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Sulfhydryl Groups in Hemoglobin. A New Molecular Probe at the $\alpha_1\beta_1$ Interface Studied by Fourier Transform Infrared Spectroscopy†

George H. Bare,‡ James O. Alben,* and Philip A. Bromberg

ABSTRACT: Infrared absorption bands due to sulfhydryl groups (ν_{SH}) of α-104(G11) and β-112 (G14) cysteine residues of human carboxyhemoglobin (HbCO) have been observed near 2560 cm^{-1} by use of Fourier transform infrared (FTIR) spectroscopy. The β-93 cysteine SH groups absorb infrared radiation so weakly that they are not distinguished from background. Only single SH absorption bands due to the α-104 cysteines of pig and horse hemoglobin are observed. The SH absorption bands from human HbCO disappear in alkali, are broadened by detergent or guanidinium chloride, and show a complex titration curve, and an isotopic frequency shift (ν_{SD}/ν_{SH} = 0.7267) virtually identical with that reported for methanethiol. The integrated absorption coefficient (ϵ_{mM}(area)) for 0.1 *M* ethanethiol increased with H-bond acceptor solvents in the order: CCl_4 (0.07), water (0.21), acetone (0.43), and *N,N*-dimethylacetamide (1.35 mM^{-1} cm^{-2}). Comparison of the integrated absorption coefficients for the α-104 cysteine SH (2.43), and the β-112 SH (0.80), of human HbCO with those of ethanethiol solutions suggested specifically H-bonded structures with peptide carbonyl groups 4 (or 3) residues back in the G helices. This was found to agree with a molecular model of the α-chain G helix of horse HbO_2 built to coordinates from M. F. Perutz.

Fourier transform infrared interferometry has provided a considerable enhancement of signal/noise ratio over previous infrared spectroscopic methods. This has made it possible to extend the infrared studies of small molecules to include biologically native proteins such as hemoglobin. Our earlier studies have explored the effects of local molecular structure on strongly absorbing groups such as carbon monoxide (Alben and Caughey, 1968; Caughey et al., 1969) or azide (Alben and Fager, 1972) coordinated to the heme groups in hemoglobin or myoglobin. We have now investigated the sulfhydryl groups of cysteine residues in hemoglobins from man, horse, pig, and cow, and have defined absorption bands due to sulfhydryl groups at the $\alpha_1\beta_1$ interface (α-104 and β-112 cysteine). These absorption bands provide a molecular probe into a region of hemoglobin which previously has been relatively inaccessible.

X-Ray crystallographic data indicate (Perutz, 1969) that the $\alpha_1\beta_1$ contact of hemoglobin includes 32 amino acid residues in deoxyhemoglobin and 34 residues in the oxygenated form. Most of these are nonpolar van der Waals contacts, with only four or five hydrogen bonds, all of which are probably in contact with the aqueous surface. In contrast to the $\alpha_1\beta_2$ interface, relatively small movements have been described at the $\alpha_1\beta_1$ interface as a consequence of ligand binding by hemoglobin. Many studies have stressed the importance of the $\alpha_1\beta_2$ region to the control of oxygenation, whereas the $\alpha_1\beta_1$ region of contact has been assumed to the relatively inert. Our studies of vibrational absorption bands of sulfhydryl groups at the $\alpha_1\beta_1$ interface provide a new probe of native hemoglobin structure and its conformational alterations. We will demonstrate in a later paper (Alben et al., to be published) the sensitivity of the sulfhydryl groups at the $\alpha_1\beta_1$ interface to the state of ligation of the hemes.

In this paper, we report the direct spectroscopic observation of absorption bands due to sulfhydryl vibrational transitions in aqueous solutions of native carboxyhemoglobins by Fourier transform infrared interferometry. Evidence to support the assignment of these bands includes comparison with spectra of small molecule sulfhydryls, isotopic shift in D_2O, pH titration, and comparison with animal hemoglobins which lack one or more cysteine residues. Quantitative measurements have allowed assignment of particular human hemoglobin absorption bands to SH stretching modes of the α-104 and β-112 cysteine residues, respectively. Band shapes and integrated intensities are interpreted in terms of molecular interactions present at the $\alpha_1\beta_1$ interface. In preliminary reports of some of this work, we proposed a model with intrachain H-bonding of the α-104 and β-112 sulfhydryl groups (Alben et al., 1974; Bare et al., 1974a,b).

