amino terminals may be advisable. In addition to its action on tyrosine, iodine converts imidazole in proteins to mono and diiodo derivatives (Cohen, 1968) and, in certain cases, oxidizes the indole nucleus to an oxindole (Hartdegen and Rupley, 1967). *N*-Bromosuccinimide has been shown to cleave tyrosyl-peptide bonds (Wilson and Cohen, 1963) as well as those of tryptophan and histidine (Witkop, 1961). Whether *N*-iodosuccinimide has a similar action on proteins is currently under investigation.

References

Barnes, J. H., Borrows, E. T., Elks, J., Hems, B. A., and Long, A. G. (1950), *J. Chem. Soc.*, 2824.
Cohen, L. A. (1968), *Ann. Rev. Biochem. 37*, 698.
Cohen, L. A., and Jones, W. M. (1962), *J. Amer. Chem. Soc. 84*, 1629.
Cook, C. D., Nash, N. G., and Flanagan, H. R. (1955), *J. Amer. Chem. Soc. 77*, 1783.
Farber, L., and Cohen, L. A. (1966), *Biochemistry 5*, 1027.
Hartdegen, F. J., and Rupley, J. A. (1967), *J. Amer. Chem. Soc. 89*, 1743.
Isoe, S., and Cohen, L. A. (1968), *Arch. Biochem. Biophys. 127*, 522.
Matsuura, T., Nishinaga, A., Matsuo, K., Omura, K., and Oishi, Y. (1967), *J. Org. Chem. 32*, 3457.
Seliwanoff, T. (1893), *Ber. 26*, 985.
Wilson, J. G., and Cohen, L. A. (1963), *J. Amer. Chem. Soc. 85*, 564.
Witkop, B. (1961), *Advan. Protein Chem. 16*, 221.

Circular Dichroism of Isolated and Recombined Hemoglobin Chains*

Giuseppe Geraci† and Ting-Kai Li‡

ABSTRACT: The optical activity of the heme bands of hemoglobin has been used as a probe to study the alterations of the structure of the isolated α and β chains on recombination to form the normal tetrameric molecule. In all oxidation and ligand states, the circular dichroism spectra of the two chains differ both in the Soret and in the visible region and are readily distinguishable from each other by this technique. The circular dichroism bands exhibit distinctive peak positions, molar ellipticities, and rotational strengths, reflecting a difference in the environments of the heme in the α from that of the β chains. Chain recombination induces a circular dichroism spectrum identical with that of native hemoglobin, but different from that of the noninteracting mixture, indicating an alteration of the heme environments upon recombination. The largest variations are observed upon recombination of the deoxy chains. Chain recombination when studied in the region of absorption of the peptide chromophore does not appear to indicate a change in the conformation of the molecule except in the case of the deoxy derivative, where there is an increase in the depth of the 233-nm Cotton effect trough comparable with that observed upon deoxygenation of hemoglobin.

The ligand binding properties of the isolated subunits of hemoglobin change dramatically upon recombination, and the properties of the recombined material are identical with those of the native molecule (Antonini *et al.*, 1965, 1967). The physicochemical basis of these phenomena has been investigated by a variety of techniques, and recent studies have demonstrated that some of the spectral properties of the isolated chains differ from those of the native and of the reconstituted molecules. The molar absorptivities in the Soret region of both the α and the β chains in the deoxy form are lower than that of deoxyhemoglobin (Benesch *et al.*, 1964; Antonini *et al.*, 1965), and absorptivity increases when the chains are mixed (Antonini *et al.*, 1966; Brunori *et al.*, 1968). Brunori *et al.* (1967) demonstrated that the amplitude of the Cotton effect trough at 233 nm increases upon deoxygenation of hemoglobin and concluded that the change is dependent upon chain interaction since no change occurs with the isolated *p*-hydroxymercuribenzoate subunits. Beychok *et al.* (1967) found that the α and β chains have different molar ellipticities both in the 260-nm region and between 410 and 415 nm while hemoglobin has intermediate values. Moreover,

* From the Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts. *Received December 10, 1968.* This work was supported by Grants-in-Aid HE-07297 and GM-15003 from the National Institutes of Health of the Department of Health, Education, and Welfare.

† On leave of absence from the International Laboratory of Genetics and Biophysics of Consiglio Nazionale delle Richerche, Naples, Italy.

‡ Markle Scholar in Academic Medicine.

they found that the negative circular dichroism band at 285 nm, present in deoxyhemoglobin, is absent in the deoxy chains.

