IMMUNOLOGICAL AND BIOCHEMICAL STUDIES ON SERUM HAPTOGLOBIN*

By BONG-SOP SHIM,‡ M.D., AND ALEXANDER G. BEARN, M.D.

(From The Rockefeller Institute)

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Haptoglobin is an α_2-protein present in the serum of many species which has the specific capacity to bind hemoglobin stoichiometrically *in vivo* and *in vitro* (1–3). In 1955, Smithies (4, 5) reported the existence of genetically determined variations in haptoglobin using starch gel electrophoresis. Two haptoglobin alleles designated Hp^1 and Hp^2 determined the three common haptoglobin phenotypes Hp 1-1, Hp 2-1, and Hp 2-2. Additional genetical heterogeneity in the haptoglobin 1-1 phenotype was observed by Connell and his collaborators (6) when, following reductive cleavage of the haptoglobin, electrophoresis was performed in urea-mercaptoethanol-formate gels. The pattern obtained enabled the Hp 1-1 phenotype to be subclassified into Hp 1F-1F, Hp 1S-1S, Hp 1F-1S. In addition Hp 2-1 individuals may be of type Hp 2-1S or Hp 2-1F. Reduction appears to cleave the haptoglobin molecule into two types of chains which have been labeled the α- and β-chains. Only the α-chain varies in the six haptoglobin phenotypes.

Family studies (7) indicated that all the phenotypes are controlled by three co-dominant alleles designated Hp^{1S}, $Hp,^{1F}$ and Hp^2. Chemical studies on the isolated α-chains from each type of haptoglobin (8) led to the development of a hypothesis for evolution of the haptoglobin genes and predicted the existence of certain other alleles belonging to Hp^2 group (9). These predictions were confirmed when certain of the postulated alleles were recognized in a Brazilian population (10). Thus far, five normal haptoglobin alleles have been found (Hp^{1S}, Hp^{1F}, $Hp^{2(FS)}$, Hp^{2FF}, Hp^{2SS}).

The molecular structure underlying the polymorphism of each genetic type of haptoglobin has been examined by several investigators using a variety of physicochemical and immunological techniques (1, 11–18). A recent report on the occurrence of antigenic differences among human haptoglobins (19) suggested that further immunochemical studies might add to the understanding of the molecular differences in the genetically determined variations in the haptoglobin molecule. The present paper reports chemical and immunochemical studies on human and non-human primate haptoglobin which provide additional information on the properties and structure of the haptoglobin 1-1 molecule.

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‡ Present address: Catholic Medical College, Seoul, Korea.

Materials and Methods

Serum from human and non-human primates was kept frozen until immediately before use. Three of the sera which showed immunologically atypical reactions were kindly supplied by Dr. L. Korngold, The Hospital for Special Surgery, the New York Hospital–Cornell Medical Center, New York. The plasma for the purification of type 1-1 haptoglobin was obtained from Knickerbocker Blood Bank, New York, after preliminary haptoglobin typing by starch gel electrophoresis.

Starch Gel Electrophoresis.—Vertical starch gel electrophoresis was performed as described by Smithies (20). Subtyping of haptoglobin and the identification of isolated α- and β-polypeptide chains of haptoglobin was performed in a urea-formate gel after reduction by mercaptoethanol essentially as described by Smithies and coworkers (7). However, the pH of the buffer in the gel was raised to pH 3.1, and electrophoretic separation was carried out at 13 v/cm for 6 hours in the cold (+4°C) (21).

Isolation of Type 1-1 and Type 2-1 Haptoglobin.—Purified haptoglobin was prepared from 200 ml of normal plasma using the procedure described by Connell and Shaw (22). Small amounts of contaminating proteins were removed by starch block electrophoresis in barbital buffer pH 8.6, ionic strength 0.1 (23) and by gel filtration on sephadex G-200. Sephadex G-200 columns (3 x 65 cm) were equilibrated with tris (0.1 M)–HCl buffer pH 8.0, containing 1.0 M sodium chloride. Gel filtration (24) was carried out at +4°C with the same tris buffer with a flow rate of 4.0 ml/hour.

3-ml fractions were collected and the effluent protein determined by measuring the optical density at 280 mμ in the Beckman spectrophotometer. Individual effluent fractions were characterized by starch gel electrophoresis (20) and microimmunoelectrophoresis as described by Scheidegger (25) using horse anti-human antiserum. The gel filtration eliminated most of the contaminants with a molecular weight greater than haptoglobin 1-1. Following gel filtration on sephadex G-200, the haptoglobin-rich peak was divided into three fractions, and each concentrated fraction was again passed through sephadex G-200. The second filtration eliminated the remainder of the larger molecular weight substances and a number of small molecular weight substances which migrated in the postalbumin region in starch gel electrophoresis. The immunoelectrophoretically pure haptoglobin fractions were pooled, dialyzed against distilled water for 48 hours, lyophilized, and stored at −20°C. The isolated type 1-1 haptoglobin was found to be 1F-1S. The recovery was estimated to be approximately 60 per cent. Purification of type 2-1 haptoglobin was performed by a similar procedure.