Methods

Preparation of Carboxyhemoglobins. Fresh human, pig, horse, and cow (Holstein) blood samples (35–50 ml each)

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were collected in heparin or 0.013 *M* sodium citrate. Red cells were washed three times with 1% saline and hemolyzed with 1 volume of H_2O and 0.5 volume of toluene. After centrifugation, hemolysates were passed through a 2.5 × 100 cm Sephadex column equilibrated with 0.05 *M* bis-tris[1] buffer (pH 7.1) containing 0.1 *M* total chloride. The resulting solutions were concentrated by pressure ultrafiltration (Amicon) to a heme concentration of 11–18 m*M* and then exposed to pure CO gas for at least 3–4 hr before use. The foregoing preparations were conducted at 5–10°.

Preparation of Human HbCO in D_2O. A hemolysate of fresh human red cells was prepared as described above except that D_2O was used in the hemolysis step instead of H_2O. The hemolysate was concentrated by pressure ultrafiltration to half its original volume and diluted with an equal volume of D_2O. This step was repeated and the solution was again concentrated. The resulting 18 m*M* (heme) solution was equilibrated with CO gas. The replacement of H_2O by D_2O was estimated to be greater than 90% since the near-infrared absorption of water at 1.43 μm was not observed in this preparation.

Effect of pH on Denatured Human HbCO. A fresh hemolysate of human red cells was equilibrated with CO gas and ultrafiltered to a heme concentration of 18 m*M*. Aliquots of this solution were dialyzed against 200 volumes of a 0.1 *M* carbonate–0.025 *M* borate buffer titrated to various pH values between 7.0 and 11.5. Crystalline guanidinium chloride (Eastman) was dissolved in aliquots of the solutions to a final concentration of 6 *M*. The clear, viscous solutions were immediately loaded into infrared cells and visible, near-infrared, and infrared spectra were taken. The pH of the remaining solution was then measured.

Denatured HbCO solutions resulting from the addition of sodium dodecyl sulfate (Sigma) to a concentration of 0.5 *M* were gelatinous and frequently contained gas bubbles which made them unsuitable for infrared spectroscopy. Only those samples which contained no detectable bubbles were used for quantitative studies.

Determination of Reactive SH Groups of Human and Cow HbCO. Reactive SH groups of human and cow HbCO and of 0.04 m*M* β-mercaptoethanol were determined by the method of Boyer (1954) in 0.1 *M* phosphate buffer (H 6.0).

Preparation of Sperm Whale Metmyoglobin and Cytochrome c. Sperm whale metmyoglobin (Miles Seravac) and Type III oxidized horse heart cytochrome *c* (Sigma) were each dissolved in H_2O to a heme concentration of about 11 m*M*. The measured pH of the solutions was 6.0 and 8.0, respectively.

Spectroscopy. Infrared spectra at 2-cm^{-1} resolution were taken of samples in infrared cells with CaF_2 windows and a 0.2-mm path. A Digilab Model FTS-14 interferometer was equipped with a liquid N_2 cooled InSb detector or with a cooled (Hg–Cd)Te detector for spectra of myoglobin, cytochrome *c*, and carboxyhemoglobin in D_2O. Spectra were taken of samples at ambient temperature (28°). All infrared frequencies are corrected to vacuum. Band areas were determined by planimetry (Alben and Fager, 1972).

Visible and near-infrared spectra were taken in our infrared cells with a Perkin-Elmer Model 4000A spectrophotometer. Hemoglobin concentrations were measured as HbCN assuming $\epsilon_{540} = 11.5$ (m*M* heme)$^{-1}$ cm^{-1}.