Recently, it has become possible to prepare conveniently chains which contain free SH groups, are stable, and recombine even in the ferric state (G. Geraci, L. J. Parkhurst, and Q. H. Gibson, 1969, in preparation). Hence, we have examined the absorption and circular dichroism spectra of the isolated chains and compared them directly with those of their recombination products in both oxidation states of iron, ligand and unliganded. The results show that the α and β chains have different spectral properties and these are altered when the chains recombine.

Materials and Methods

Human hemoglobin was prepared from freshly drawn blood. The erythrocytes were washed several times with 0.9% NaCl solution, packed by centrifugation for 10 min at 12,000*g*, and lysed with 1.5 volumes of distilled water. Red cell ghosts were precipitated by the addition of 0.1 volume of a 1.0 M solution of sodium phosphate (pH 7) and removed by centrifugation for 30 min at 40,000*g*. When toluene was employed to remove the red cell ghosts, the chains recombined slowly or not at all. Mixtures of these chains with those prepared by the phosphate procedure showed that the β chains from toluene-treated hemoglobin failed to recombine with α chains.

Isolated chains of hemoglobin with free SH groups were prepared by the method of G. Geraci, L. J. Parkhurst, and Q. H. Gibson (1969, in preparation) as follows: hemoglobin was split into chains by adding *p*-hydroxymercuribenzoate (Bucci and Fronticelli, 1965). The solution was equilibrated with 10 mM phosphate (pH 8.0) and applied to a DEAE-cellulose (Whatman DE-23) column, equilibrated with the same buffer. The *p*-hydroxymercuribenzoate α chains,[1] which do not bind, were eluted, brought to pH 6.7, and applied to a CM-cellulose column equilibrated with 10 mM phosphate (pH 6.7). Both columns were washed with four column volumes of 10 mM β-mercaptoethanol in the respective equilibrating buffers to remove *p*-hydroxymercuribenzoate from the SH groups of the chains, followed by the equilibrating buffers alone to remove β-mercaptoethanol. The α chains were eluted from the CM-cellulose column and the β chains from the DEAE-cellulose column with 0.1 M phosphate (pH 7.0). The chains thus isolated had the following properties: α chains contained 1.0 $\pm$ 0.05 and β chains 2.0 $\pm$ 0.1 SH groups per mole of heme, as measured by titration with *p*-hydroxymercuribenzoate (Boyer, 1954) and employing the molar absorptivities given below for the oxy and cyanomet derivatives of the chains. The amount of *p*-hydroxymercuribenzoate remaining in the chains, as determined by mercury analysis with atomic absorption spectroscopy (M. Suzuki, T. L. Coombs, and B. L. Vallee, to be published), corresponded to less than 1% of the SH groups of the subunits. Starch gel electrophoresis of the isolated chains using the discontinuous buffer of Poulik (1957) revealed single bands for each of the chains and a 1:1 mixture of chains migrated as a single band with the same mobility as that of native hemoglobin. When stored as the oxygenated derivative in 50 mM phosphate buffer, pH 7.0, 4°, the subunits retained the capacity to recombine for at least 1 week and were not denatured when oxidized to methemoglobin with ferricyanide or nitrite.

Carbon monoxy-liganded chains were prepared by passing over the solution for 10 min a gentle stream of CO saturated with water. Met derivatives were prepared by adding a fivefold molar excess of ferricyanide or nitrite to solutions of oxygenated chains and removing the excess reagent by filtration through a Sephadex G-25 column equilibrated with 50 mM phosphate, pH 7.0, 4°. The procedure was completed within 5 min. The met α chains obtained in this manner were stable for up to 24 hr in 50 mM phosphate buffer, pH 7.0, 4°, as evidenced by their spectral properties. However, the spectrum of *n* met β chains, under the same conditions, changed slowly both in the visible and in the Soret region and mixing the met chains 30 min after the preparation of β chains failed to give the peak amplitude of the Soret band expected for an equivalent concentration of methemoglobin. For this reason, the met β chains were mixed with the met α chains as soon as possible after their preparation. The cyanomet derivatives were prepared by the addition of an excess of KCN to solutions of the met chains. Deoxygenated chains were prepared by injecting a solution of sodium dithionite into solutions of chains in syringes. The deoxygenated solutions were slowly injected into sealed cuvets, previously flushed with N_2, and then evacuated through a needle. Protein concentrations were determined spectrophotometrically using the following molar absorptivities for heme: A_{576} 15.4 $\times$ 10^3 (Drabkin, 1946) for the oxy and A_{540} 11.5 $\times$ 10^3 for the cyanomet derivatives (Antonini *et al.*, 1965; Tyuma *et al.*, 1966). The concentrations of met chains and of methemoglobin were determined after conversion into the cyanomet form. All measurements were performed in 50 mM phosphate buffer (pH 7.0), unless otherwise indicated.

