pH-Dependent Soret Difference Spectra of the Deoxy and Carbonmonoxy Forms of Human Hemoglobin and Its Derivatives†

Shiv K. Soni and Lutz A. Kiesow*

ABSTRACT: The transition from deoxy to oxystructure of hemoglobin A (Hb) is accompanied by the breaking of the salt bridges formed by C-terminal residues in deoxy-Hb. This, in turn, changes the state of the heme. The switch between these different allosteric forms can be followed by changes in the optical absorbance spectra (Perutz, M. F., Ladner, J. E., Simon, S. R., and Ho, C. (1974), *Biochemistry 13,* 2163). Using difference spectroscopy in the Soret region, pH-dependent spectral changes of Hb and its derivatives (carbamylated at both the α-NH_2 groups, $\alpha_2^c\beta_2^c$; *N*-ethylsuccinimide hemoglobin, NES-Hb) in their deoxy and carbonmonoxy forms were measured. From these measurements, the p*K* values of histidine-146β and valine-1α in deoxy-Hb were determined to be 8.6 ± 0.2 and 7.7 ± 0.1, respectively. In carbonmonoxy-Hb a p*K* value of 6.3 ± 0.1 was found.

The existence of salt bridges formed by C-terminal residues of deoxyhemoglobin represents the major difference between deoxy and oxy structure of hemoglobin (Hb[1]). In deoxy-Hb, the C-terminal histidine-146β interacts with lysine-40α and aspartate-94β, while C-terminal arginine-141α forms salt bridges with valine-1α and aspartate-126α of the other α chain (Muirhead and Greer, 1970; Perutz, 1970). More recently, these salt bridges have elicited great interest because they confer on the Hb molecule unique properties which are essential for its normal structure and function. Any alterations in these interactions due to amino acid substitutions lead to the development of clinical symptoms by changing the Hb-oxygen affinity (Morimoto et al., 1971; Perutz and Lehmann, 1968; Hamilton et al., 1969; Wajcman et al., 1975). The functional properties have also been shown to change in modified Hb's in which residues forming salt bridges are either blocked or removed (Kilmartin et al., 1975; Kilmartin and Hewitt, 1971; Kilmartin and Wootton, 1970; Kilmartin et al., 1973a; Kilmartin and Rossi-Bernardi, 1969). Based on studies with modified and mutant Hb's, the phenomenon of cooperativity between binding sites of the Hb molecule has been suggested to involve these salt bridges (Perutz and TenEyck, 1971). Two of the groups, i.e., histidine-146β and valine-α, which are involved in these salt bridges, have particular physiological significance. Histidine-146β contributes to the Bohr effect only while the α-NH_2 group of valine-1α, in addition to contributing to the Bohr effect, also binds CO_2, and approximately 10% of the physiologically produced CO_2 is transported by Hb through its binding with this group (Baldwin, 1975). On the basis of specific chemical modifications and enzymatic cleavage of these groups, along with x-ray studies of hemoglobin, histidine-146β and valine-α are clearly identified as alkaline Bohr groups (Hamilton et al., 1969; Kilmartin and Wootton, 1970; Kilmartin and Rossi-Bernardi, 1969; Riggs, 1961; Benesch and Benesch, 1961, 1962; Kilmartin and Rossi-Bernardi, 1971; Kilmartin et al., 1973b; Perutz et al., 1969; Muirhead and Greer, 1970). These groups contribute approximately 70% to the total alkaline Bohr effect by virtue of their p*K* change on ligation.