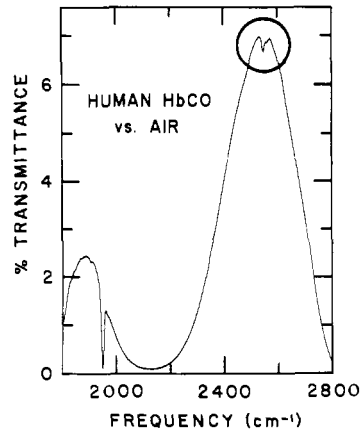

FIGURE 1: Infrared transmittance spectrum of aqueous human carboxyhemoglobin vs. air. The hemoglobin SH absorptions occur in the center of the circled area. The spectrum was taken of 17.0 m*M* (heme) HbCO in a cell with 0.2-mm optical path and CaF_2 windows using a Digilab Model FTS-14D interferometer equipped with a liquid nitrogen-cooled InSb detector. Four sets of 64 coherently coadded interferograms were independently subjected to Fourier transformation and were, in turn, coadded to yield a digital single beam spectrum at 2-cm^{-1} resolution. This was ratioed against a similarly obtained spectrum of air derived from four sets of 256 interferograms and plotted in transmittance with respect to frequency.

Experimental Results and Discussion

Spectra of aqueous solutions were measured by Fourier transform infrared interferometry with use of a relatively long light path through the sample (0.2 mm). At this path length, water absorbs nearly all of the incident radiation but has a transmittance maximum of about 7% of the incident radiation in the spectral region where sulfhydryl groups are known (Gordy and Stanford, 1940; de Alencastro and Sandorfy, 1972) to absorb (Figure 1). The small absorption peak observed in the region of maximum transmission will be shown to be due to the sulfhydryl groups of hemoglobin. Absorbance spectra, computed from data similar to those of Figure 1, are shown in Figure 2. The absorption bands observed with human carboxyhemoglobin A are not observed in cow CO-hemoglobin, sperm whale metmyoglobin, horse heart cytochrome *c*, or water. Thus the absorption bands in question are absent in water or in heme proteins (myoglobin and cytochrome *c*) known to contain no sulfhydryl groups. Our failure to observe sulfhydryl absorptions in cow hemoglobin was somewhat surprising since cysteic acid (which was only assumed to represent cysteine) has been assigned solely to the β-93 positions in this hemoglobin (Schroeder et al., 1967a,b). *p*-Mercuribenzoate titration (Figure 3) showed that our sample of cow hemoglobin contains two reactive sulfhydryl groups per tetramer which must be assigned to the β-93 cysteine residues. The β-93 sulfhydryl groups must therefore contribute very little to the infrared sulfhydryl spectrum observed in human hemoglobin A.[2] The sulfhydryl groups of β-93 cysteines react readily with water-soluble reagents, and are therefore probably solvated by rapidly exchanging water molecules. To this extent, they

[1] Abbreviations used are: bis-tris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; Hb, hemoglobin; HbASSG, the disulfide adduct of glutathione with hemoglobin A at the β-93 cysteine residues; Gdn · Cl, guanidinium chloride; SDS, sodium dodecyl sulfate.

[2] A sample of hemoglobin A, which was reacted with oxidized glutathione to yield HbASSG (Birchmeier et al., 1973) gave an SH infrared spectrum indistinguishable from that of fresh HbA.

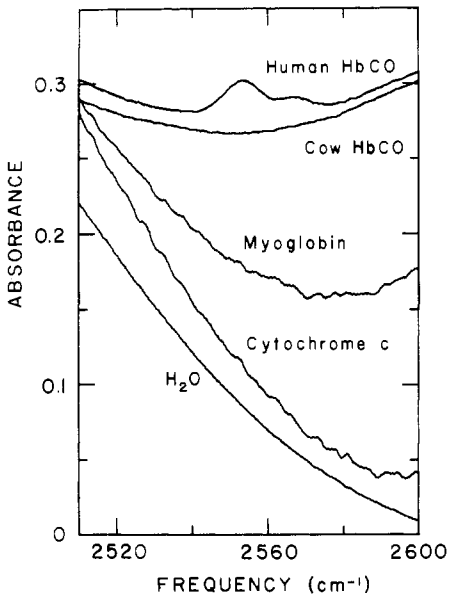

FIGURE 2: Infrared absorbance spectra, each vs. air, of (from top to bottom) human carboxyhemoglobin (17 mM heme), cow carboxyhemoglobin (16 mM heme), sperm whale met myoglobin (11 mM), horse heart cytochrome c (10 mM), and water. Base-line positions are arbitrarily shifted for convenience of presentation. Infrared spectra of samples were taken as described in the legend to Figure 1 except that a liquid nitrogen cooled (Hg-Cd)Te detector was used to collect the myoglobin and cytochrome c spectra.