Absorption spectra were measured with a Cary 15 spectrophotometer. Circular dichroism and optical rotatory dispersion were measured with a Cary 60 spectropolarimeter, equipped with circular dichroism attachment, at 23°. The slit width was programmed for constant energy. Cuvets of 2-, 1-, 0.2-, and 0.01-cm light paths were used for the different concentration ranges investigated. The instrument was calibrated with standard solutions of *d*-10-camphorsulfonic acid. Bichromate solutions of absorbancy 2.0–3.0 were employed to ensure that the instrument did not produce spurious circular dichroism readings with high absorbancy.

To compare the circular dichroism spectra of the isolated chains with those of their mixtures, two cuvets of 1-cm light path were fixed to each other forming a single linear unit of two separate chambers. The spectra of the isolated chains were recorded by filling both chambers

[1] α or β chains of hemoglobin with the SH groups blocked by *p*-hydroxymercuribenzoate.

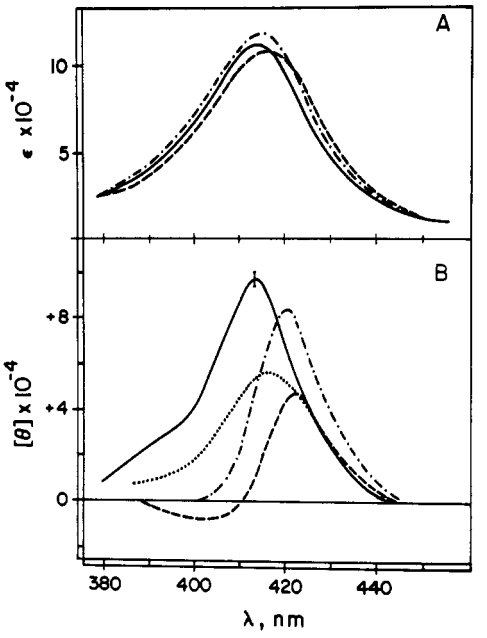

FIGURE 1: Absorption (A) and circular dichroism (B) spectra in the Soret region of oxy α chains (—), oxy β chains (– – –), recombined oxy chains (–·–·–) in 50 mM sodium phosphate (pH 7.0). The mean of the circular dichroism spectra of the isolated α and β chains (· · ·) is measured as an optical mixture as described in Methods.

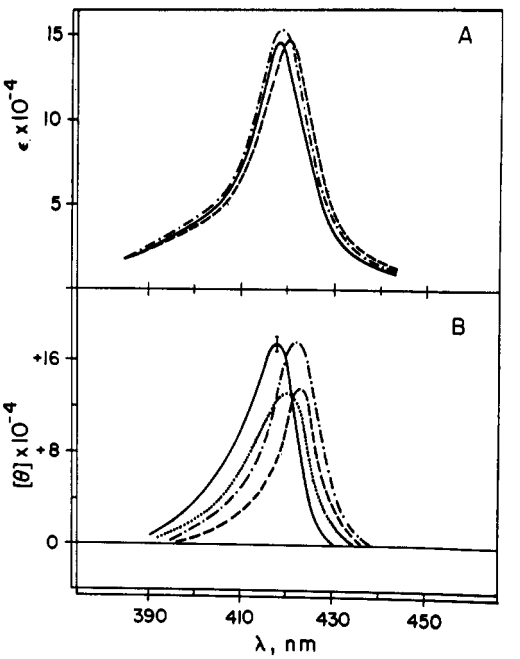

FIGURE 2: Absorption (A) and circular dichroism (B) spectra in the Soret region of CO α chains (—), CO β chains (– – –), recombined CO chains (–·–·–) in 50 mM sodium phosphate (pH 7.0). The mean of the circular dichroism spectra of the isolated chains (· · ·) is measured as an optical mixture as described in Methods.

with solutions of either α or β chains at equimolar concentrations, and those of the noninteracting mixture by filling one chamber with the solution of α chains and the other with that of β chains. These latter spectra corresponded to the mean value of those of the isolated chains. Lastly, the spectrum of the interacting mixture was recorded by filling both chambers with a 1:1 mixture of the two chains. Thus, the spectra of the individual solutions, their optical mixtures, and the recombined chains were obtained in a manner eliminating the possibility of errors due to manipulation.