Immunologic Techniques.—Specific antisera against type 1-1 haptoglobin were obtained by immunizing four New Zealand albino rabbits with isolated type 1-1 haptoglobin in complete Freund's adjuvant (Difco Laboratories, Inc., Detroit) according to the method described by Mills (26). 8 mg of lyophilized type 1-1 haptoglobin was dissolved in 2.0 ml of 0.9 per cent saline and emulsified with 2.0 ml of complete Freund's adjuvant. 0.1 ml of this mixture was injected into each foot-pad of four rabbits. 1 week later another injection was given, and 2 weeks from the second injection, the rabbits were bled from the central ear artery. The antisera contained antibodies against minor components, which were removed by adding 0.3 ml of ahaptoglobinemia serum per 1.0 ml of antiserum. 50 microliters of a saturated solution of phenylmercuric nitrate was added as a preservative to each ml of the absorbed antiserum. The equivalence ratio of absorbed antiserum to antigen (normal human serum) was approximately 2 to 1. The antisera were stored at +4°C.

Microimmunoelectrophoresis was performed according to Scheidegger (25) and starch gel immunoelectrophoresis by the method described by Poulik (27). The double gel diffusion technique was performed by the method of Ouchterlony (28) or by a modification of the micro method described by Wadsworth (29).

Isolation of α- and β-Polypeptide Chains of Type 1-1 Haptoglobin.—35.0 mg of the purified

lyophilized haptoglobin 1-1 was dissolved in 1.0 ml of a borate buffer mixture (prepared by adding 2.0 ml of 0.5 M boric acid–0.2 M NaOH buffer, 4.0 ml of deionized water, 4.8 gm urea, and 0.2 ml of 2-mercaptoethanol, Matheson, Coleman and Bell, East Rutherford, New Jersey, bp 153–157°C). Final concentration was equivalent to 8 M urea and 0.5 M mercaptoethanol. After 1 hour at room temperature, iodoacetamide (Aldrich Chemical Co., Inc., Milwaukee) was added to a final concentration of 1 M, and the mixture left in the dark for 1 hour. The samples were immediately applied to a sephadex G-75 column (2 x 60 cm) previously equilibrated with 1 M acetic acid. Gel filtration was carried out at +4°C with a flow rate of 4.0 ml/hour with 1 M acetic acid solution. Approximately 2.5 ml fractions were collected and the protein determined by measuring the optical density at 680 mμ by the modified Folin-Ciocalteu procedure. After separation each peak was pooled or divided into several parts and concentrated by ultrafiltration or sephadex G-25. Each chain was identified by urea-gel electrophoresis as described above. For immunological studies on the α- and β-chains, the Hp 1-1 was reduced with 0.1 M mercaptoethanol in the absence of urea at +4°C overnight and then alkylated with iodoacetamide (final concentration 0.15 M) for 3 hours at +4°C. After overnight dialysis against 0.5 M propionic acid, gel filtration was performed on a G-75 column.

Sialic Acid Analysis.—Sialic acid analysis on the fractionated α- and β-chains was performed using the modified Bial orcinal reaction (30, 31). Treatment of the purified haptoglobin with neuraminidase was performed by incubating purified type 2-1 haptoglobin at 37°C for 48 hours with neuraminidase (Behringwerke, A. G., Marburg, West Germany) to give 2 mg/ml concentration. Treatment of serum with neuraminidase was performed in a similar fashion except that 0.4 ml of neuraminidase solution was added to 0.2 ml of serum.

High Voltage Paper Electrophoresis of Tryptic Digest of Type 1-1 Haptoglobin.—10 mg of lyophilized type 1-1 haptoglobin was dissolved in 4 ml of deionized water and denatured with heat in a 100°C water bath for 15 minutes. Using an automatic pH-stat (Radiometer, Copenhagen, Denmark), the protein solution was adjusted and maintained at pH 8.0 with 0.1 NaOH. Trypsin (1 mg) was added, and the reaction was carried out for 1 hour and 45 minutes under a nitrogen atmosphere. After digestion the pH was adjusted to 3.5, and the solution was centrifuged at 25,000 RPM for 45 minutes. After concentrating the digest *in vacuo*, electrophoresis was performed with pyridine:acetic acid:water buffer (1:10:189), pH 3.5 in a varsol tank at 40 v/cm. After electrophoresis a guide strip was cut from the main paper and stained with 0.2 per cent ninhydrin in acetone and with a sulfur reagent (platinic iodide). The remaining major part of the strip was exposed overnight to performic acid vapor at room temperature to cleave the disulfide bonds. The paper was dried under a fume hood and in a vacuum desiccator before it was sewed (using a domestic sewing machine) on a new piece of Whatman 3 MM paper. The second electrophoretic separation was carried out in the same buffer at right angles to the first separation. Peptides cleaved by oxidation migrated off the resulting diagonal line of unchanged peptides.

RESULTS

Immunologic Studies on Human and Non-Human Primate Haptoglobin.—The immunoelectrophoretic pattern of the purified type 1-1 haptoglobin is illustrated in Fig. 1. This preparation also gave a single band in the starch gel system. The reaction of haptoglobin antiserum with various normal haptoglobin types is shown in Fig. 2. Each genetic type of haptoglobin, including subtypes, showed complete reaction of interference against rabbit anti-human Hp 1-1 serum. The reaction of atypical haptoglobins will be described later. In agreement with the findings of Korngold and van Leeuwen (19, 32), differences in

the position and curvature of the precipitin zones among each type of haptoglobin were observed depending upon the molecular size.