Any quantitative analysis of the Bohr effect is critically dependent upon the precise determination of p*K* values of the groups involved. By comparing the proton resonance spectra at different pH values of normal and des-histidine-146β human deoxy-Hb, the p*K* value of histidine-146β was determined to be 8.1 at 25 °C (Kilmartin et al., 1973a). Greenfield and Williams (1972) had estimated a value of about 8.1 at 32 °C, but the assignment of the resonance peak to the C2 proton of histidine-146β in deoxy-Hb was very tentative. Furthermore, these values are not in agreement with the value of 7.4 obtained by Huestis and Raftery (1972). Edsall and Wyman (1958) have given the p*K* value of 7.7–8.2 for the α-NH_2 group. Based on detailed study of pH dependence of carbamylation of terminal amino groups, Garner et al. (1975) have calculated a p*K* value of 7.8 for valine-1α in human deoxy-Hb. This value agrees with that of Kilmartin et al. (1973a). The present investigation is an effort to confirm p*K* values of Bohr groups by changes in optical absorbance. The underlying principle of the optical method is the same as recently reported by Perutz et al. (1974). The transition between the two allosteric forms of Hb is brought about by the breaking of the salt bridges. This switch between oxy and deoxy structures changes the electronic state of heme, which can be studied quantitatively by changes in the optical absorbance spectra. Using difference spectroscopy, we investigated the pH-dependent spectral changes in the Soret region of Hb and its derivatives (carbamylated at both α-NH_2 groups of α and β chains, $\alpha_2^c\beta_2^c$; *N*-ethylsuccinimide hemoglobin, NES-Hb) in their deoxy and carbonmonoxy forms.

Materials and Methods

Hemolysates of adult human Hb were prepared as mentioned elsewhere (Soni and Hill, 1975). The Hb solution was concentrated in an Amicon ultrafiltration cell (Model 202) under oxygen or carbon monoxide instead of nitrogen to avoid any formation of ferri-Hb during concentration. The oxy and carbonmonoxy forms of the Hb derivative $\alpha_2^c\beta_2^c$ were prepared by the method of Williams et al. (1975) with the fol-

† From the Department of Experimental Medicine, Naval Medical Research Institute, Bethesda, Maryland 20014. *Received September 29, 1976.* Supported by a National Research Council–Naval Medical Research and Development Command Research Associateship to S.K.S.

[1] Abbreviations used are: Hb, hemoglobin; $\alpha_2^c\beta_2^c$, hemoglobin carbamylated at both α-NH_2 groups of α and β chains; NES-Hb, *N*-ethylsuccinimide hemoglobin; COHb, carbonmonoxyhemoglobin; DEAE, diethylaminoethyl; TEA, triethanolamine hydrochloride; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

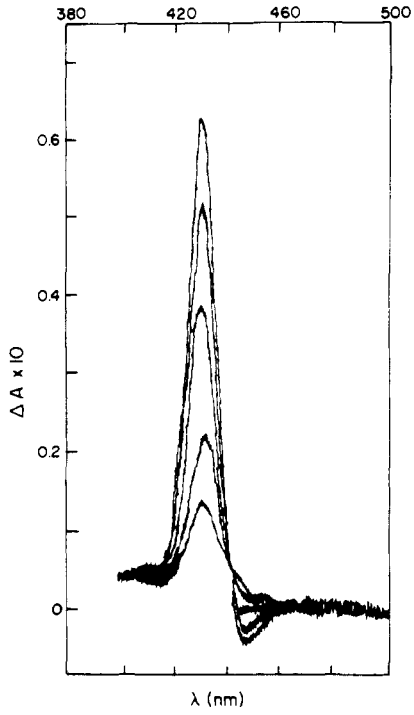

FIGURE 1: pH-dependent difference spectra of 3.86 μM human deoxyhemoglobin at 25 °C in 0.05 M triethanolamine hydrochloride, (Cl^-) = 0.1 M. Reference pH 9.5, sample pH corresponding to each increasing peak at 430 nm is 8.84, 8.65, 8.40, 7.75, and 7.26, respectively.

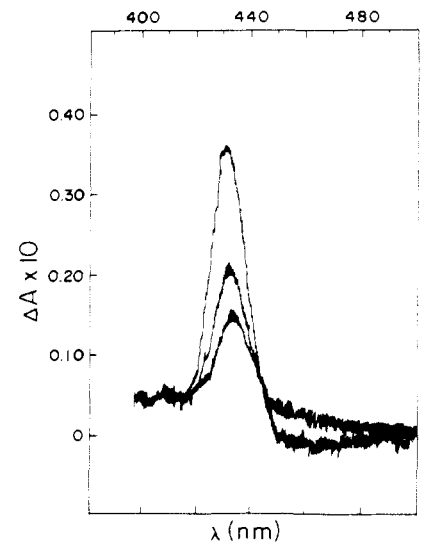

FIGURE 2: pH-dependent difference spectra of 3.60 μM human carbamylated deoxyhemoglobin ($\alpha_2^c\beta_2^c$) under experimental conditions of Figure 1. Reference pH 9.5, sample pH corresponding to each increasing peak at 430 nm is 8.80, 8.52, and 8.20, respectively.