Circular dichroism is expressed as molar ellipticity in deg cm^2 per dmole of heme. The areas under the ellipticity bands are compared by weighing the paper corresponding to the bands in tracings of the circular dichroism spectra plotted as $[\theta]$ *vs.* λ, nm.

Results

Absorption Spectra. The Soret absorption bands of deoxygenated α and β chains differ from that of deoxyhemoglobin both in absorptivity and in the location of the spectral maximum (Antonini *et al.*, 1965; Brunori *et al.*, 1968). Less pronounced differences are observed for the oxy, CO, and met derivatives. The absorption maximum of oxy-β chains is at 415 nm and that of oxy α chains at 413 nm (Figure 1A). That of the recombined mixture is at a wavelength intermediate between those of the isolated chains but is of larger amplitude. A similar relationship is observed for the CO derivative of the isolated chains and of their mixture (Figure 2A). In the met form, the absorption maxima of the two chains are 4 nm apart and the absorptivity of α chains is about 20% higher than that of the β chains (Figure 3A). In contrast to the ferrous derivatives, the absorptivity of the recombined met chains is intermediate between those of the isolated chains.

Circular Dichroism Spectra. SORET REGION. The circular dichroism spectra of the isolated chains and of their mixtures reveal pronounced differences. The oxy α chains exhibit a positive peak at 413 nm, coincident with the absorption maximum (Figure 1B). The positive peak of oxy β chains is situated at 422 nm and has a molar ellipticity lower than that of the oxy α chains. In addition, there is a negative band with an apparent extremum at about 405 nm. The positive circular dichroism maximum of the recombined chains is located at 420 nm and does not coincide in spectral location with that of the mean value measured for the sum of the spectra of the individual chains. It also has a higher molar ellipticity.

Qualitatively similar findings are obtained with the CO, met, and deoxy derivatives (Figures 2B, 3B, and 4) with the exception that the CO β chains do not exhibit the negative band seen with the other derivatives. This is the only instance in which the β chains do not show a negative band in the Soret region. In all cases, the positive circular dichroism maxima of the β chains are at a wavelength longer than the corresponding absorption maxima and have lower ellipticities than those of the α chains. At its maximum, the ellipticity of the positive Soret circular dichroism band of the deoxygenated recombined product is almost twice that of the measured mean value for the individual deoxy chains.

The circular dichroism spectra of the recombined chains are indistinguishable from those of the native

TABLE I: Peak Position and Molar Ellipticity of the Positive Soret Circular Dichroism Bands of the Isolated and Recombined Chains of Human Hemoglobin in Different Oxidation and Ligand States.[a]

	Oxy		CO		Deoxy		Met		CN-met	
	λ_{max} (nm)	$[\theta] \times 10^{-3}$	λ_{max} (nm)	$[\theta] \times 10^{-3}$	λ_{max} (nm)	$[\theta] \times 10^{-3}$	λ_{max} (nm)	$[\theta] \times 10^{-3}$	λ_{max} (nm)	$[\theta] \times 10^{-3}$
α	414	96	417	176	434	100	406	80	419	64
β	421	44	421	123	435	72	414	64	425	32
$\alpha\beta$ or Hb	420	98	421	176	433	168	410	90	425	64

[a] Values obtained from the spectra of three different preparations of chains recorded at different protein concentrations. The maximum deviation from the reported values is 7%. $[\theta]$ is molar ellipticity in deg cm^2 per dmole.

hemoglobin in all oxidation and ligand states. Over a concentration range of 0.1–40 mg/ml, the differences between the mean values of the circular dichroism spectra of individual oxy chains and those of their recombination products remain constant. The λ_{max} and molar ellipticities for the derivatives examined are summarized in Table I. The total areas under the ellipticity bands which are proportional to rotational strength are also influenced by subunit recombination (Table II): the differences between the areas for the recombined chains and that of the measured mean values for the isolated chains are small for the oxy and met states but are well above the limit of error for the other derivatives.