During an immunological survey of a Nigerian population by the double gel diffusion method, several instances of an immunologically atypical haptoglobin

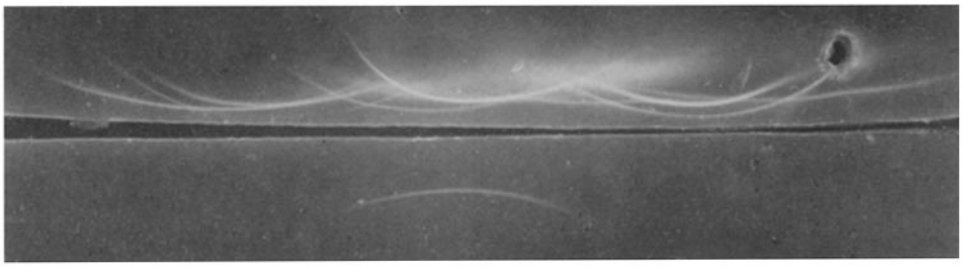

FIG. 1. Immunoelectrophoretic pattern of the purified type 1-1 haptoglobin. Upper pattern, type 1-1 serum; lower pattern, purified type 1-1 haptoglobin preparation.

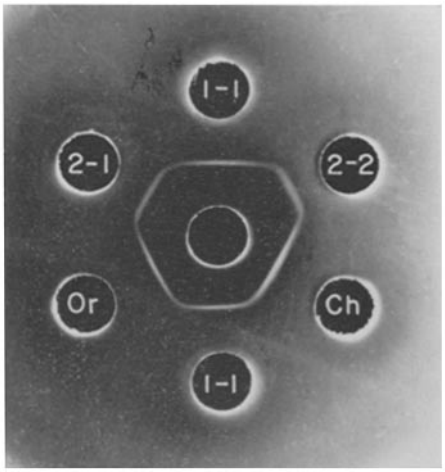

FIG. 2. The reactions of anti–Hp 1-1 serum (central well) with human type 1-1, 2-1, and 2-2 haptoglobin; chimpanzee (*Ch*) and orangutan (*Or*) haptoglobin. Chimpanzee and orangutan showed identical reaction with human haptoglobin.

were observed. These immunologically atypical haptoglobins showed spur formation between normal haptoglobin which appeared independent of the genetic type of the atypical haptoglobin. These observations indicated a difference in antigenic determinants in the atypical haptoglobin molecule (Fig. 3), in agreement with the results of Korngold (19), who used a rabbit anti-human Hp 2-2 serum. The atypical haptoglobins showed a completely normal pattern in conventional starch gel electrophoresis (Fig. 4) except in those cases

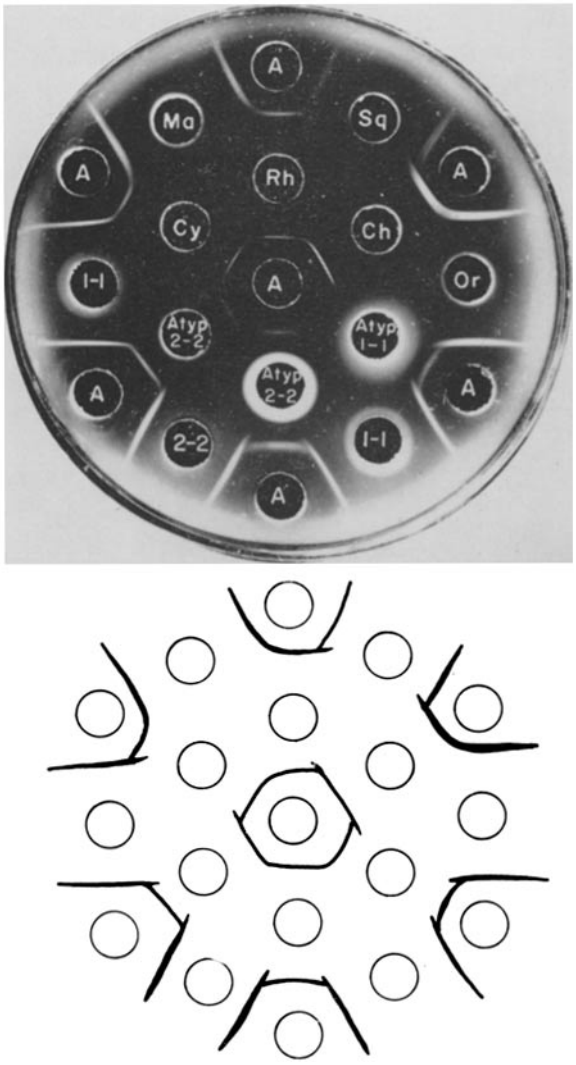

FIG. 3. The reactions of anti-Hp 1-1 serum (*A*) with normal, atypical human haptoglobins, and various non-human primate haptoglobins. Spur formation could be seen between normal 1-1 or 2-2 and atypical human haptoglobins (atypical 1-1 or atypical 2-2). *Ch*, chimpanzee; *Cy*, cynomolgus; *Ma*, short tail macaque; *Or*, orangutan; *Rh*, rhesus; *Sq*, squirrel.

in which the haptoglobin concentration was too low or the protein denatured. Urea-formate starch gel electrophoretic patterns were also normal, provided the haptoglobin level in the serum was sufficient for subtyping. It is thus apparent that the rabbit anti-human Hp 1-1 serum can also be used for the detection of atypical haptoglobin (19).