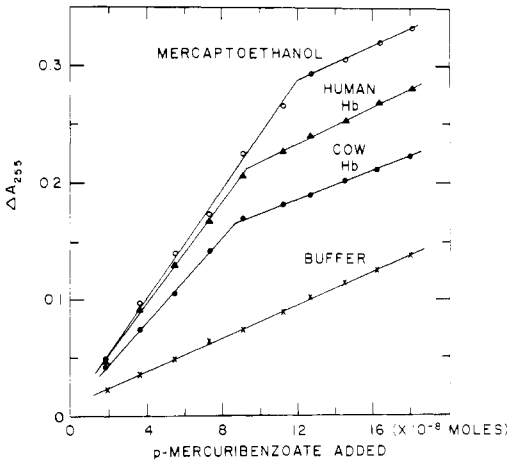

FIGURE 3: Titration of human and cow HbCO β-93 SH groups and mercaptoethanol with p-mercuribenzoate (PMB). The absorbance at 255 nm was monitored as 10-μl aliquots of 1.8 mM PMB were added to 3 ml of 0.05 mM human and cow HbCO, to 0.04 mM β-mercaptoethanol, and to the buffer (pH 6.0, 0.1 M phosphate). Hemoglobin concentrations were determined after the titration as cyanmethemoglobin. The end points correspond to 2.3 ± 0.3 and 2.2 ± 0.4 reactive SH groups/Hb tetramer for human and cow Hb, respectively, and to 1.0 ± 0.03 SH groups/mol of β-mercaptoethanol.

may be similar to sulfhydryl groups of aqueous ethanethiol. If their integrated infrared absorption coefficients (see below and Table III) are also similar, then they should absorb less than 10% as intensely as the bands that are observed with hemoglobin A, and thus should not be observed under these conditions of measurement.

In order to more firmly establish that these absorption bands are in fact due to sulfhydryl groups, we measured isotope effects on spectral frequency. Isotopic mass effects on absorption frequencies and intensities have been discussed by several authors (Herzberg, 1945; Wilson et al., 1955;

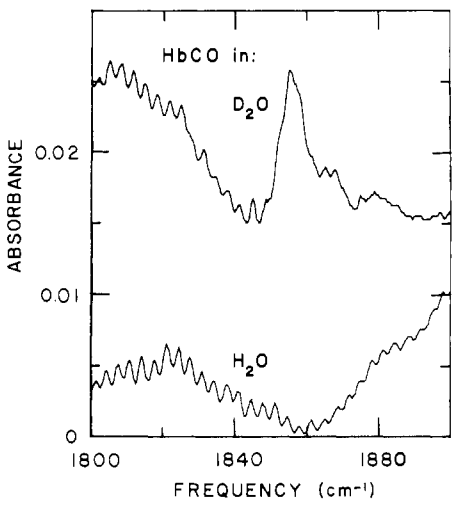

FIGURE 4: Infrared spectra of human HbCO prepared in D_2O and H_2O, vs. D_2O and H_2O, respectively. Single beam spectra were collected as in Figure 1 except that the (Hg-Cd)Te detector was used and four sets of 256 interferograms were collected. Single beam spectra were initially ratioed vs. air. Absorbance spectra of D_2O or H_2O were digitally subtracted from the sample spectra. A straight line was also subtracted from the digital absorbance spectrum of HbCO in D_2O to improve the flatness of the base line. Hemoglobin heme concentrations were 18 mM (in D_2O) and 13 mM (in H_2O).