VISIBLE ABSORPTION REGION. The isolated chains and their recombination product have distinctive circular dichroism features not only in the Soret absorption region but also in the visible. Oxy α chains exhibit two positive circular dichroism bands with maxima at 576 and 542 nm, coincident with the absorption maxima (Figure 5A). Oxy β chains have three circular dichroism bands in this wavelength region, two positive ones located at 576 and 550 nm and a negative one at 525 nm. The band at 576 nm is of lower molar ellipticity than that of the oxy α chains. The circular dichroism spectra of the two chains differ markedly between 560 and 500 nm since there appears to be splitting of the 540-nm absorption band of the oxy β chains. However, the circular dichroism spectrum of their recombination product is very similar to that of the oxy α chains with the exception that the molar ellipticity of the 540-nm band is lower.

Effect of Blocking of SH Groups. It has been shown

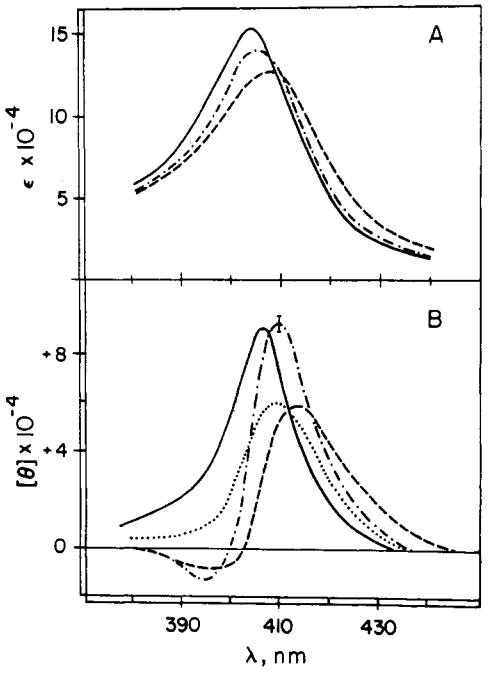

FIGURE 3: Absorption (A) and circular dichroism (B) spectra in the Soret region of met α chains (—), met β chains (– – –), recombined met chains (–·–·–) in 50 mM sodium phosphate (pH 7.0). The mean of the circular dichroism spectra of the isolated chains (· · ·) is measured as an optical mixture as described in Methods.

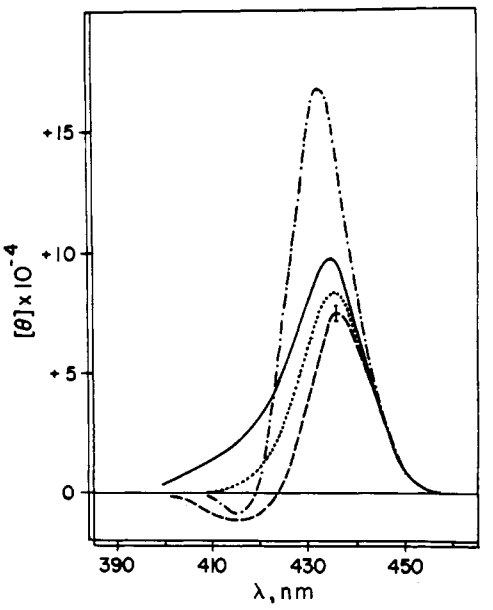

FIGURE 4: Circular dichroism spectra in the Soret region of deoxy α chains (—), deoxy β chains (– – –), recombined deoxy chains (–·–·–) in 50 mM sodium phosphate (pH 7.0). The mean of the circular dichroism spectra of the isolated chains (· · ·) is measured as an optical mixture as described in Methods.

TABLE II: Areas under the Soret Ellipticity Bands of the Isolated and of the Recombined Chains of Human Hemoglobin in Different Oxidation and Ligand States.[a]

	α (mg)	β (mg)	$\alpha\beta$ (mg)	$\alpha + \beta$ (mg)	α/β (mg)	$\alpha\beta \times \frac{2}{\alpha+\beta} \times 100$ (%)
Oxy	225	88	161	156	2.56	103
CO	270	173	261	221	1.56	118
Deoxy	216	124	254	170	1.75	149
Met	180	122	163	151	1.47	108
CN met	142	56	129	99	2.58	130

[a] $\alpha\beta$ is the recombined product of α and β chains or Hb. $\alpha + \beta$ is the optical mixture of α and β chains. Values are obtained from the spectra of three different preparations of chains recorded at different protein concentrations. The average deviation is 10% on all values. See Methods for details.