In some samples of atypical haptoglobin the antigenicity was very weak (Fig. 5). Furthermore, one sample of atypical 1-1 type haptoglobin seemed to be more different in antigenic determinants than the chimpanzee and orangutan haptoglobins tested (Fig. 3).

Absorption of rabbit anti-human Hp 1-1 (1F-1S) serum with 1S-1S, 1F-1S or 1F-1F type serum removed the reactivity with human haptoglobin. Antisera absorbed with chimpanzee, orangutan, and rhesus monkey serum still retained the reactivity toward normal and atypical human haptoglobins even though the antiserum absorbed with rhesus monkey serum lost its reactivity toward chimpanzee and orangutan haptoglobin.

The sera from non-human primates in the present study reacted with rabbit anti-human Hp 1-1 serum to a differing degree. Although chimpanzee and orangutan haptoglobin showed reactions identical with human haptoglobin

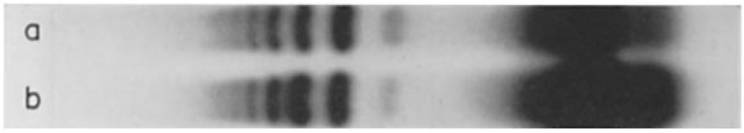

FIG. 4. Starch gel electrophoretic pattern of immunologically atypical 2-2 haptoglobin. (Borate buffer, pH 8.9; benzidine staining for haptoglobin-hemoglobin complex). (*a*) Normal 2-2 type. (*b*) Atypical 2-2 type.

in Ouchterlony plates (Fig. 2), haptoglobin obtained from other monkeys differed from human haptoglobins. Chimpanzee and orangutan showed reactions of identity with cynomolgus monkey. Orangutan showed a reaction of identity with rhesus monkey but not with the short tail macaque and squirrel monkey (Fig. 6). These antigenic interrelationships are summarized in Table I.

Antigenic Properties of Modified Human Haptoglobin and Isolated α- and β-Chains.—In agreement with results obtained by earlier workers, it was found that the binding of haptoglobin with hemoglobin (33, 34) and the treatment of haptoglobin with neuraminidase (35–37) did not effect the antigenicity of the haptoglobin molecule. Incomplete digestion of the purified haptoglobin with trypsin without heat denaturation completely abolished the precipitability by antiserum. Brief treatment (10 minutes) with 8 M urea and 0.1 M mercaptoethanol at room temperature altered the precipitin line as shown in Fig. 7. A new, weak precipitin line nearer to the antiserum well was observed which raised the possibility of an immunologically reactive smaller molecular subunit (Fig. 8). The same phenomenon was observed when purified haptoglobin or whole serum was treated with 0.1 M mercaptoethanol in the absence of urea and later acidified to pH 2.0 with acetic acid or alkylated with iodoacetamide (final concentration 0.2 M) in borate buffer, pH 8.9.

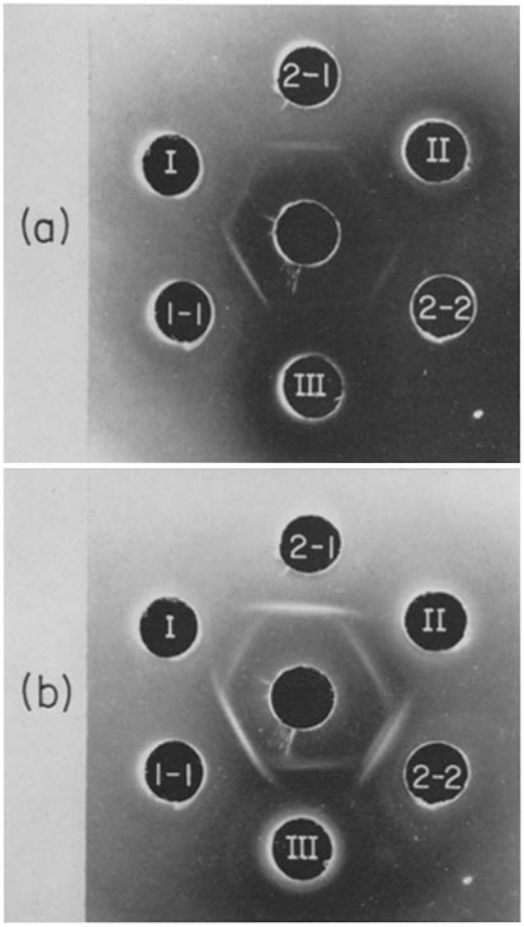

FIG. 5. The reactivity of atypical human 1-1 type haptoglobin with anti-Hp 1-1 serum. 100 μl of antiserum was applied to the central well, and 30 μl of normal 1-1, 2-1, and 2-2 type serum was applied to each well. 30, 50, and 100 μl of same atypical 1-1 type haptoglobin serum were applied to well *I*, *II*, and *III* respectively. Photographs were taken after (*a*) 48 and (*b*) 96 hours.