lowing modifications. In the reaction of Hb with potassium cyanate, the Hb concentration was 6–7 g % instead of 2–3 g %. The pH gradient for the DEAE-cellulose (DE-52 Whatman) column was between pH 7.0 ± 0.02 and 8.0 ± 0.02 instead of 7.6 and 8.1 with 0.05 M Tris buffer. The reaction product of Hb and cyanate was then filtered thru Millipore filters (pore diameter 0.45 ± 0.03 μm) and dialyzed against three changes of 0.05 M Tris buffer, pH 8.0 ± 0.02, to remove excess potassium cyanate. The elution pattern is identical to that obtained by Williams et al. (1975). The use of DEAE-50 Sephadex prior to the use of DEAE-cellulose (DE-52 Whatman) was abandoned because of its extremely long elution time under the conditions of the experiment. The rest of the procedure was identical with that of the above reference. The final product was dialyzed against three changes of 0.1 M NaCl, stored under CO or O_2, and used as promptly as possible.

N-Ethylmaleimide (Aldrich, Gold label) was used without further purification. NES-Hb was prepared by the reaction of *N*-ethylmaleimide (NEM) with cysteine-93β (Benesch and Benesch, 1962; Guidotti and Konigsberg, 1964), to yield a hemoglobin derivative in which histidine-146β does not form salt bridges with aspartate-94β.

The pH values of the individual solutions were measured with a Corning digital research pH meter (Model 112). Hb samples of different pH values were prepared in 0.05 M triethanolamine hydrochloride containing NaCl to provide final Cl^- concentrations of 0.1 M. The volume ratio of this buffer to protein solution was between 50 and 100. The pH values of deoxygenated samples were measured by an indwelling combination electrode at 25 °C. The pH of the buffered protein solution was essentially the same as that of the buffer. The pH of the reference solution was kept constant at pH = 9.5, while the pH of the sample solution was varied.

Oxy-Hb and its derivatives were deoxygenated at room temperature by passing H_2O saturated, ultrapure, nitrogen for 40 min over Hb solutions contained in a Warburg flask, which was shaken on a shaker platform. Two identical Hb samples, which only differed in their pH values, were deoxygenated simultaneously. The anaerobic transfer of the solution was accomplished by means of a polyethylene capillary tubing with one end attached to the side arm of the flask and the other end placed in the cuvette to which sample was subsequently transferred (Kiesow et al., 1971). Nitrogen gas used for deoxygenation also flushed the cuvette. Cuvettes had a capillary diffusion stopper (3 cm) to avoid any oxygenation of the sample during the experiment. Both cuvettes contained a few crystals of sodium dithionite to assure continued and complete deoxygenation of the samples.

Difference spectra were measured at 25 ± 0.2 °C in a thermostated cell adapter using a Cary 14 recording spectrophotometer with an 0.1 absorbance slide wire. The temperatures of sample and reference cells were maintained by a Haake, Model FSE, circulating thermostat. Pairs of matched stoppered cells were always used. Quartz absorption cells (10-mm Zeiss) were used at low Hb concentrations. The light path of 10-mm cells was reduced to 1 mm with two-way silica inserts for spectral measurements at higher Hb concentrations. The slit width varied between 0.3 and 0.5 mm, depending upon the Hb concentration. A high-intensity light source and a scanning speed of 2.5 Å/s were used in all measurements. Prior to all measurements, the Hb solutions were filtered through Millipore filters (pore diameter 0.45 ± 0.03 μm) in order to remove suspended impurities. To avoid any error in delivering protein solutions, the same Lang–Levy micropipet (accuracy ± 0.3%, Fisher Scientific Co.) was used for any particular series of experiments. For spectral measurements, the volume of buffer delivered was corrected by weight measurements on a Mettler analytical balance (Model S) before the protein solution was added and mixed with the buffer. Buffer was 0.05 M triethanolamine hydrochloride (TEA) containing NaCl to provide a final Cl^- concentration of 0.1 M. The protein solution was dialyzed against 0.1 M NaCl to keep it isomolar with the buffer Cl^- concentration and to eliminate anions, except chloride ions.