Table I: Isotopic Frequency Dependence of Sulfhydryl Groups of Carboxyhemoglobin in Water or Deuterium Oxide.[a]

	ν_{SH} (cm^{-1})	ν_{SD} (cm^{-1})	ν_{SD}/ν_{SH}
Human HbA(CO)			
α-104 cys[b]	2552.6	1855.0	0.7267
Methanethiol			
Vapor[c]	2605	1893	0.7267
Liquid[c]	2550	1854	0.7271

[a] The natural frequency of a diatomic harmonic oscillator is $\nu = (1/2\pi)\sqrt{k/\mu}$, where k is a force constant and μ is the reduced mass, $m_1m_2/(m_1 + m_2)$. If the force constant is assumed to be independent of isotopic mass, the ratio of frequencies with isotopic substitution is $\nu^*/\nu = \sqrt{\mu/\mu^*}$. This leads to a mass-only value of $\nu_{SD}/\nu_{SH} = 0.71772$. Deviations from this ratio are usually ascribed to anharmonicity. [b] Center frequencies were measured by comparison with computed Lorentzian functions, as in Figure 5. [c] Data from May and Pace (1968).

Pinchas and Laulicht, 1971). Spectra of carboxyhemoglobin A equilibrated with D_2O (Figure 4) show an absorption band due to the S-D stretching vibration which is absent in H_2O solution. The spectral frequency shift due to deuterium substitution is nearly the same as that calculated for a harmonic oscillator, due to mass effect only (Table I) and is virtually identical with the frequency shifts that are reported for liquid or gaseous methanethiol (May and Pace, 1968). The observed intensity (cf. Table III) is in reasonable agreement with the predicted value for deuterium substitution. The deuterium exchange established that these absorption bands involved a proton, and the virtual identity of the isotopic frequency shift with that of methanethiol strongly supports their assignment to an S-H stretching vibration.

Assignment of S-H Absorption Bands to α-104 and β-112 Cysteines. Assignments to specific cysteine residues are based on a comparison of S-H spectra of human and animal carboxyhemoglobins (Figure 5). Cow carboxyhemoglo-

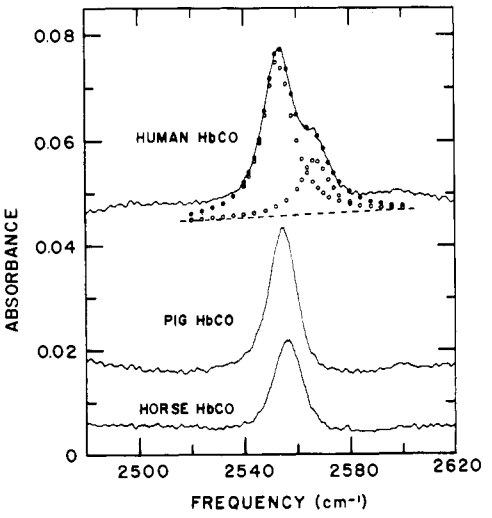

FIGURE 5: Infrared absorbance difference spectra of human (17 mM heme), pig (16 mM), and horse (11 mM) carboxyhemoglobins, each vs. cow carboxyhemoglobin as reference. Single beam spectra were collected as described in the legend to Figure 1 and initially ratioed against a single-beam spectrum of air. A similarly obtained digital absorbance spectrum of water was then subtracted from each sample spectrum. The water content of the samples was determined from a near-infrared absorption band of water at 1.92 μm. The spectrum of cow hemoglobin was treated similarly, and after normalization to the appropriate hemoglobin concentration, was subtracted from each of the human, pig, and horse absorbance spectra. This produced absorbance spectra containing only the hemoglobin SH absorptions of interest. Hemoglobin solutions were buffered to pH 7.1 with 0.05 M bis-tris and contained 0.1 M chloride. A sum of two Lorentzian (Cauchy) functions (●) (Ramsey, 1952), computed to fit the observed spectrum of human HbCO, was used to estimate individual SH absorption bands (○) contributed by the α-104 and β-112 cysteines.