Following urea gel electrophoresis (7, 21) of purified reduced and alkylated haptoglobin, the gel was sliced into two pieces, and the bottom slice was stained with amidoblack to visualize the location of α- and β-chains. The starch gel immunoelectrophoretic technique of Poulik (27) was employed for the localization of the antigenic determinants in the haptoglobin molecule. Using this technique, distinct precipitin zones were observed in the β-chain region after 3 to 4 days. No clear cut precipitin zone could be recognized in the α-chain area even after standing over 1 week. The failure to obtain a precipitin line with the α chain may have been due to antibody excess.

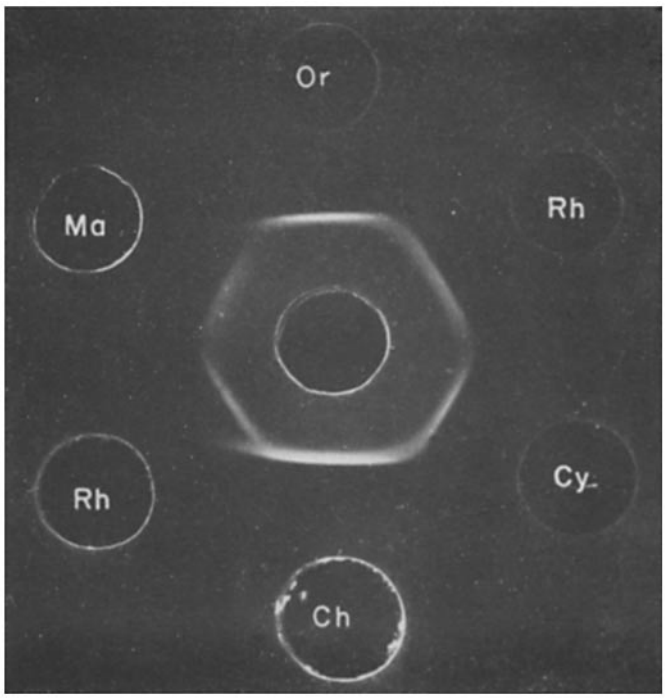

FIG. 6. The reaction of non-human primate haptoglobins with rabbit anti-human Hp 1-1 serum. Spur was seen between chimpanzee (*Ch*) and rhesus monkey (*Rh*), between orangutan (*Or*) and short tail macaque monkey (*Ma*), but not between rhesus and short tail macaque monkey. *Cy*, cynomolgus monkey.

TABLE I

Antigenic Relationships of Human and Non-Human Primates' Serum Haptoglobin as Revealed by Rabbit Anti-Human Haptoglobin (Type 1-1) Serum

	Human	Chimpanzee	Orangutan	Cynomolgus	Rhesus	Short tail macaque	Squirrel
Human	−	−	−	+	+	+	+
Chimpanzee	−	−	−	−	+	+	+
Orangutan	−	−	−	−	−	+	+
Cynomolgus	+	−	−	−	−	−	−
Rhesus	+	+	−	−	−	−	+
Short tail macaque	+	+	+	−	−	−	−

−, complete fusion; +, spur formation.

The antigenic properties of the isolated α- and β-polypeptide chains from type 1-1 haptoglobin were destroyed after reductive cleavage with 8 M urea and 0.1 M mercaptoethanol. Loss of immunologic activity in the presence of urea has also been reported with γ-globulin (38–40). However, reduction of type

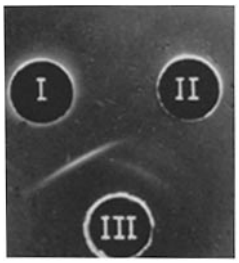

FIG. 7. The reactivity of reduced haptoglobin with anti–Hp 1-1 serum. *I*, type 1-1 serum; *II*, purified type 1-1 haptoglobin treated with 8 M urea and 0.1 M mercaptoethanol for 10 minutes; *III*, anti-serum. A fast, diffuse precipitin line could be recognized in addition to the weak line in the position corresponding to the control serum.

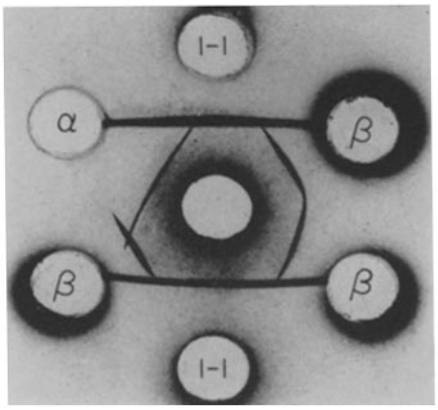

FIG. 8. Immunological reactions of α- and β-polypeptide chains of type 1-1 haptoglobin isolated by gel filtration with sephadex G-75 column after reductive cleavage with 0.1 M mercaptoethanol followed by alkylation with iodoacetamide in the absence of urea. Both β- and α-chains have antigenic determinants. The photograph has been retouched to bring out the essential features.

