Purification and Some Properties of the Three Common Genetic Types of Haptoglobins and the Hemoglobin-Haptoglobin Complexes

HIDEO HAMAGUCHI[1]

Human haptoglobin (Hp) exhibits genetic polymorphism determined by allelic genes at an autosomal locus (Smithies 1955; Smithies and Walker 1955, 1956; Smithies et al. 1962*a*). All three common types of Hp consist of two kinds of polypeptide chains, the α and β chains, linked by interchain disulfide bonds, and the genetic variability of Hp is based on structural differences of α polypeptides controlled by the Hp_α locus (Connell et al. 1962; Smithies et al. 1962*a*, 1966). Three common alleles of the Hp locus, Hp^{1F}, Hp^{1S}, and Hp^2, determine the structure of the α chains, and partial gene duplication of the Hp^1 locus was proposed as a mechanism for the origin of the Hp^2 gene (Smithies et al. 1962*b*). Recently, this hypothesis was confirmed by the complete amino acid sequences of the α chains of the common variants of human Hp (Black and Dixon 1968). In addition, Black and Dixon (1968) suggest the structural homology between haptoglobins and 7S immunoglobulins. On the other hand, the β chains of the three common Hp types, which are involved in the binding of hemoglobin (Hb), are similar, possibly identical (Connell et al. 1962; Gordon and Bearn 1966; Cleve et al. 1967; Gordon et al. 1968).

A noteworthy property of Hp is its ability to bind Hb stoichiometrically in vivo and in vitro (Jayle and Moretti 1962). It has been indicated by several investigators that the Hb-binding property of Hp is involved in the biological role of Hp (Allison and Rees 1957; Laurell and Nyman 1957; Murray et al. 1961; Nakajima et al. 1963). Therefore, comparisons of properties among the three common types of the hemoglobin-haptoglobin complexes (Hb-Hp complexes) as well as Hps are considered to be important in clarifying the mechanism maintaining the Hp polymorphism.

In this communication, purification and some properties of the three common genetic types of Hp and the Hb-Hp complexes are described with particular reference to comparisons among the three common types. Data presented here include circular dichroism (CD) spectra in the 200–240 mμ region of Hp; molecular weights of the Hb-Hp 1-1 intermediate and the Hb-Hp 1-1 complex; and, for the three common types of Hb-Hp complexes, heme and nitrogen contents, CD spectra in the Soret region, effects of dithionite, and the rate of autoxidation. From the data obtained, it

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[1] Department of Clinical Genetics, Institute for Medical Genetics, Tokyo Medical and Dental University, 3 Chome, Yushima, Bunkyo-ku, Tokyo, Japan. Present address: Department of Medicine, New York Hospital–Cornell Medical Center, 525 E. 68th St., New York, New York 10021.

is suggested that (1) Hp 1-1 has secondary structures similar to those of gamma globulin and there are significant differences in conformation between Hp 1-1 and Hp 2-2; (2) each genetic type of Hp can bind Hb in the ratio of half a molecule of Hb to one Hp α chain and one β chain; (3) there is a difference in the heme environment or a difference in the tightness of heme attachment, or both, between the Hb-Hp complexes and Hb but no significant difference among the three common Hb-Hp complexes; (4) the heme of each genetic type of Hb-Hp complex is easily degraded by dithionite under aerobic conditions at *p*H 7.0; (5) all three common Hb-Hp complexes have a rate of autoxidation similar to that of the hemoglobin dimers. Some of this work was reported previously in preliminary communications (Hamaguchi 1967, 1968; Hamaguchi and Sasazuki 1967; Hamaguchi, Isomoto, and Nakajima 1969).

MATERIALS AND METHODS

Chemicals

Sephadex G-100, Sephadex G-200, and DEAE-Sephadex A-50 were purchased from Pharmacia, Uppsala. All other reagents were of reagent-grade materials from commercial sources.

Biological Materials

Unhemolyzed adult human blood containing acid-citrate-glucose solution was used as the starting materials. Whole blood was allowed to stand for several days in the cold until the cells were well settled, and the plasma was then aspirated. Individual samples of serum were classified according to haptoglobin type by polyacrylamide gel electrophoresis (Davis 1964; Ornstein 1964).

Preparation of Hb

Human adult oxyhemoglobin was prepared by the method of Drabkin, but without employing $AlCl_3$ (Drabkin 1946). Oxyhemoglobin was then dialyzed against 0.05 M phosphate buffer, *p*H 7.0, for 12 hr. The ferric derivative of Hb was obtained from the oxygenated derivative by addition of 5-fold excess of potassium ferricyanide followed by exhaustive dialysis against 0.05 M phosphate buffer, *p*H 6.5.

Purification of Hp and the Hb-Hp Complexes

All steps of purification were performed at *p*H 7.0, at 3°–4° C.

1. Partial purification of Hp. Batches of 150–200 ml of plasma were fractionated using a saturated solution of ammonium sulfate, adjusted to *p*H 7.0 with concentrated sodium hydroxide. The precipitate obtained between 0.40–0.60 saturation was dissolved in a minimum volume of distilled water by adding a small amount of distilled water and by dialyzing against distilled water for one hour. Approximately 24 ml of solution was usually obtained and the solution was passed through a Sephadex G-100 column, 5 × 75 cm, packed and equilibrated with 0.05 M phosphate buffer, *p*H 7.0. When each fraction was tested for Hb-binding activity by polyacrylamide gel electrophoresis, 120–140 ml of the first fraction was active in every Hp type. This fraction was applied to a DEAE-Sephadex column, 3.2 × 25 cm, equilibrated with

0.05 M phosphate buffer, *p*H 7.0. After the column was washed with 600 ml of 0.08 M phosphate buffer, *p*H 7.0, in the case of Hp 1-1 and 2-1, and the same volume of 0.09 M phosphate buffer, *p*H 7.0, in the case of Hp 2-2, partially purified Hp was eluted with 0.12 M phosphate buffer, *p*H 7.0.