that the kinetic and physicochemical properties of mixtures of *p*-hydroxymercuribenzoate chains vary as a function of the pH of the solutions (Antonini *et al.*, 1966). Hence, the single SH group of α chains and the two SH groups of β chains were blocked by the stoichiometric addition of *p*-hydroxymercuribenzoate. The mixture of chains treated in this manner shows two bands corresponding to the individual chains when analyzed by electrophoresis or column chromatography. At both pH 7.0 and 8.0, the *p*-hydroxymercuribenzoate α chains exhibit a Soret circular dichroism spectrum similar to that of the corresponding unmodified chains but the maximum is blue shifted 1–2 nm and the molar ellipticity is about 20% lower (Figure 6A). The *p*-hydroxymercuribenzoate β chains, on the other hand, show more pronounced differences. The Soret maximum is blue shifted about 4–418 nm, and the negative band observed at the low-wavelength side of the positive peak of unmodified β chains is absent. In the visible wavelength region, circular dichroism spectra of the *p*-hydroxymercuribenzoate chains are virtually identical with those of the corresponding chains whose SH are unblocked. The circular dichroism spectra of 1:1 mixtures of the *p*-hydroxymercuribenzoate chains differ at pH 7.0 from the mean values of the spectra of the individual chains in both wavelength regions (Figures 5A and 6A). In contrast, at pH 8.0, the spectra of the mixtures closely approximate the mean values of the spectra of the chains (Figures 5B and 6B).

Absorption Region of the Peptide Bonds. Circular dichroism measurements were employed to study the optical activity of the heme bands since this technique allows the isolation of the individual bands. To study the effect of subunit recombination and ligand binding in the region of peptide absorption, optical rotatory dispersion measurements were made in order to compare the present with previous results (Brunori *et al.*, 1967).

Recombination of the subunits in the oxygenated state does not alter the amplitude of the 233-nm Cotton effect trough nor does deoxygenation of the isolated chains. Recombination of deoxygenated chains results in a 5–8% increase in the amplitude of the trough, a change comparable with that observed upon deoxygenation of recombined oxy chains and of native hemoglobin (Brunori *et al.*, 1967). Thus conformational changes occur not only upon deoxygenation of hemoglobin, but also upon recombination of deoxygenated chains.

Discussion

The α and β chains of hemoglobin employed in this study are stable and recombine even when they are in the ferric state implying properties different from those of chains prepared by other methods (Bucci and Fronticelli, 1965; Tyuma *et al.*, 1966). The recombinant of the oxy chains is indistinguishable from native oxyhemoglobin, and similarly those of chains in either oxidation state of iron, liganded and unliganded, are indistinguishable from the corresponding hemoglobin derivatives. The spectra, therefore, characterize chains that have this functional capacity.

The absorption and circular dichroism spectra of the α and β chains differ and are distinctive both in the Soret and visible wavelength regions (Figures 1 and 5A). Tyuma *et al.* (1966) already reported a difference in the wavelength of the maxima of the α-absorption bands of the two oxy chains. In all the derivatives here examined, the Soret absorption maxima of the β chains occur at wavelengths longer than those of the corresponding maxima of α chains (Figures 1A and 3A). However, the absorptivities at the maxima of the two types of chains are almost identical. Among all those studied, the met derivatives are the only exceptions: the absorptivity of the met β chains is lower by about 20% than that of the met α chains (Figure 3A). Circular dichroism measurements emphasize the differences between the chains. In contrast to that of α chains, the extremum of the predominant circular dichroism band of β chains in the Soret region is located at the long-wavelength side of the absorption maximum (Figures 1–4). Moreover, the number of circular dichroism bands is distinctive for the two types of subunits both in the Soret and in the visible wavelength regions (Figures 1B, 3B, 4, and 5A),