1-1 haptoglobin with 0.1 M mercaptoethanol in the absence of urea retains the immunologic activity of α- and β-chain; both α- and β-chains showed partial identity with the intact haptoglobin 1-1 molecule. However, both chains showed a weaker activity than the intact molecule (Fig. 8). Thus far, recombination of the α- and β-chains has been unsuccessful.

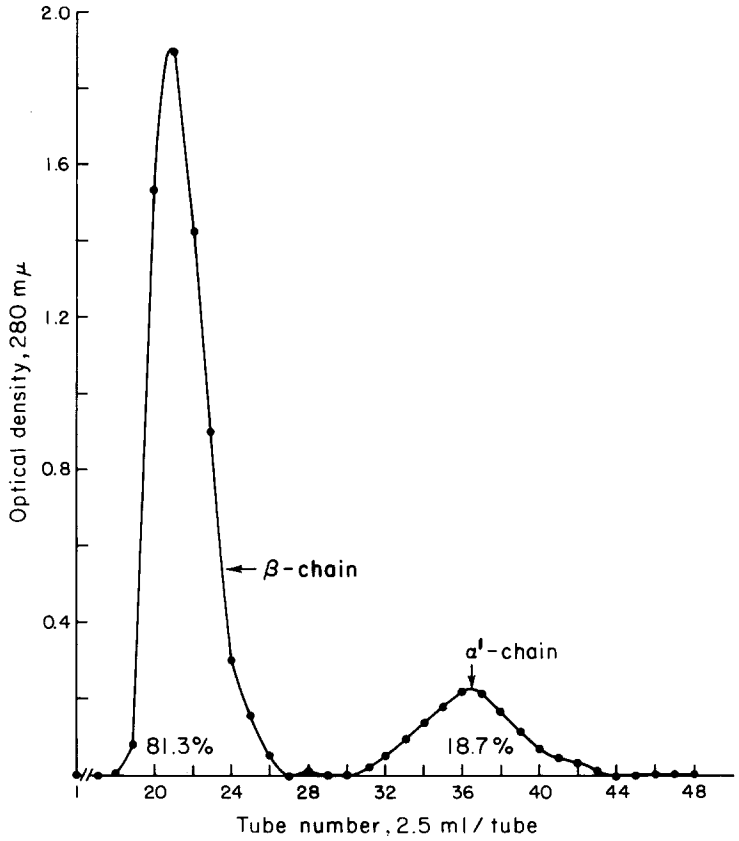

FIG. 9. Gel filtration of reduced and alkylated type 1-1 haptoglobin with sephadex G-75. First peak contained β-chains and the second minor peak contained α-chains.

Isolation of α- and β-Polypeptide Chains from Type 1-1 Haptoglobin and Some Chemical Studies.—The α- and β-polypeptide chains of type 1-1 haptoglobin were isolated by gel filtration on sephadex G-75 column following reductive cleavage of the disulfide bonds with 0.1 M mercaptoethanol in the presence of 8 M urea and alkylation with iodoacetamide (Fig. 9). The main peak was divided into four subfractions and the second peak pooled. After concentration of each fraction with sephadex G-25, urea gel electrophoresis was performed to identify

the α- and β-chains (Fig. 10). The main peak is composed of β-chains and the second minor peak composed of α-chains. The α-chains comprised 18.7 per cent of total as estimated by absorbancy at 280 mμ and 19.1 per cent as judged by the modified Folin reaction.

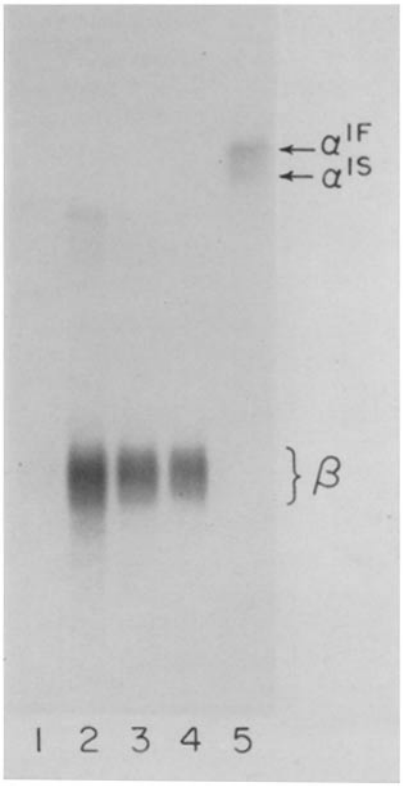

FIG. 10. Urea gel electrophoresis (formate buffer, pH 3.1) of the isolated β- and α-polypeptide chains of type 1-1 haptoglobin by gel filtration. The main peak (see Fig. 9) was divided into four fractions (*1*, *2*, *3*, and *4*) and minor peak was pooled (*5*). α-Chain composed of α^{1F}- and α^{1S}-chains.