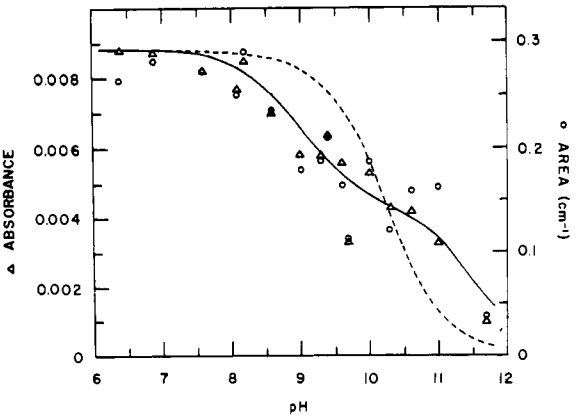

FIGURE 6: pH dependence of S–H infrared intensity of human carboxyhemoglobin in 6 M Gdn · Cl. Spectra were collected similarly to those in Figure 5 except that both sample and reference (cow hemoglobin) contained Gdn · Cl at the appropriate pH. The absorbance maximum (Δ) and integrated intensity (○) were measured from each spectrum and gave similar results. The dashed line is a calculated pH titration curve for a single pK of 10.25. The solid line represents two simultaneous equilibria with pK values of 9.0 and 11.5. The SH absorption band is half-maximal at pH 10.25 (the midpoint), but the titration is nearly complete at the highest pH observed (11.7).

Table II: Correlation of Infrared S–H Absorption Bands with Cysteine Residues in Hemoglobins.

Species	Cysteine Residues/$\alpha\beta$ Dimer α-104	β-93	β-112	No. of S–H Bands Observed
Cow	0	1	0	0
Horse	1	1	0	1
Pig	1	1	0	1
Human	1	1	1	2

bin, which possesses only the β-93 cysteines, is the reference for all three spectra, so that no contribution from this S–H group can be observed. Horse and pig hemoglobins contain, in addition, α-104 cysteine, and their S–H spectra consist of a single, symmetrical absorption band of similar intensity and frequency to the lower frequency, higher intensity absorption band of human carboxyhemoglobin (Table II). We therefore assign the band at 2552.6 cm^{-1} from human HbCO to α-104 (G11) cysteine. The addition of the β-112 cysteine residue in human Hb is accompanied by the appearance of a new S–H band at 2566.3 cm^{-1} which appears as a shoulder in Figure 5. In order to account for the unusually intense peak absorptions and integrated absorption areas of the α-104 and β-112 cysteine S–H groups, we have proposed (Alben et al., 1974) that both S–H groups are strongly hydrogen bonded to main chain amide carbonyl oxygen atoms in the respective α-G and β-G helices.

Properties of Hemoglobin S–H Absorptions under Dissociating Conditions. The S–H absorption bands, due to cysteine residues at the $\alpha_1\beta_1$ interface, should be sensitive to dissociating conditions. Guanidinium chloride (Gdn · Cl) or

Table III: Infrared Absorption Bands of Sulfhydryl Groups in Carboxyhemoglobins from Humans and Animals, and Ethanethiol in Several Solvents.[a]

	ν_{SH} (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	a_{mM} (mM^{-1} cm^{-1})	ϵ_{mM} (area) (mM^{-1} cm^{-2})
Human carboxyhemoglobin				
in H_2O: α-104	2552.6	13.5	0.17	2.43
β-112	2566.3	12.5	0.055	0.80
in D_2O: α-104 (S-D)	1855.0	9.5	0.06	0.57
in 0.5 M SDS	2559.4	27.2	0.083	2.52
in 6 M Gdn·Cl	2558.6	32.0	0.069	2.05
Pig carboxyhemoglobin α-104	2554.1	11.9	0.178	2.58
Horse carboxyhemoglobin α-104	2555.6	12.5	0.144	2.00
Ethanethiol (0.1 M)				
in carbon tetrachloride	2579.8 (2584,[b] 2580[c])	24.2	0.0018	0.070 (0.037,[b] 0.100[c])
in water	2573.6	39.5	0.0073	0.210
in acetone	2564.8 (2570[d])	30.2	0.0132	0.429 (0.62[d])
in dimethylacetamide	2534	58	0.021	1.35

[a] Absorbance, $A = \log(I_0/I)$, was used to calculate millimolar absorptivity, $a_{mM} = A/(cl)$, and the integrated absorption coefficient, ϵ_{mM} (area) $= [1/(cl)] \int A\, d\nu$. These are "practical units" (Wexler, 1967) except that millimolarity is used for concentration. [b] Bulanin et al., 1959. [c] Spurr and Byers, 1958. [d] Data for 5 M n-propylthiol in acetone from Bulanin et al., 1959.