The oxygen equilibrium curves were measured by a Hem-

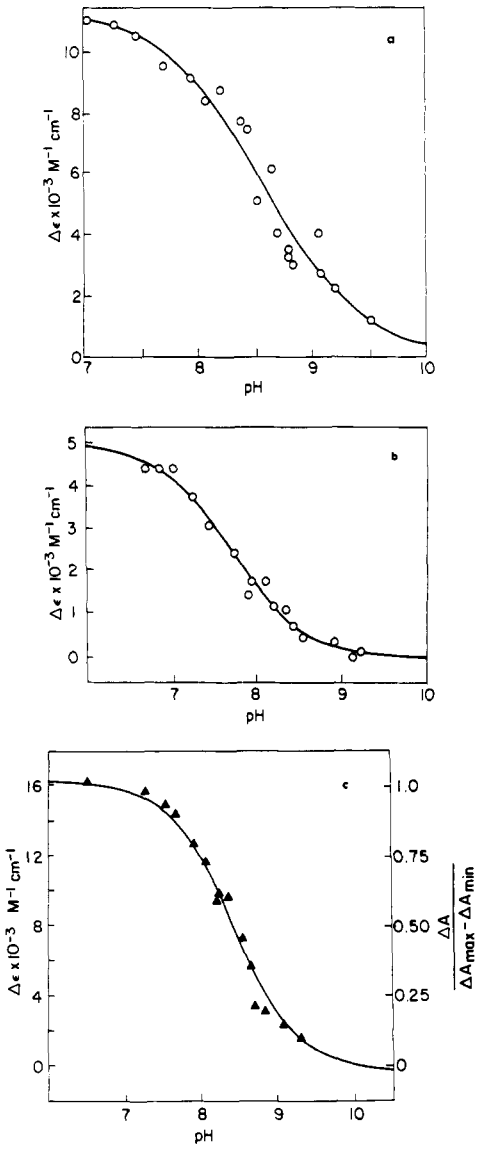

FIGURE 3: The change in extinction coefficient at 430 nm as a function of pH. (a) Deoxy-$\alpha_2^c\beta_2^c$ and (b) deoxy-NES-Hb. The solid line in a and b is the least-square fit of the data points based on a single proton reaction. (c) Deoxy-Hb; the solid line in c is the least-square fit based on a two-proton reaction. See text for details.

O-Scan oxygen-dissociation analyzer (American Instrument Co.). The essentials of this method were published previously by Kiesow et al. (1972, 1974).

Results

pH-Dependent Difference Spectra of Deoxy-Hb's. pH-dependent difference spectra of Hb and its derivatives in their deoxy forms were recorded in the Soret region at 25 °C. These are shown in Figures 1 and 2. Deoxy-Hb and deoxy-NES-Hb have absorbance maxima at 430 nm and minima at 445 nm with an isosbestic point at 440 nm. The isosbestic point does not lie on the baseline because of an instrument-dependent nonlinearity of the baseline at this wavelength range. pH-dependent spectral changes in deoxy-NES-Hb (not shown) with regards to wavelength location and isosbestic point (except for the intensity) are similar to those of deoxy-$\alpha_2^c\beta_2^c$ or deoxy-Hb. For the sake of clarity, only a few difference spectral curves are shown in Figures 1 and 2. However, similar Soret difference spectra between T and R states of deoxy-Hb have been observed by Perutz et al. (1974) and Sawicki and Gibson

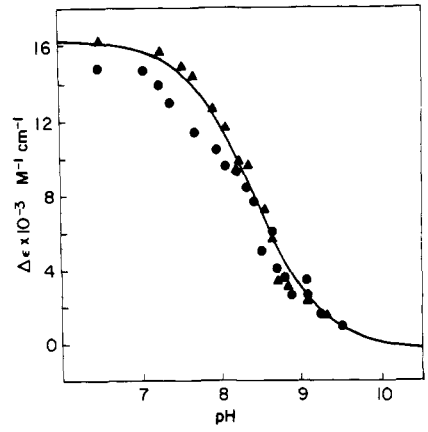

FIGURE 4: The sum ($\alpha_2^c\beta_2^c$ + NES-Hb) (●) of the spectral changes with pH from Figure 3a,b, and deoxy-Hb curve (▲) from Figure 3c, for comparison.

(1976). In our investigation, the magnitude of the difference spectrum for the R → T transition has been found to be the same as reported by Perutz et al. (1974) and Brunori et al. (1968).