2. Purification of Hp. The partially purified Hp solution was diluted with equal amounts of distilled water to give a buffer concentration of approximately 0.06 M. It was then applied to a DEAE-Sephadex column, 3.2 × 20 cm, equilibrated with 0.05 M phosphate buffer, *p*H 7.0, and rechromatography was performed in the same way as described above.

3. Purification of the Hb-Hp complexes. In order to obtain the solution containing the Hb-Hp complex, approximately 500 mg of oxyhemoglobin was added to the partially purified Hp solution and then a 1:2 dilution of the sample in distilled water was prepared. The solution thus obtained was applied to a DEAE-Sephadex column, 3.2 × 20 cm, equilibrated with 0.05 M phosphate buffer, *p*H 7.0, and the column was washed with 200 ml of the same buffer. Free Hb was passed through this column. The Hb-Hp 1-1 and the Hb-Hp 2-1 complexes were eluted with 0.08 M phosphate buffer, *p*H 7.0, and the Hb-Hp 2-2 complex was eluted with 0.09 M phosphate buffer, *p*H 7.0.

4. Purification of the Hb-Hp 1-1 intermediate. Oxyhemoglobin was added to the partially purified Hp 1-1 solution to 40% saturation of its hemoglobin binding capacity (HBC). The solution was diluted with equal amounts of distilled water and then applied to a DEAE-Sephadex column, 3.2 × 27 cm, equilibrated with 0.05 M phosphate buffer, *p*H 7.0. Elution was performed with 0.08 M phosphate buffer, *p*H 7.0. The fraction containing the Hb-Hp 1-1 intermediate was determined by polyacrylamide gel electrophoresis. When needed, concentration of each purified sample was achieved by applying it to a DEAE-Sephadex column, 2.5 × 5 cm, equilibrated with 0.05 M phosphate buffer, *p*H 7.0, after the dilution of the sample with distilled water to give the buffer concentration of approximately 0.05 M and then by eluting with 0.2 M phosphate buffer, *p*H 7.0. Every purified sample was dialyzed against 0.05 M phosphate buffer, *p*H 7.0, for 12 hr. The ferric derivative of the Hb-Hp complexes was obtained from the oxygenated derivative as described for the preparation of hemoglobin.

Analytical Methods

Sedimentation patterns were obtained using the Hitachi model UCA-1A analytical ultracentrifuge with a rotor speed of 55,430 rpm at 10° C. Sedimentation equilibrium experiments were carried out on a Beckman model E analytical ultracentrifuge, equipped with the standard schlieren optical system, by the short-column equilibrium techniques of Yphantis (1960). Temperature was maintained at 20° C on all runs. Molecular weight determinations were made on the sample dissolved in 0.05 M phosphate buffer, *p*H 7.0, at 6166 rpm. For molecular weight calculations, the partial specific volume of proteins was assumed to be 0.750. The CD measurements were performed at 20° on a Jouan dichrograph. A cell with a path length of 0.5 mm and Hp concentrations between 0.15 and 0.20 mg/ml were used for the measurements in the 200–240 mμ region. A cell with a path length 1.0 mm and heme concentrations of

the Hb-Hp complexes and Hb between 100 and 150 μM were used for the measurements in the Soret region. The solvent used was 0.05 M phosphate buffer, *p*H 7.0 or *p*H 6.5. Molar ellipticities of Hp are given on a mean residue weight basis taken as 110 for each Hp type. Molar ellipticities of the Hb-Hp complexes and Hb are given on a heme basis.

The experiments for the effect of dithionite on the Hb-Hp complexes and Hb under aerobic conditions were carried out as follows: A minimum amount of dithionite was added to 2 ml of the oxygenated Hb-Hp complexes or Hb in 0.05 M phosphate buffer, *p*H 7.0, and then the reaction mixture in a test tube was shaken gently, exposing it to atmospheric oxygen for one minute. Rates of autoxidation of the oxyhemoglobin-haptoglobin complexes and oxyhemoglobin were measured by following the absorbance at 542 mμ and 578 mμ in 0.05 M phosphate buffer, *p*H 7.0, or in the same buffer in 2 M NaCl. Heme concentrations of samples between 25 and 35 μM were used. Reactions were carried out in a Thunberg-type cuvette filled with oxygen at 1 atm and kept in a 38° water bath. The experiment was terminated by adding sufficient sodium nitrite to convert all the sample to the ferric form.

Polyacrylamide gel electrophoresis was carried out following the procedure of Ornstein and Davis (Davis 1964; Ornstein 1964). The HBC was determined by polyacrylamide gel electrophoresis on mixtures of increasing amounts of Hb with a constant volume of Hp solutions followed by benzidine staining. The magnitude of the differences between the concentration of Hb added varied from 2 to 5 mg/100 ml. Protein estimations were made by the Lowry method (Lowry et al. 1951). The estimation of nitrogen was by the micro-Kjeldahl method. The concentration of heme was determined by spectral analysis after conversion to reduced pyridine-hemochromogen. The concentration of heme was calculated from the absorbance at 557 mμ, using the millimolar extinction coefficient for the reduced pyridine-hemochromogen of 34.7 (Paul et al. 1953). Spectrophotometric measurements were performed either with a Cary 14 recording spectrophotometer or with a Hitachi spectrophotometer model EPU-2A.