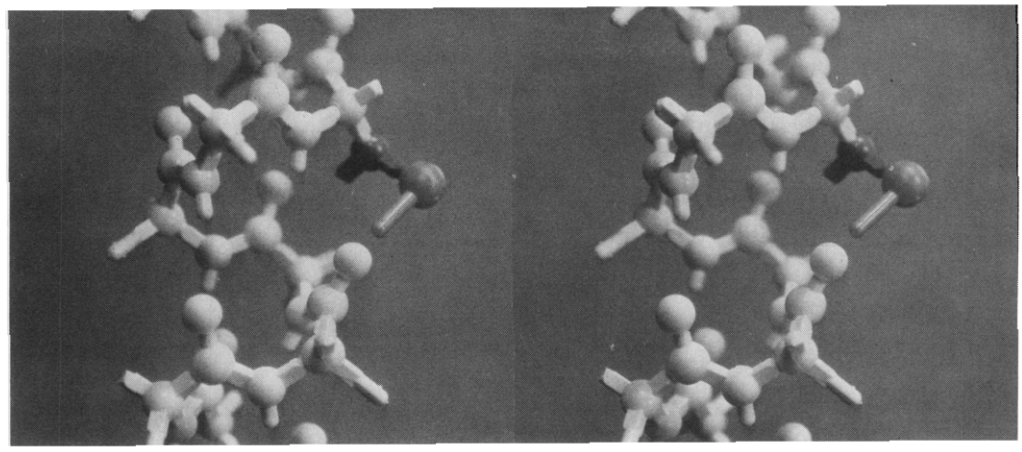

FIGURE 7: Stereo photograph of a molecular model of the α-chain G helix, which was built from coordinates (Perutz, 1968) for horse oxyhemoglobin (Labquip Corp., Caversham, England). The α-104 cysteine S–H group is arbitrarily rotated to illustrate the proposed H-bonding to the peptide carbonyl of α-100 leucine.

sodium dodecyl sulfate (SDS) was therefore added to samples of carboxyhemoglobin at neutral and alkaline pH. The spectra show broad absorption bands in the presence of the detergent which diminish with increasing pH and disappear under highly alkaline conditions. The absorption bands are strongly overlapped and are not resolved into contributions from separate S–H groups. The broadened band shape (cf. $\Delta\nu_{1/2}$, Table III) indicates that the α and β chains are dissociated under these conditions, and that the S–H groups are exposed to exchanging solvent molecules. Based on intensity measurements with small molecules (see below), the relatively large integrated absorption coefficients (Table III) are interpreted to indicate that the S–H groups remain hydrogen-bonded and highly polarized in detergent, as in the native molecule; i.e., that at least the secondary structure of the G helices is still intact. It should be noted that the guanidinium group can act only as a H-bond donor, so that the H-bond acceptor group must come from the same chain as the S–H group under these dissociating conditions.

A pH titration of carboxyhemoglobin in 6 *M* Gdn · Cl (Figure 6) was undertaken to determine whether the S–H groups were titratable in the pH range (6.5–10.8) reported by Jocelyn (1972) for small molecule thiols, and to compare the results with the intrinsic pK' value of 9.1 assumed for the β-93 S–H (Bucci et al., 1968; Tanford and Nozaki, 1966). It previously has not been possible to observe specific dissociation of an S–H group in proteins, uncomplicated by other dissociating groups. We found that some of the broad underlying absorption bands, due to other groups in the protein, also changed with pH, producing a corresponding change in infrared baseline absorptions. This was adequately corrected by use of cow carboxyhemoglobin at appropriate pH values in 6 *M* Gdn · Cl, as a reference. The titration curve has a midpoint near pH 10.25, but is not well-fitted by a curve computed for a single titratable group. A much better fit is obtained by use of two pK' values of 9 and 11.5. Although it is premature to assign structural meaning to our apparent pK values, the very high pH required for complete titration suggests persistent strong H-bonding of S–H groups in Gdn · Cl.