The titration curves of Figures 3a,b,c were constructed from difference spectra as those exemplified in Figures 1 and 2. The solid line represents the least-square fit of the data points based on a single proton reaction, expressed by eq 1.

$$\Delta A = \frac{\Delta A_{min} + K[H^+]\Delta A_{max}}{1 + K[H^+]} \quad (1)$$

where ΔA is the change in differential absorbance at various pH values, while K is an association constant. The titration curves of deoxy-$\alpha_2^c\beta_2^c$ (Figure 3a) and deoxy-NES-Hb (Figure 3b) yielded pK values of 8.6 ± 0.2 and 7.7 ± 0.1, respectively.

Computational Analysis of Soret Absorbance Changes in Deoxy-Hb. Figure 3c represents the titration curve of unmodified deoxy-Hb. The absorbance changes in this curve appear, therefore, to originate from two groups, histidine-146β and valine-1α. This can be explained on the basis of eq 2 involving two association constants, K_1 and K_2, for these groups, which are obtained from the individual titration curve of deoxy-$\alpha_2^c\beta_2^c$ and NES-Hb by the use of eq 1.

$$\alpha x \left(\frac{[HbH^+]}{[THb]} + \frac{[HbH_2^+]}{[THb]}\right) = \frac{\Delta A}{\Delta A_{max} - \Delta A_{min}} \quad (2)$$

where $[HbH^+] = K_1[Hb][H^+]$; $[HbH_2^+] = K_1K_2[Hb][H^+]^2$; $[THb] = [Hb] + [HbH^+] + [HbH_2^+]$; α is the fraction of the total absorbance change that is caused by the HbH^+ species. [THb] is the total deoxy-Hb; $[HbH^+]$ and $[HbH_2^+]$ are the concentrations of singly and doubly protonated Hb species.

By nonlinear least-squares estimation on unmodified Hb, the value of α was estimated to be 0.8 ± 0.03. This is in good agreement with the experimentally found value of about 0.7, i.e., the total absorbance change in Figure 3a divided by the total absorbance change of Figure 3a plus Figure 3b. At this point, it may be recalled that the pH of the reference solution is 9.5; therefore, the protonated group in singly protonated Hb is histidine-146β. This group is responsible for the total spectral change in $\alpha_2^c\beta_2^c$ (Figure 3a), which is about 70–75% of the total spectral change in unmodified Hb, or to that of Figure 3a plus Figure 3b.

The sum of the spectral changes observed with two deoxy-Hb derivatives is also nearly identical to the spectral changes observed with deoxy-Hb alone, as shown in Figure 4,

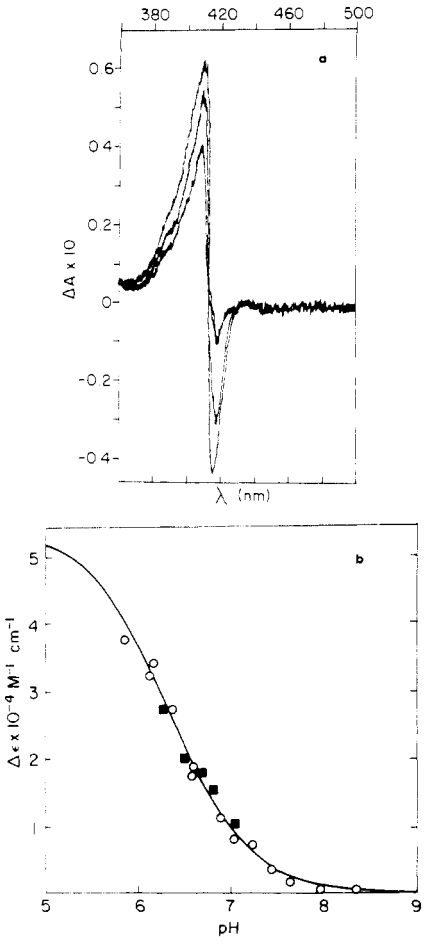

FIGURE 5: The spectral changes of carbonmonoxyhemoglobin as a function of pH under the experimental conditions described for Figure 1: (a) Difference spectra were obtained at a concentration of 32.6 μM at pH values 6.60, 6.40, and 6.15, which correspond to each increasing peak at 412 nm, respectively. b shows the changes in extinction coefficients between maximum absorbance at 412 nm and minimum absorbance of 422 nm. The solid line is the least-squares fit as in Figure 3a or 3b. The reference pH is 8.6. Concentration: (○) 32.6 μM; (■) 2.89 M.

indicating that the absorbance difference spectrum of the unmodified Hb is the result of protonation of at least two groups, namely, the imidazole group of histidine-146β and α-NH_2 group of valine-1α.