RESULTS

Purification

The results of purification of Hp 2-1 and the Hb-Hp 2-1 complex are shown in table 1 and figures 1–4. The results of purification of Hp 2-2 were similar to those of Hp 2-1 and the results of purification of the Hb-Hp 1-1 and 2-2 complexes were similar to those of the Hb-Hp 2-1 complex. However, the yield of purified Hp 1-1 was less than one-half that of Hp 2-1. A typical elution curve for purification of the Hb-Hp 1-1 intermediate by chromatography on a DEAE-Sephadex column was shown previously (Hamaguchi 1967). The purity of each purified sample was tested by polyacrylamide gel electrophoresis. The purity of the Hb-Hp 1-1 intermediate and the Hb-Hp 1-1 complex was also tested by ultracentrifugal analyses. Figures 5 and 6 show the results of polyacrylamide gel electrophoresis. Almost no contaminant is visible in the case of Hp 2-1 and 2-2 and all three common types of the Hb-Hp complexes. In the case of Hp 1-1 and the Hb-Hp 1-1 intermediate, however, minor contaminants are

TABLE 1

TYPICAL RESULTS OF PURIFICATION OF HP 2-1 AND THE HB-HP 2-1 COMPLEX

Fraction	Volume (ml)	Protein Concentration (mg/ml)	Total Protein (mg)	HBC (mg/dl)	Total HBC (mg)	Specific Activity (mg HBC/mg protein)	Yield (%)
Plasma	180	64.0	11,520	123	221	1.9×10^{-2}	100
$(NH_4)_2SO_4$ fractionation and Sephadex G-100	130	4.84	628	112	145	23×10^{-2}	65
First DEAE-Sephadex	220	1.04	229	61	134	58×10^{-2}	60
Second DEAE-Sephadex (purified Hp 2-1)	290	0.48	139	39	113	81×10^{-2}	51
Second DEAE-Sephadex (purified Hb-Hp 2-1)	360	0.68	245	.....	.....	.....	.....

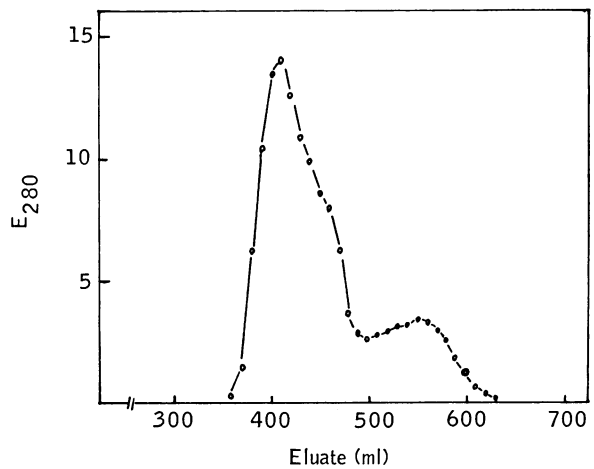

FIG. 1.—The partial purification of Hp 2-1 by gel filtration on a Sephadex G-100 column. Flow rate: 70–90 ml/hour.

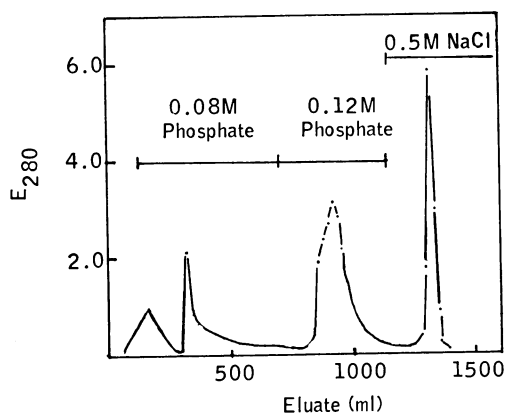

FIG. 2.—The partial purification of Hp 2-1 by chromatography on a DEAE-Sephadex column. Flow rate: 35–50 ml/hour.

visible which did not exceed 10% of the total protein, judging by the staining intensity. The Hb-Hp 1-1 intermediate as well as the Hb-Hp 1-1 complex are homogeneous by ultracentrifugation (fig. 7). Polymers of lower orders of magnitude and higher orders of magnitude of the Hb-Hp 2-2 complex were obtained by applying 1.0 ml of the purified Hb-Hp 2-2 complex to a 2.6 × 88 cm column of Sephadex G-200 equilibrated with 0.05 M phosphate buffer, *p*H 7.0, and by eluting with the same buffer. The eluate was divided into three equal fractions. Polyacrylamide gel electrophoresis revealed the presence of the larger polymers in the first fraction and the smaller polymers in the third fraction, but the separation was not complete (fig. 8).

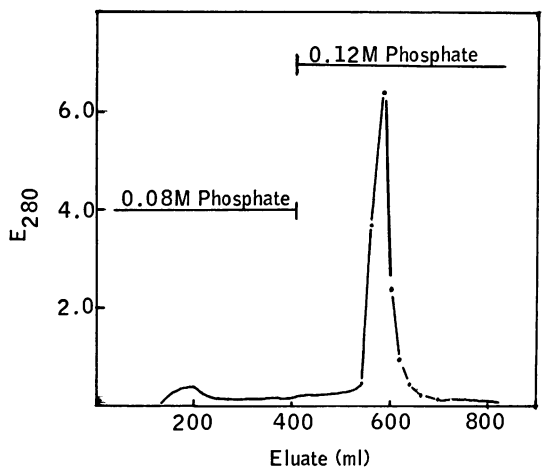

FIG. 3.—The purification of Hp 2-1 by rechromatography on a DEAE-Sephadex column. Flow rate: 40–50 ml/hour.