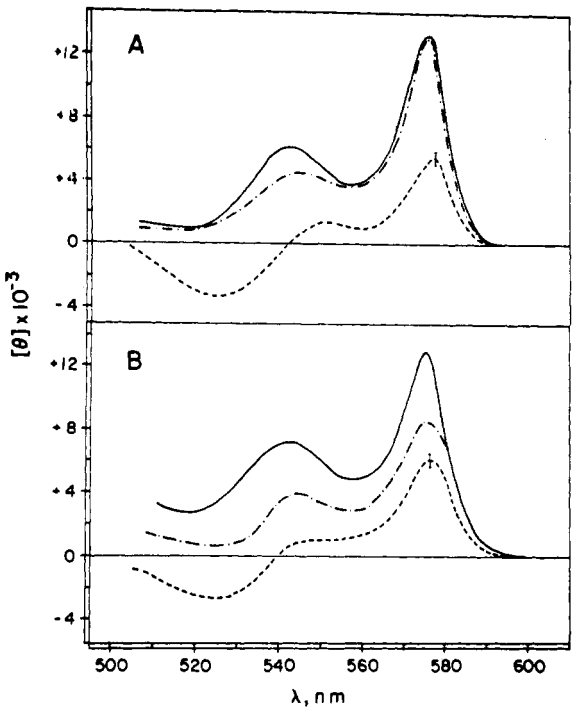

FIGURE 5: Visible circular dichroism spectra. (A) Oxy chains with free SH groups or with SH groups blocked by *p*-mercuribenzoate. α chains (—), β chains (– – –), and recombined chains (–·–·–) in 50 mM sodium phosphate (pH 7.0). (B) Visible circular dichroism spectra of oxy α *p*-mercuribenzoate chains (—), oxy β *p*-mercuribenzoate chains (– – –), and 1:1 mixture of the two chains (–·–·–) in 50 mM sodium phosphate (pH 8.0).

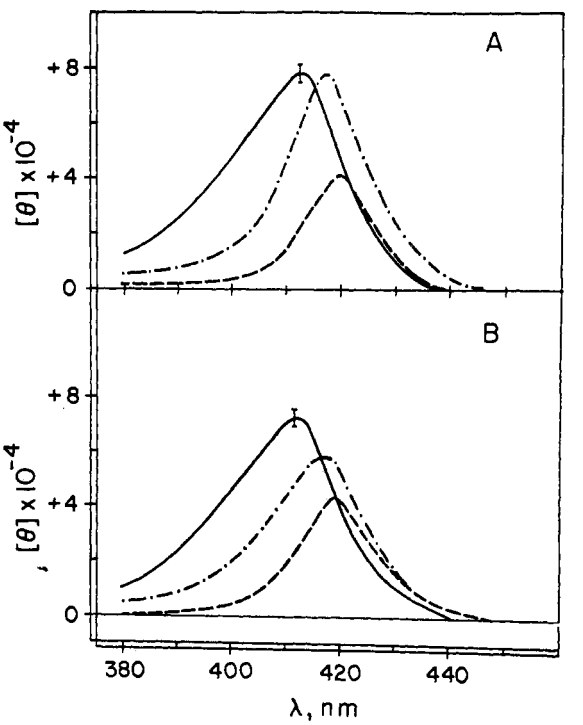

FIGURE 6: Circular dichroism spectra in the Soret region at pH 7.0 (A) and at pH 8.0 (B) of oxy α *p*-mercuribenzoate chains (—), oxy β *p*-mercuribenzoate chains (– – –), and the 1:1 mixture of the two chains (–·–·–) in 50 mM sodium phosphate.

representing qualitative differences. The molar ellipticities and the areas under the circular dichroism bands are smaller for β chains than for α chains (Table II) in agreement with the data for the oxy- and CN-met derivatives in the region between 410 and 415 nm reported by Beychok *et al.* (1967). Thus it appears that the differences in the environment surrounding the heme groups in the α and β chains (Perutz *et al.*, 1968) manifest not only in kinetic behavior (Antonini *et al.*, 1966) but are revealed also in their absorption and circular dichroism properties. The previous finding that blocking of the SH groups with *p*-hydroxymercuribenzoate perturbs the kinetic properties (Antonini *et al.*, 1966) together with the present data showing that the circular dichroism spectra of the chains are also perturbed by *p*-hydroxymercuribenzoate (Figure 6) are in accord with the hypothesis that both parameters are expressions of the heme environments.

The recognition of such characteristic spectral properties of the chains provides an approach to study the physicochemical basis of the functional changes that accompany subunit recombination. Changes in the Soret absorption spectrum have been reported to occur upon deoxy chain recombination (Antonini *et al.*, 1966; Brunori *et al.*, 1967). Recombination alters both the circular dichroism (Figure 4) and absorption spectra of deoxy chains to give rise to spectra indistinguishable from those of deoxyhemoglobin. Identical results are obtained with the different liganded forms of the chains and with chains in the met state (Figures 1–3). The deoxy chains undergo the largest change since the molar ellipticity of the Soret band doubles and its area increases by 50% (Tables I and II). The effect of subunit interaction is also observed with mixtures of *p*-hydroxymercuribenzoate chains. At pH 7.0, where the mixture behaves like hemoglobin (Antonini *et al.*, 1966), there is an alteration of the circular dichroism spectra (Figures 5A and 6A), but at pH 8.0, where the *p*-hydroxymercuribenzoate chains do not interact (Antonini *et al.*, 1966), there is practically no change (Figures 5B and 6B). Thus, the characteristic ligand binding properties of hemoglobin generated through subunit interaction have an easily identifiable, physical counterpart in the heme circular dichroism spectra. The kinetics of the circular dichroism changes may provide useful information concerning the process of chain recombination.