Analysis for sialic acid by the modified orcinol reaction (31) indicated that each fraction of β-chain contained 7.3 to 7.6 per cent sialic acid. No sialic acid was detectable in the α-chain. Confirmatory results were obtained by urea gel electrophoresis of the purified 2-1 type haptoglobin before and after treatment with neuraminidase when the β-chain migrated more rapidly toward the cathode after enzymatic removal of the negatively charged sialic acid residues, although the mobility of the α-chain remained unchanged (Fig. 11). These results indicate that sialic acid is present only in the β-chain.

High voltage paper electrophoresis of the tryptic digest for type 1-1 haptoglobin with pyridine:acetic acid:water buffer, pH 3.5, gave at least twenty ninhydrin-positive spots migrating toward the cathode in addition to material at the origin. Four peptides migrating toward the cathode showed a positive sulfur stain with platinic iodide. After oxidation of the same unstained paper strip with performic acid vapor overnight, the second dimensional electrophoresis at right angles to the first direction was performed with the same buffer to disclose additional peptides produced by oxidation. Nine peptides were altered by oxidation among which four showed more than one new peptide; the others showed only one new peptide.

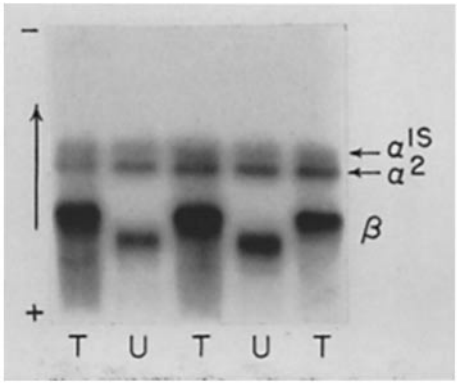

FIG. 11. Urea gel electrophoretic patterns of purified haptoglobin 2-1 before and after treatment with neuraminidase for 24 hours at +37°C. *U*, untreated control sample; *T*, neuraminidase-treated sample.

DISCUSSION

Burtin and coworkers (41) were the first to demonstrate that haptoglobins are good antigens. It seemed possible that the various genetic types of haptoglobin differing in their physicochemical characteristics might show different antigenic properties. Some attempts have been made to demonstrate such an antigenic difference using antisera against isolated haptoglobins by Fine and others (42), or using antisera against the haptoglobin-hemoglobin complex by Bearn and Franklin (12). In the present study no difference in the antigenic properties of each genetic subtype against the present rabbit human Hp 1-1 serum was observed. Moreover, normal human haptoglobin showed a reaction identical with chimpanzee and orangutan haptoglobin, species known to have an α-chain which is clearly different from the human α^1-chain (21). It has been shown by Smithies and coworkers (9) that the α^{1S}-chain differs from the α^{1F}-chain by one amino acid. The present study indicates that both have common

antigenic determinants. It was further demonstrated that α- and β-chains have distinct antigenic determinants (Fig. 8). Recently, Korngold (19) has suggested that 2-2 and 2-1 type haptoglobins have more antigenic determinants than haptoglobin 1-1. The only difference between haptoglobin 2-2 and 1-1 thus far known is the difference in the α-chain. The present study indicates that the α-chain also has antigenic determinants. The present anti-Hp 1-1 serum does not contain antibodies toward the additional determinants in the α^2-chain. This explains why the present antiserum does not form a spur between 1-1 and 2-2 types.

Korngold recently (19) described some immunologically atypical haptoglobins which lacked certain antigenic determinants found in the usual 1-1 type haptoglobin. The present rabbit anti-human Hp 1-1 serum could not usually reveal antigenic differences among the normal genetic type of haptoglobins. However, using this antiserum it was possible to disclose certain immunologically atypical haptoglobins which were different in antigenic determinants and which were independent of the haptoglobin types. Most of the atypical haptoglobin sera showed normal patterns in conventional starch gel electrophoresis and in urea gel electrophoresis. Absorption experiments of rabbit anti-human Hp 1-1 serum with atypical human haptoglobin and various non-human primates haptoglobin indicate that human haptoglobin has additional human-specific antigenic determinants besides the primates' common determinants. Some of the atypical haptoglobins still showed a spur with normal haptoglobin when tested with antisera absorbed with non-human primate serum.

Goodman (43, 44) has emphasized that it is necessary to use antiserum from very closely related species if small differences in protein structure are to be detected. For example, using chicken antihuman albumin serum, human, chimpanzee, and orangutan albumin showed an identical immunological reaction, whereas with rhesus monkey anti-human albumin serum, gorilla and man were indistinguishable immunologically, while chimpanzee and orangutan clearly differed from man. Similar results were observed with ceruloplasmin, transferrin, and certain other serum proteins. The results of the present experiments enable some tentative phylogenetic relationships between the haptoglobins of closely related species to be depicted (Fig. 12). The results obtained with rabbit antiserum are consistent with those obtained using antisera from more closely related species (43).