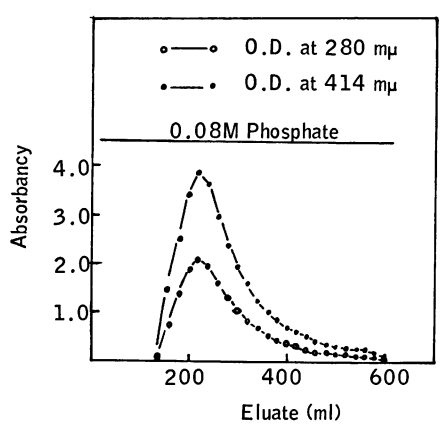

FIG. 4.—The purification of the Hb-Hp 2-1 complex by chromatography on a DEAE-Sephadex column. Flow rate: 40–50 ml/hour.

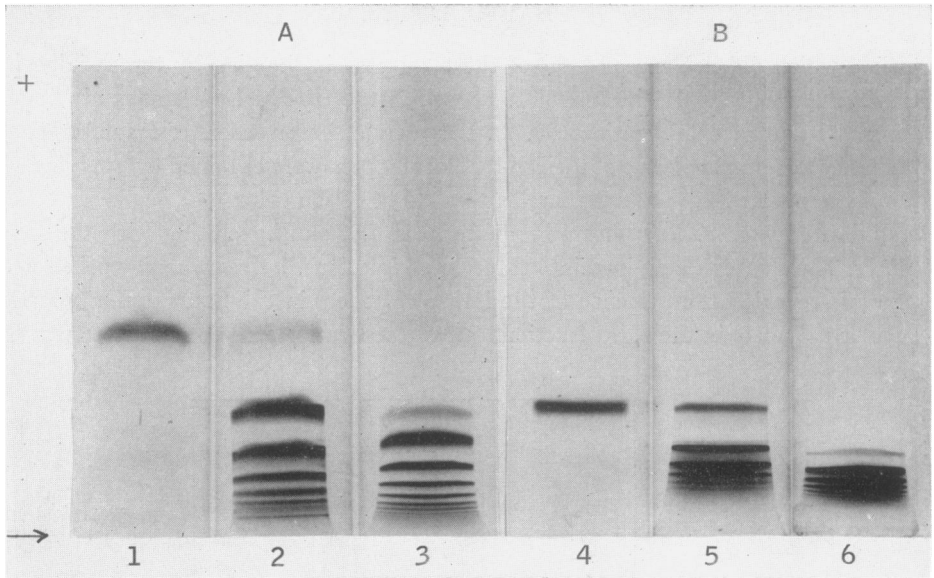

FIG. 5.—Polyacrylamide gel electrophoretic patterns of the purified haptoglobins (*A*) and Hb-Hp complexes (*B*): *1*, Hp 1-1; *2*, Hp 2-1; *3*, Hp 2-2; *4*, Hb-Hp 1-1; *5*, Hb-Hp 2-1; *6*, Hb-Hp 2-2. Amido black 10B staining.

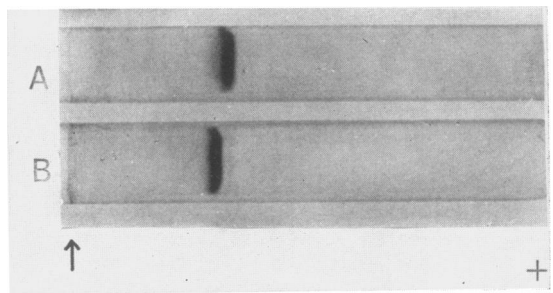

FIG. 6.—Polyacrylamide gel electrophoretic patterns of the purified Hb-Hp 1-1 intermediate (*A*) and Hb-Hp 1-1 complex (*B*). Amido black 10B staining.

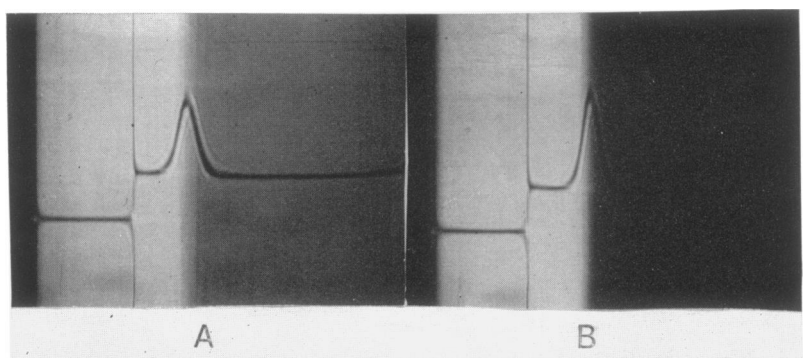

FIG. 7.—Sedimentation patterns of the purified Hb-Hp 1-1 intermediate (*A*) and Hb-Hp 1-1 complex (*B*). *A*, 36 min after starting; *B*, 35 min after starting.

Circular Dichroism of Haptoglobins

Figure 9 shows the CD spectra in the 200–240 mμ region of the three common types of Hp. It is worthy of note that the CD spectrum of Hp 1-1 presented here is very similar to that of gamma globulin from rabbit serum reported by Sarkar and Doty (1966). In addition, the CD behavior of all three common types of Hp suggests the presence of beta structure in human Hp, and the absence of a two-band feature in the CD spectra seems to eliminate the helical form. It must be also pointed out that there is a significant difference in the CD spectra between Hp 1-1 and Hp 2-2, which suggests the presence of some differences in conformation between these two Hp types. The CD spectrum of Hp 2-1 resembles that of Hp 2-2.