Intensity Measurement. In order to account for the unusually intense absorption of the α-104 and β-112, in contrast to the β-93, hemoglobin sulfhydryl groups, we studied the relation between nearest neighbor molecular interactions and absorption intensity. The increase in integrated absorption intensity (band area) with H-bonding is well established in small molecules by the concentration dependence of the S–H absorption of alkyl thiols (Spurr and Byers, 1958), and by the dependence of absorption intensity on the ortho substituent, but not on the para substituent of thiophenols (Mori et al., 1971). Bulanin et al. (1959) have demonstrated that S–H absorption intensity increases with polarity of H-bond acceptor solvents. Theoretical interpretations of solvent interactions (Buckingham, 1960) and H-bonding (Sandorfy, 1972) have been presented. We have measured the absorption intensities of ethanethiol in CCl_4, H_2O, acetone, and dimethylacetamide (Table III). The integrated intensity of the S–H absorption band is about three times greater in water than in CCl_4, about twice as great in acetone as in water, and three times greater in dimethylacetamide than in acetone. Thus, the stronger H-bond acceptor leads to greater polarization of the S–H bond along the internuclear axis, and therefore to a greater integrated absorption intensity.[3]

The observed integrated absorption coefficient (Table III) for the α-104 S–H group is about twice that of 0.1 *M* ethanethiol in dimethylacetamide, while that of the β-112 cysteine is approximately half as large. We conclude that the S–H group of the α-104 cysteine is very strongly polarized in native human hemoglobin A, while that of the β-112 cysteine is somewhat less polarized, but still strongly H-bonded. As previously noted, the β-93 cysteine in carboxyhemoglobin is exposed to the aqueous solvent. Its S–H group is presumably weakly and more randomly hydrogen bonded to solvent water molecules, thus producing a relatively broad and very weak absorption which is unobservable under our conditions.

We constructed molecular models (Labquip Co., Caversham, England) of horse oxyhemoglobin with coordinates from Perutz (1968). A model of the horse β-chain G helix, which contains no cysteine, shows the peptide carbonyl and amide groups reasonably well aligned into a good α-helical structure. The horse α-chain G-helix model (Figure 7) also shows the peptide groups reasonably well aligned except for

[3] Integrated absorption intensity, or area under an absorption band ($\int A d\nu = \epsilon_{mM}(\text{area})cl$), is a function of the integrated absorption coefficient, $\epsilon_{mM}(\text{area})$, which in turn is proportional to $(\partial\mu/\partial Q)^2$, and thus depends upon the change in dipole moment (μ) with the vibrational coordinate Q.

the carbonyl of the G-7 (α-100) leucine, which appears to be bent outward from the helical axis so that it is not aligned with the corresponding amide (G-11, α-104 cys), but is ideally positioned to H-bond with the S–H group of that residue. Such a H-bond would be stabilized by the surrounding nonpolar protein matrix and would also account for the high peak intensity of the infrared S–H absorption band. A similarly strong H-bond structure would account for the α-104 cysteine S–H absorption of human hemoglobin, and a somewhat weaker H-bond is suggested for the β-112 S–H absorption. The S–H absorption intensities of human hemoglobin A in Gdn · Cl or SDS (Table III) provide support for the presence of intrachain H-bonds, since the S–H groups remain strongly polarized (absorb intensely) even though the α and β chains are dissociated and their absorption bands are broadened by solvent.

Conclusions

We have described the first spectroscopic observation of sulfhydryl groups in a protein. These observations are especially important in the case of human hemoglobin A, since they provide a spectroscopic probe at the $\alpha_1\beta_1$ interface, which previously has been inaccessible. These sulfhydryl absorptions have been shown (Alben et al., 1974; Alben et al., to be published) to be highly sensitive to the state of ligation, and to the tertiary or quaternary structure of the protein.

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