Connell and others (6, 8) have demonstrated the existence of two kinds of polypeptide chain in the haptoglobin molecule by electrophoresis in urea gel, a rapidly migrating α-chain and a slowly moving β-chain. However, no information is available on the number of chains in the type 1-1 haptoglobin molecule. The molecular weight of the β-chain is unknown, whereas the molecular weight

of the α^1-chain was estimated to be 8860 ± 400 (9). The molecular weight of type 1-1 haptoglobin was first reported as 85,000 by Jayle and Boussier (1), but more recent ultracentrifugal analysis by Herman-Boussier and coworkers (45) suggest a molecular weight of 100,000. Estimates derived from the known molecular weight of the hemoglobin-haptoglobin complex and hemoglobin combining capacity gave a molecular weight of 89,000. The present analytical data obtained from gel filtration of the reduced and alkylated type 1-1 haptoglobin indicate that 18.7 to 19.1 per cent of type 1-1 haptoglobin molecule is α^1-chain. This suggests the presence of two α^1-chains in the haptoglobin 1-1 molecule. If we assume the molecular weight of the α^1-chain to be 8860 and the molecular weight of haptoglobin to be either 89,000 or 100,000, then the α^1-chain should represent 19.9 or 17.7 per cent of the total haptoglobin molecule respectively.

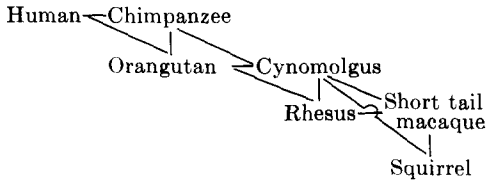

FIG. 12. Diagrammatic illustration of the tentative phylogenetic relationships of human and some non-human primate haptoglobins by the immunologic reactivity with rabbit anti-human Hp 1-1 serum. Those species connected directly with line show identical reactions.

The N-terminal amino acid analysis of the α-chain was shown to be valine by Smithies *et al.* (9), and Smith and coworkers (46) have demonstrated that each genetic type of haptoglobin has equimolar amounts of valine and isoleucine as the N-terminal amino acids. These results indicate that the N-terminal amino acid of the β-chain is isoleucine, and moreover that there exist an equal number of α- and β-chains in each genetic type of haptoglobin. Since the number of α-chains was shown to be two, this would suggest the presence of two β-chains in type 1-1 haptoglobin. According to the most recent amino acid analysis on human type 1-1 haptoglobin (47), 2.3 per cent of the molecule is comprised of half-cystine residues. This corresponds to 16 to 18 residues in haptoglobin 1-1 depending on the assumed molecular weight. It has been shown by Robert *et al.* (48) that about nine sulfhydryl groups could be released in the presence of guanidine hydrochloride. This suggests that three to four disulfide bonds may be present in the intact molecule.

The electrophoretic pattern of the tryptic digest of type 1-1 haptoglobin before and after oxidation suggests the existence of at least three disulfide bonds. One can construct a tentative model in which one disulfide bond connects two symmetrical halves of the molecule, probably between the two β-chains. The

remaining two disulfide bonds are connected between the α- and β-chains. The disulfide containing peptides designated A—S—S—B, C—S—S—B, C—S—S—D, and A—S—S—D should each appear as two new peptides after oxidation and A—SH, B—SH, C—SH, and D—SH each exist as single new peptides after oxidation. The electrophoretic results are in agreement with the above interpretation. The evidence cited, in conjunction with observations published by others, enable a tentative model of the secondary structure of the type 1-1 haptoglobin molecule to be constructed (Fig. 13). It should be emphasized that the number of interchain disulfide bonds and the position of the

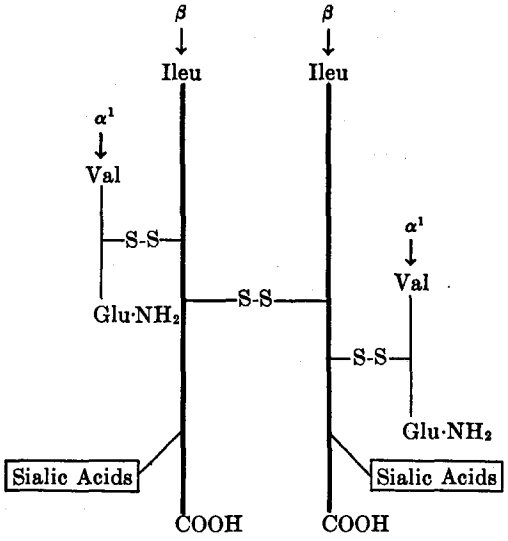

FIG. 13. Tentative model of the secondary structure of human type 1-1 haptoglobulin.

SH groups remain somewhat uncertain and it seems likely that the details of the model will require modification as more information accumulates.

SUMMARY

Antibody to purified type 1-1 human serum haptoglobin was obtained in rabbits. Using this antiserum it was demonstrated that the antigenic determinants of the haptoglobin molecule reside in both α- and β-chains. No immunological difference between the normal α^{1F}- and α^{1S}-chains of haptoglobin could be detected.

A number of healthy individuals whose haptoglobin pattern appeared normal by starch gel electrophoresis were shown to possess a haptoglobin which could be distinguished immunologically from the common haptoglobin types. These

immunological atypical haptoglobins have been observed in individuals of types 1-1, 2-1, and 2-2. Combined immunological and chemical studies of purified haptoglobin have enabled the construction of a tentative model for the structure of human type 1-1 haptoglobin.

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