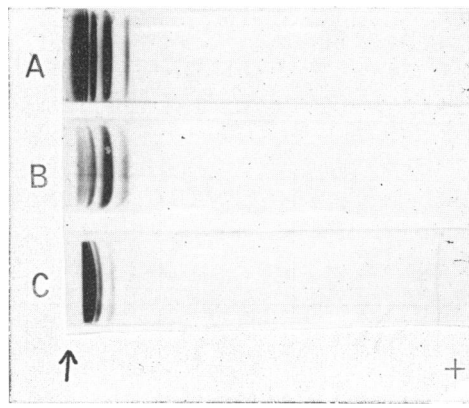

FIG. 8.—Polyacrylamide gel electrophoretic patterns of the separated polymers of the Hb-Hp 2-2 complex. *A*, starting sample; *B*, polymers of lower orders of magnitude; *C*, polymers of higher orders of magnitude. Amido black 10B staining.

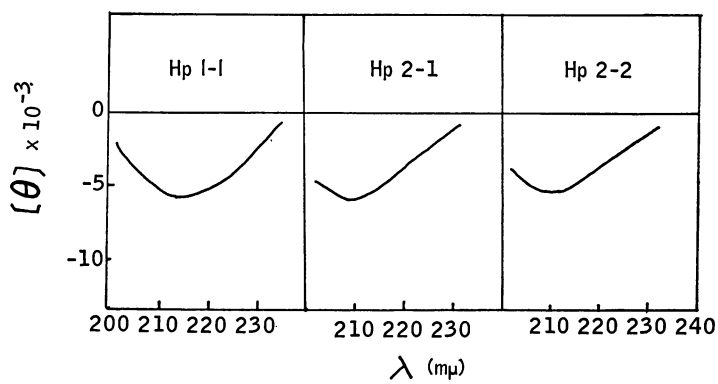

FIG. 9.—Circular dichroism in the 200–240 mμ region of the three common types of haptoglobins in 0.05 M phosphate buffer, *p*H 7.0.

Molecular Weights

Molecular weight determinations were made on solutions with protein concentrations of 3.0 mg/ml and 4.5 mg/ml in the case of the Hb-Hp 1-1 intermediate and 4.2 mg/ml in the case of the Hb-Hp 1-1 complex. The values obtained were 135,000 and 137,000 for the Hb-Hp 1-1 intermediate and 167,000 for the Hb-Hp 1-1 complex. The value of 167,000 for the Hb-Hp 1-1 complex is very similar to the molecular weight of the Hb-Hp 1-1 complex reported previously (Herman-Boussier et al. 1960). The molecular weight of Hp 1-1 has been estimated to be 100,000 (Herman-Boussier et al. 1960). Therefore, the data presented here suggest that the Hb-Hp 1-1 intermediate consists of one molecule of Hp 1-1 and half a molecule of Hb, and that the Hb-Hp 1-1 complex consists of one molecule of Hp 1-1 and one molecule of Hb. These suggestions are also supported by the data on heme and nitrogen contents of the Hb-Hp 1-1 intermediate and the Hb-Hp 1-1 complex (table 2).

TABLE 2

HEME AND NITROGEN CONTENTS OF THE THREE COMMON TYPES OF THE HB-HP COMPLEXES

Substance	Grams of Nitrogen per 2 Moles of Heme	Grams of Nitrogen of Hp Moiety per 2 Moles of Heme	Moles of Heme per 1 Mole of Hp α and 1 Mole of β Chain
Hb-Hp 1-1 intermediate	19,900	14,100	1.0
Hb-Hp 1-1 complex	13,500	7,700	1.8
Hb-Hp 2-1 complex	14,200	8,400	1.9
Hb-Hp 2-2 complex	15,200	9,400	1.8
Polymers:			
Lower orders	15,100	9,300	1.8
Higher orders	15,100	9,300	1.8

NOTE.—Each value represents the average of five to six analyses.

Heme and Nitrogen Contents

Table 2 summarizes the results of estimation of heme and nitrogen contents of the Hb-Hp complexes. The estimated heme and nitrogen contents of the Hb-Hp complexes are given as grams of nitrogen per 2 moles of heme (column 1 in table 2). The grams of nitrogen of the Hp moiety per 2 moles of heme of the Hb-Hp complex are calculated from the values in column 1, table 2, and the theoretical grams of nitrogen per 2 moles of heme of hemoglobin A which are obtained from the data on amino acid sequences of hemoglobin A (col. [2] in table 2). Since Smith et al. (1962) suggest that there exists an equal number of α and β chains in each genetic type of haptoglobin, in order to determine the HBC of the three common types of Hp, the value of moles of heme per one mole of α and one mole of β chain of each genetic type of the Hb-Hp complex was calculated from the values in column (2) in table 2. Calculation in grams of nitrogen of one mole of Hp α chain was based on the data on amino acid sequences of Hp α chains reported by Black and Dixon (1968). Calculation in grams of nitrogen

of one mole of Hp β chain was based on the data on the amino acid analyses of Hp β chains described by Gordon et al. (1968) and the data on carbohydrate contents of Hp reported by Heimburger et al. (1964). The results thus obtained are also presented in table 2, column (3). These results indicate that each genetic type of Hp can combine with Hb in the ratio of half a mole of Hb to one mole of α and one mole of β Hp chains.