On the basis of current knowledge, interaction of the heme groups with one another, conformational alterations of the protein environments surrounding the hemes, or both might be considered to underlie these phenomena. Direct heme–heme interaction has been suggested to account for the variations of the circular dichroism spectra upon aggregation of the heme peptides of cytochrome *c* (Urry, 1967; Urry and Pettegrew, 1967). The spectral changes observed upon hemoglobin chain recombination do not seem to comply with this type of interaction, particularly since the circular dichroism spectra both of the recombination products and of native hemoglobin do not vary as a function of concentration over a range in which there is dissociation of

hemoglobin into dimers (Brunori *et al.*, 1967). Alteration of the protein environments of the hemes, on the other hand, would appear more likely to account for these spectral changes since different heme environments manifest in different spectral properties of the hemes as shown for the α and β chains and their *p*-hydroxymercuribenzoate derivatives. The increase of the amplitude of the 233-nm Cotton effect trough upon interaction of deoxy chains is consistent with the occurrence of a change in protein conformation. A recent electron paramagnetic resonance study employing a spin label bound to the SH groups of hemoglobin subunits (Ohnishi *et al.*, 1968) shows that when the label is bound to the SH group in position 93 of the β chains in close proximity to the heme site, subunit recombination induces a large perturbation of the electron paramagnetic resonance spectrum. Minor alterations are also observed with the label on the SH group of α chains far from the heme site. These findings, indicating conformational changes also when the chains are liganded, are consistent with the alterations of heme spectra but contrast with the absence of measurable change of the 233-nm Cotton effect trough. Apparently, the protein conformational changes thus far observed upon recombination of liganded chains are more localized than those with the deoxy chains.

The data here reported further document the interrelationship of the conformational state of hemoglobin and the physical and kinetic properties of the heme group. The distinctive spectral features offer another approach for the elucidation of the mechanism regulating the reactivity of hemoglobin with its ligands.

References

Antonini, E., Bucci, E., Fronticelli, E., Chiancone, E., Wyman, J., and Rossi-Fanelli, A. (1966), *J. Mol. Biol. 17*, 29.

Antonini, E., Bucci, E., Fronticelli, E., Wyman, J., and Rossi-Fanelli, A. (1965), *J. Mol. Biol. 12*, 375.

Antonini, E., Chiancone, E., and Brunori, M. (1967), *J. Biol. Chem. 242*, 4360.

Benesch, R., Gibson, Q. H., and Benesch, R. E. (1964), *J. Biol. Chem. 239*, PC 1668.

Beychok, S., Tyuma, I., Benesch, R. E., and Benesch, R. (1967), *J. Biol. Chem. 242*, 2460.

Boyer, P. D. (1954), *J. Am. Chem. Soc. 76*, 4331.

Brunori, M., Antonini, E., Wyman, J., and Anderson, S. R. (1968), *J. Mol. Biol. 34*, 357.

Brunori, M., Jurgen, E., and Schuster, T. M. (1967), *J. Biol. Chem. 242*, 773.

Bucci, E., and Fronticelli, C. (1965), *J. Biol. Chem. 240*, PC 551.

Drabkin, D. L. (1946), *J. Biol. Chem. 164*, 703.

Ohnishi, S., Maeda, T., Ito, T., Hwang, K. J., and Tyuma, I. (1968), *Biochemistry 7*, 2662.

Perutz, M. F., Muirhead, H., Cox, J. M., and Goaman, L. C. G. (1968), *Nature 219*, 131.

Poulik, M. D. (1957), *Nature 180*, 1479.

Tyuma, I., Benesch, R. E., and Benesch, R. (1966), *Biochemistry 5*, 2957.

Urry, D. W. (1967), *J. Biol. Chem. 242*, 4441.

Urry, D. W., and Pettegrew, J. W. (1967), *J. Am. Chem. Soc. 89*, 5276.