Oxygen Equilibrium Parameters. Hill's plots of oxygen equilibrium curves of Hb and its NES-Hb and $\alpha_2^c\beta_2^c$ derivatives were calculated in order to assure that no extensive structural alterations had occurred as a result of the chemical modifications. The values of Hill's constant, n, and the P_{50} (oxygen partial pressure at which Hb is half saturated) of normal and the two chemically modified hemoglobins at two pH values are summarized in Table I. The values of n were found to be in the normal range, indicating the lack of major structural changes due to chemical modification. This finding is supported by the work of Kilmartin et al. (1973b) and Perutz and TenEyck (1971) who have shown that neither chemical modifications of hemoglobin at the α-amino groups of both chains nor the steric attachment of NEM to cysteine-93β result in any significant conformational changes.

A small decrease in the value of n with an increase in pH or with the cleavage of the salt bridge could be expected due to a change of the allosteric equilibrium constant, L. However, the values shown in Table I reflect little change in n. The reason for this could be that a Hill constant near its normal value does

TABLE I: Oxygen Equilibrium Parameters.

	Temp 25 °C; pH 7.2		Temp 30 °C; pH 8.5	
Hemoglobin Derivative	$P_{50} \pm 0.2$ (mm Hg)	$n \pm 0.1$	$P_{50} \pm 0.2$ (mm Hg)	$n \pm 0.1$
Hb	7.0	2.8	3.7	2.7
NES-Hb	4.2	2.9	2.9	2.5
$\alpha_2^c\beta_2^c$	2.7[a]	2.7	1.7	2.7

[a] pH = 7.27.

not change even with large variations in the value of L (Baldwin, 1975).

pH-Dependent Difference Spectra of COHb. pH-dependent difference spectra of COHb at concentrations of 32.6 μM are shown in Figure 5a. The changes in the extinction coefficient at 422 nm plus 412 nm of COHb at two concentrations are shown in Figure 5b. These changes are identical at 2.9 and 32.6 μM COHb. However, at a COHb concentration of 2.9 μM, dimers are present at significant concentrations and are in equilibrium with tetrameric molecules (Kellet, 1971). This leads us to suggest that COHb dimers show spectral changes which are identical with those exhibited by tetrameric COHb. A similar observation has also been made previously by Anderson et al. (1971) with deoxy-Hb in the R structure.

The titration of COHb yielded a pK value of 6.3 ± 0.1, which is much lower than the pK values of histidine-146β and valine-1α in deoxy-Hb. Since in liganded Hb the salt bridges are broken, this clearly indicates that these spectral changes do not originate from these groups which form the salt bridges in the unliganded state. The possibility of histidine-146β and valine-1α contributing to the spectral changes is again ruled out on the basis of their higher pK values (in the range of 7) determined with COHb by other investigators (Kilmartin et al., 1973a; Greenfield and Williams, 1972; Garner et al., 1975; Hill and Davis, 1967).

Discussion

As mentioned earlier, the deoxy structure of hemoglobin is stabilized by various salt bridges. The transition from the deoxy to the oxy structure is accompanied by the breaking of the salt bridges of the C-terminal residues in deoxy-Hb which involve α-NH_2 groups of α chains and C-terminal histidines in β chains. These groups will lose their protons as the pH is raised, breaking their bonds with other groups. Conversely, these groups will be protonated as the pH is lowered forming bonds with their partner groups. Perutz et al. (1974) have attributed the R → T spectral transition to the movement of iron atoms out of the planes of the porphyrin rings. This is stereochemically equivalent to increasing the tension on the heme due to structural changes in the globin, which can be followed by changes in the optical absorbance spectra. In deoxy-NES-Hb, the SH group of cysteine-93β is substituted by N-ethylmaleimide, which prevents histidine-146β from forming a salt bridge with aspartate-94β and thus renders it free in solution preventing any contribution to the pH-dependent difference spectra. A link between α-NH_2 and C-terminal carboxyl groups of two α chains still exists at low pH and will contribute to the spectral change upon raising the pH. Thus, the titration of deoxy-NES-Hb yielded a pK of 7.7, which can be attributed to the α-NH_2 group of valine-1α. In the carbamylated hemoglobin, α-NH_2 groups of α and β chains cannot contribute to the spectral change; here, the spectral changes occur due to a titration of the salt bridge between histidine-146β and aspar-

tate-94β. The pK value of 8.6 resulting from the titration curve of $\alpha_2^c\beta_2^c$ is therefore attributed to histidine-146β.