Circular Dichroism of the Hb-Hp Complexes

Figure 10 shows the CD spectra in the Soret region, respectively, of the oxygenated and the ferric Hb-Hp complexes and adult Hb. Comparison of the spectra in figure 10 shows that, although the corresponding derivatives of the Hb-Hp complex and adult

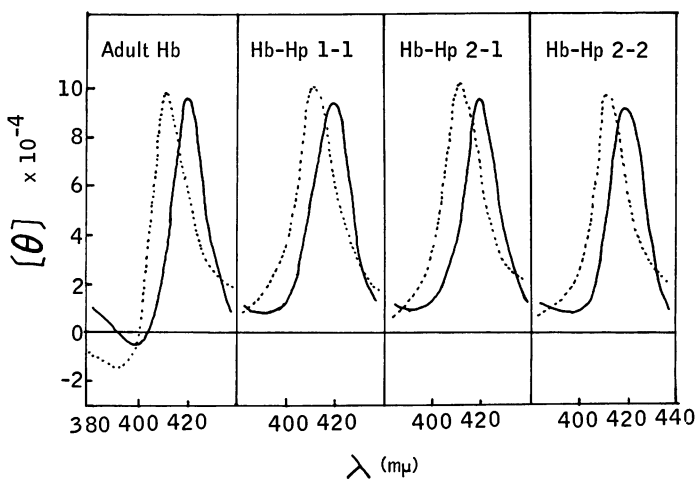

FIG. 10.—Circular dichroism in the Soret region of the three common types of the Hb-Hp complexes and adult Hb in 0.05 M phosphate buffer. The oxygenated derivative (*p*H 7.0): ———; the ferric derivative (*p*H 6.5): ..... .

hemoglobin have similar magnitudes of positive extremum at the same wavelength, at around 420 mμ in the case of the oxygenated derivative and at around 412 mμ in the case of the ferric derivative, the CD spectra of the Hb-Hp complexes are different from those of adult Hb: A negative trough in the Soret region which is observed in both derivatives of adult Hb disappears in both derivatives of the Hb-Hp complexes. As no ellipticity of Hp is observed in the region of wavelengths above 310 mμ (Hamaguchi, Isomoto, and Nakajima 1969), the spectral difference in the Soret region between the Hb-Hp complexes and adult Hb suggests the presence of a difference in the heme environment or a difference in the tightness of heme attachment, or both, between the Hb-Hp complexes and hemoglobin A. There are, however, no significant differences in the CD spectra among the three common types of the Hb-Hp complexes.

Effects of Dithionite on the Hb-Hp Complexes

Figure 11 shows the absorption spectra in the 500–700 mμ region of the Hb-Hp complexes after reduction with dithionite under aerobic conditions. In addition to the absorption band at 556 mμ which is characteristic of deoxyhemoglobin, a prominent new absorption band appears at 633–635 mμ. In relation to this absorption band at 633–635 mμ, the following phenomena are observed (Hamaguchi, Miyake, and Nakajima 1969): (1) In the case of reduction of the oxygenated Hb-Hp complex with dithionite under anaerobic conditions, no peak appears at 633–635 mμ; (2) carbon monoxide intensifies the absorption band at 633–635 mμ; (3) alkali shifts the band to 618–620 mμ; (4) carbon monoxide also reacts with the alkaline solution of the reaction mixture shifting the absorption band from 618–620 mμ to 630 mμ; (5) even in the presence of 0.1 M NaOH and 20% pyridine, in addition to the bands of reduced pyridine protohemochromogen, the absorption band at 618 mμ persists. In the case of reduction of adult Hb with dithionite under aerobic conditions, no peak appears at around 630 mμ (fig. 11). These data suggest that the absorption band at 633–635 mμ is due to the formation of a choleglobin-like substance (Lemberg et al. 1941) and that the heme of each genetic type of the Hb-Hp complex is more easily degraded under certain conditions, such as described above, than is the heme of hemoglobin A.

The Rate of Autoxidation

The rate of autoxidation of the Hb-Hp complexes is greater than for hemoglobin A (fig. 12). It is shown that each genetic type of the oxyhemoglobin-haptoglobin complex has a rate of autoxidation similar to that of oxyhemoglobin A in the presence of 2 M NaCl, which exists largely as dimers in this concentration of NaCl.

DISCUSSION

Purification of the three common types of Hp and their Hb complexes was achieved at *p*H 7.0, at around 4° C. The purities and yields were satisfactory in the case of purification of Hb 2-1 and 2-2 and all three common types of the Hb-Hp complexes. However, in the case of purification of Hp 1-1, the yield was not adequate and some minor contaminants were observed by polyacrylamide gel electrophoresis.

From the data on optical rotatory dispersion of Hp 1-1 and 2-2, Waks and Alfsen (1966) suggested a negligible helical content and the possibility of the presence of beta structure in Hp. The CD behavior in the 200–240 mμ region of the three common types of Hp presented here seems to indicate the presence of beta structure and almost no helical content in human Hp. A negligible alpha-helical content and the presence of some beta structure in gamma globulin have already been reported (Sarkar and Doty 1966). In addition, the resemblance of the CD spectra in the 200–240 mμ region between Hp 1-1 and gamma globulin from rabbit serum suggests the similarity of secondary structures between Hp 1-1 and gamma globulin. These observations are of special interest, because Black and Dixon (1968) reported a homology in sequence between a portion of the Hp α chain sequence and a series of Bence-Jones light chains and suggested that Hp and 7S immunoglobulins are structurally and functionally homologous.

