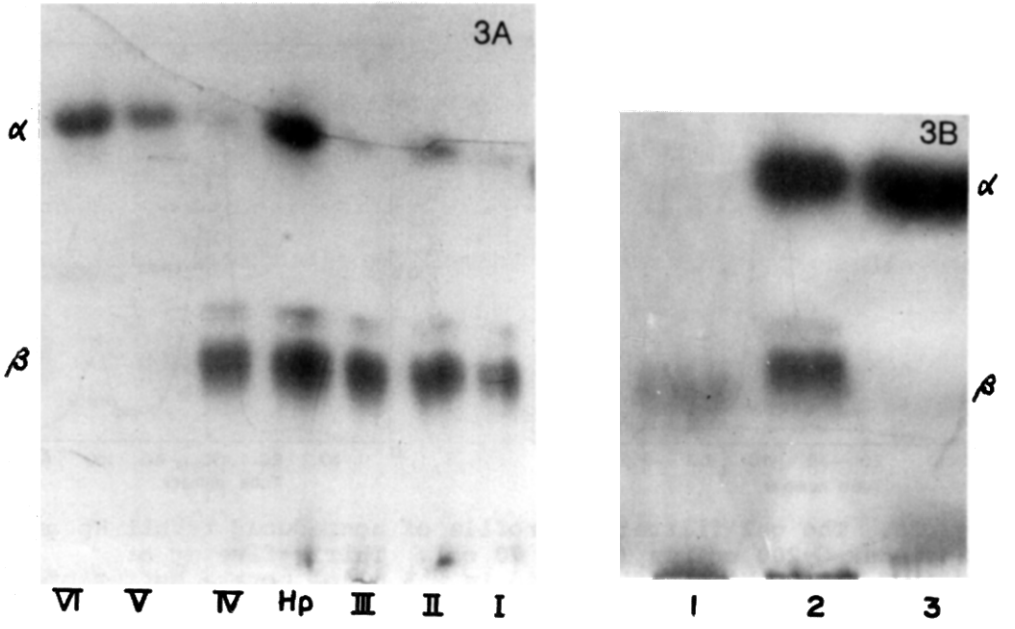

Fig. 3a. Acid urea-starch-gel electrophoretic patterns of the fractions obtained from nonreduced rabbit Hp shown in Fig. 2a. Before electrophoresis each fraction was reduced with -mercaptoethanol in borate buffer of pH 8.9. The middle slot is the control reduced sample before gel filtration. Fraction I seems to be intact Hp molecule from the elution volume, and it is clear that fraction V and VI are α-chains and fraction III and IV are chiefly β-chains. Fraction II seems to be dissociated products smaller than intact Hp. Fraction III was contaminated with fraction II. Each fraction was also checked without reduction by acid urea-starch-gel or SDS-polyacrylamide gel electrophoresis, and it was found that fraction V migrated slower than fraction VI, which is well in accord with the elution volume in gel filtration. Hence, fraction V would be α-chain dimer.

Fig. 3b. Acid urea-starch-gel electrophoretic patterns of the fractions obtained from reduced and alkylated rabbit Hp shown in Fig. 2b. The first peak was β-chain (slot 1), and the second minor peak was α-chain (slot 3). Slot 2 is the control reduced and alkylated rabbit Hp sample before gel filtration.

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(9). The faster mobility of rabbit β-chain than human β-chain in SDS polyacrylamide gel indicates that it might have smaller molecular size than human β-chain (40,000) (3). From the content of β-chain in rabbit Hp (Fig. 2b), the molecular weight of rabbit β-chain would be about 25,000. Likewise, the

molecular weight of rabbit α-chain would be around 10,000. Hence, the subunit composition of rabbit Hp will be $\alpha_2\beta_2$ as human Hp 1-1 (3), swine and canine Hps (4), or equine Hp (5).

The α- and β-chains in human, swine, canine and equine Hps are all alike held together by disulfide bonds (2-5, 10). Hence, without cleavage of the disulfide bridges, one can not isolate each chain from these Hps. Rabbit Hp, on the other hand, was dissociated into subunits only in the presence of urea or SDS without disrupting the disulfide bridges (Fig. 2a), since free α- and free β-chain were identified (Fig. 3a). Therefore, it can be said that rabbit Hp subunits are held together by noncovalent bonds. This is reminiscent of various hemoglobin molecules and $Am_2(+)$ genetic type of human IgA_2 globulin which is lacking in inter-heavy and light chain disulfide bonds (11).

Anyhow rabbit Hp has similar Hb-binding property and quaternary structure to other animal Hps even though it lacks in interchain disulfide bonds. This implies that interchain disulfide bonds of Hp are not essential either for the assembly of the molecule from their constituent chains or for the biological activity.

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