It is well known that the Hp^2 gene evolved as a consequence of partial gene duplication of the Hp^1 gene. The two common types of haptoglobin which are controlled by the Hp^2 gene, Hp 2-1 and 2-2, are a series of polymers. However, the data on heme and nitrogen content of the three common types of the Hb-Hp complexes suggest that, in spite of the formation of a series of polymers, the HBC per polypeptide chain of Hp 2-1 and 2-2 is similar to that of Hp 1-1. These results seem to indicate

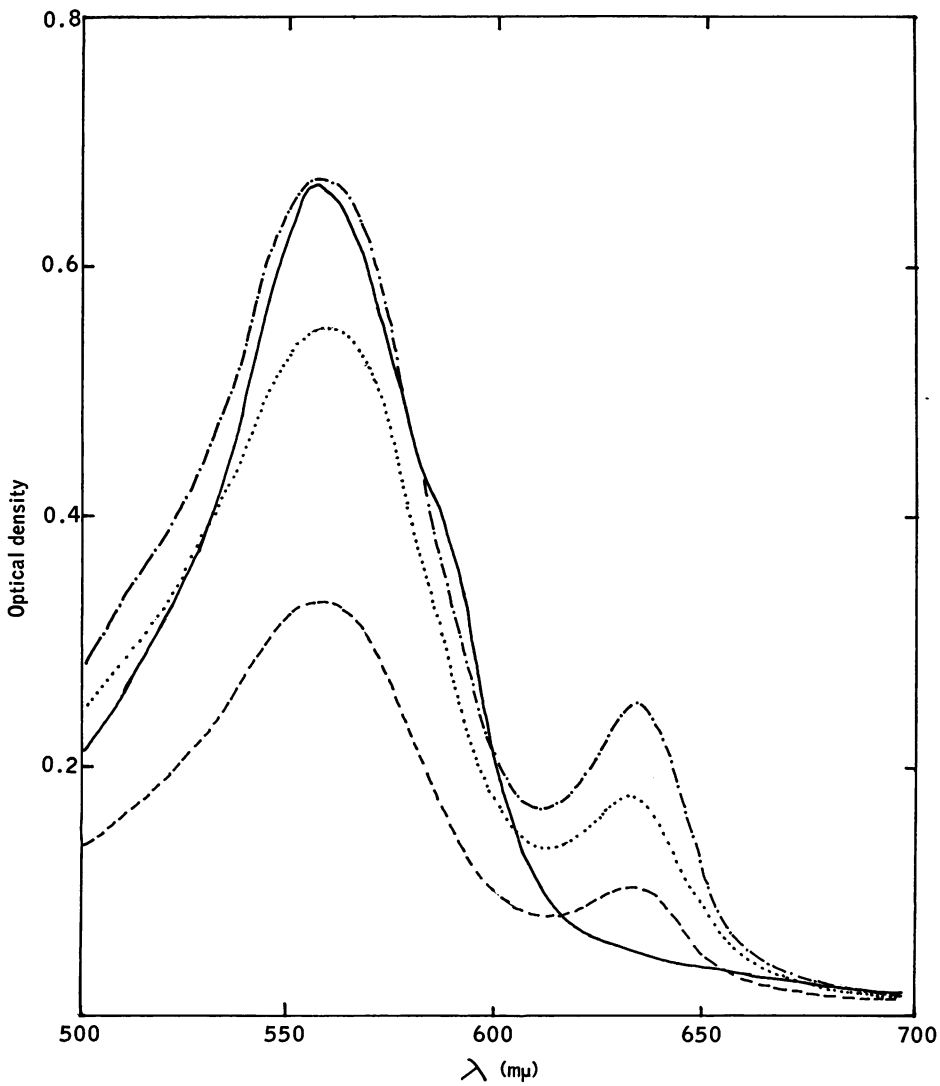

FIG. 11.—Absorption spectra of the three common types of the Hb-Hp complexes and adult Hb after reduction with dithionite under aerobic condition at *p*H 7.0. Hb-Hp 1-1: --------; Hb-Hp 2-1: —•—•—; Hb-Hp 2-2 .....; adult Hb: ———.

that almost no steric hindrance for hemoglobin linkage occurs in most of the polymers of Hp 2-1 and 2-2. In this respect, the arrangement of α and β chains in Hp 2-1 and 2-2 is interesting.

Available data seem to indicate that Hp binds hemoglobin $\alpha\beta$ dimers: (1) A complex between globin $\alpha\beta$ dimer and Hp 1-1 has been demonstrated (Giblett 1968). (2) The Hb-Hp 1-1 intermediate consists of one molecule of Hp 1-1 and half a molecule of Hb. (3) The β chain of the Hp is involved in the binding of Hb (Gordon and Bearn 1966), and each genetic type of Hp can bind Hb in the ratio of half a molecule of Hb to one α- and one β-Hp chain. (4) Hb tetramers do not react with Hp, but Hb

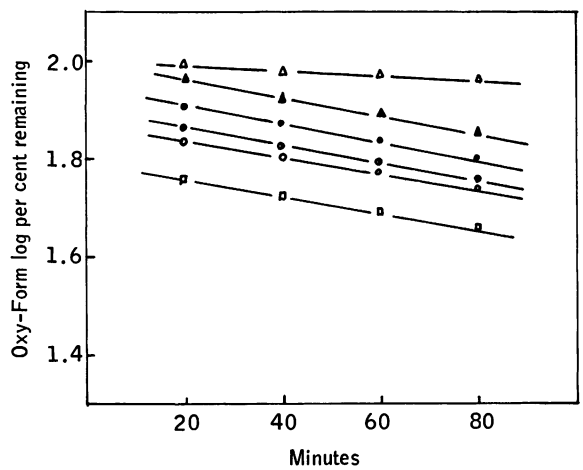

FIG. 12.—The rate of autoxidation of the three common types of the Hb-Hp complexes and adult Hb at 38° under 1 atm of O_2, in 0.05 M phosphate buffer, *p*H 7.0. Hb-Hp 1-1 intermediate: □; Hb-Hp 1-1 complex: ○; Hb-Hp 2-1 complex: ◑; Hb-Hp 2-2 complex: ●; adult Hb: △; adult Hb in 2 M NaCl: ▲.

subunits are involved (Nagel and Gibson 1967). (5) The affinity of the Hb β chains for Hp is so low that it is impossible to reach saturation, but the addition of the Hb α chains already mixed with the Hb β chains leads to complete saturation (Chiancone et al. 1968). Then, if it is assumed that the Hb $\alpha\beta$ dimer is attached to Hp and that the models of Hp proposed by Shim et al. (1965) are right, each common genetic type of the Hb-Hp complex might be represented as follows: For the Hb-Hp 1-1 intermediate, (Hp $\alpha^1\beta$) (Hp $\alpha^1\beta$–Hb $\alpha\beta$); for the Hb-Hp 1-1 complex, (Hp $\alpha^1\beta$–Hb $\alpha\beta)_2$; for the Hb-Hp 2-1 complex, (Hp $\alpha^1\beta$–Hb $\alpha\beta)_2$, (Hp $\alpha^1\beta$–Hb $\alpha\beta)_2$, (Hp $\alpha^2\beta$–Hb $\alpha\beta)_2$, (Hp $\alpha^1\beta$–Hb $\alpha\beta)_2$ (Hp $\alpha^2\beta$–Hb $\alpha\beta)_4$, (Hp $\alpha^1\beta$–Hb $\alpha\beta)_2$ (Hp $\alpha^2\beta$–Hb $\alpha\beta)_6$, . . . ; and for the Hb-Hp 2-2 complex, (Hp $\alpha^2\beta$–Hb $\alpha\beta)_4$, (Hp $\alpha^2\beta$–Hb $\alpha\beta)_6$, (Hp $\alpha^2\beta$–Hb $\alpha\beta)_8$, (Hp $\alpha^2\beta$–Hb $\alpha\beta)_{10}$, ...

A difference in the stability of the heme-globin linkage between Hb and the Hb-Hp complex has been suggested by several investigators: In intravascular hemolysis, methemalbumin forms only after Hp has been saturated with Hb (Allison and Rees 1957; Lathem and Worley 1959; Nyman 1960), and the exchange of heme among

hemoglobins and between Hb and albumin is blocked by the prior binding of Hb to Hp (Bunn and Jandl 1968). In addition, Smith and Beck (1967) suggest that Hp enhances the peroxidase activity of the heme moiety through conformational effects on the globin moiety. The CD spectra in the Soret region of the Hb-Hp complexes and Hb presented here also indicate that the heme environment or the tightness of heme attachment, or both, in Hb is altered as a consequence of the binding of Hp. The biological significance of the resulting change in the heme environment or in the tightness of heme attachment of the Hb-Hp complex remains to be elucidated.

There are no significant differences among the three common types in the properties of Hp and the Hb-Hp complexes reported here except for the CD spectra in the far ultraviolet region of haptoglobins. The peroxidase activity (Nyman 1959), the absorption spectra in the Soret region of the deoxy form (Nagel and Gibson 1966), and the oxidation-reduction potentials (Brunori et al. 1968) of the Hb-Hp complexes are also independent of the Hp type. However, some differences among the three common types of the Hb-Hp complexes in several of the properties have been pointed out: (1) The initial velocity of the conversion of heme in the Hb-Hp complexes into a possible precursor of biliverdin by heme alpha-methenyl oxygenase was greater in the Hb-Hp 2-2 complex than in the Hb-Hp 2-1 or 1-1 complex (Nakajima et al. 1963). (2) The Hb-Hp 2-2 complex exhibits a higher rate of combination with carbon monoxide than the Hb-Hp 1-1 complex (Nagel and Gibson 1966). (3) The Hb-Hp 2-2 complex was more rapidly cleared from the bloodstream than the Hb-Hp 2-1 and 1-1 complexes (Naito 1967). There are apparently differences among the three common types in molecular size, molecular shape, and polypeptide constitution of haptoglobins and the Hb-Hp complexes. These differences in molecular structure may cause a significant difference among the three common types in the interaction of Hp with Hb, or in the interaction of the Hb-Hp complexes with cells, proteins, or other substances in vivo.

SUMMARY

1. The three common genetic types of haptoglobins and their hemoglobin complexes were purified from human blood by ammonium sulfate fractionation, gel filtration on a Sephadex G-100 column, and chromatography on a DEAE-Sephadex column, at *p*H 7.0. Several properties of the purified samples are described, with particular reference to comparisons among the three common types.

2. The CD spectra in the 200–240 mμ region of the three common types of Hp have been measured. The resemblance of the CD spectra between Hp 1-1 and gamma globulin seems to indicate the similarity of secondary structures between Hp 1-1 and gamma globulin. In addition, the CD behavior of the three common types of Hp suggests a negligible helical content and the presence of beta structure in human Hp. The results also suggest that there are significant differences in conformation between Hp 1-1 and Hp 2-2.

3. From the data on heme and nitrogen content of the Hb-Hp complexes, it is shown that each genetic type of Hp can bind Hb in the ratio of half a mole of Hb to one mole of Hp α and one mole of β chains. These results suggest that, in spite of their

existence as a series of polymers, Hp 2-1 and 2-2 have a hemoglobin binding capacity per polypeptide chain similar to that of Hp 1-1.

4. The circular dichroism (CD) spectral differences in the Soret region between the Hb-Hp complexes and adult hemoglobin suggest the presence of a difference in the heme environment or a difference in the tightness of heme attachment, or both, between the Hb-Hp complexes and hemoglobin A. However, there are no significant differences in the CD spectra in the Soret region among the three common types of Hb-Hp complexes.

5. A choleglobin-like substance is easily formed from each genetic type of Hb-Hp complex by addition of a minimum amount of dithionite under aerobic conditions at *p*H 7.0.

6. The Hb-Hp complexes have a higher rate of autoxidation than hemoglobin A. The rate of autoxidation of all three common types of Hb-Hp complex is similar to that of hemoglobin in the presence of 2 M NaCl, which exists largely as Hb dimers in this solution.

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