Kilmartin et al. (1973a) have measured the pK value of histidine-146β by comparing the proton nuclear magnetic resonance spectra of normal and des-histidine-146β human Hb dissolved in D_2O at different pH and determined it to be 8.1 at 25 °C. However, these authors did not add 0.4 pH unit to the pK value in D_2O to correct for the isotope effect on the glass electrode because they assume the deuterium isotope effect on the ionization equilibrium occurs in an opposite direction and is perhaps of equal magnitude (Roberts et al., 1969). Our pK value of 8.6 is approximately 0.5 pH unit higher than the one reported by the above authors. Consequently, the reasons for this difference in pK are not yet clear.

The pK value of 7.7 of the α-NH_2 group of α chains agrees very well with the values obtained by Garner et al. (1975) and Kilmartin et al. (1973a), who reported values of 7.8 and 7.6, respectively. In contrast to the α-NH_2 of valine-1α, the same group of valine-1β is free in solution and does not make contact with neighboring groups in a manner that would perturb the heme on breaking its contact (Muirhead and Greer, 1970; and Perutz, 1970). In the pH range investigated, the possibility of this group being responsible for absorbance changes in the Soret region can again be eliminated on the basis of its low pK value of approximately 7, which does not appear to change on ligation (Garner et al., 1975).

In deoxy-Hb, the proximal and distal histidines cannot be the contributing groups. These groups are also present in myoglobin (Mb) and tadpole Hb, as well as in normal Hb; yet, Mb and tadpole Hb lack a Bohr effect (Taylor, 1939; and Riggs, 1951). This clearly indicates that these groups have unaltered proton-binding behavior in both the deoxy and oxy structure. X-ray studies (Perutz, 1970) also support this conclusion, since changes in the conformation of the heme-linked histidines which might cause alterations in their pK's were not observed between the oxy and deoxy forms. In the present study, the pK values obtained with ligated and unligated Hb are quite different, i.e., 6.3 in COHb and 8.6 and 7.7 in deoxy-Hb. Indirect evidence against proximal and distal histidines being the contributing groups to the optical change in deoxy-Hb also comes from a spectrophotometric study reported by George and Hanania (1953). They estimated by curve fitting the pK value of the proximal histidine to be 6.33. Since this pK value is identical to that obtained in our study, the possibility exists that the Soret absorbance changes in COHb involve this proximal histidine.

As a result of its close proximity to the proximal histidine and the α^1-β^2 interface, cysteine-93β is very sensitive to both heme ligation and changes in the quaternary structure (Shulman et al., 1975). The possibility that this group contributes to the spectral changes is precluded by its very high pK value, which results from the adjacent aspartic acid (94β). Based on the chemical studies of Snow (1962) and Guidotti (1967), the pK value of cysteine-93β is close to 10.

The Hb interfaces α^1-β^1 and α^1-β^2 are also very sensitive to the T $\rightarrow$ R transition. Consequently, a titration of groups along these interfaces could result in heme perturbations, which could contribute to spectral changes in the Soret region. Perutz (1969, 1970) suggested that histidine-122α at the α^1-β^1 interface may change its pK value upon ligation. Due to the close proximity of the negatively charged aspartate-126α, this group may have a higher pK in deoxy-Hb. However, on the basis of our difference-titration study between cross-linked (Macleod and Hill, 1970) and non-cross-linked COHb, a contribution from histidines along the α^1-β^1 plane can be excluded. No evidence was found that would indicate a titration of these histidines in the pH range of 6.0 to 9.5 (Soni et al., 1975). Briehl and Hobbs (1970) obtained pH-dependent ultraviolet difference spectra of Hb from which they determined pK values to be 7.0 and 7.7 in oxy- and deoxy-Hb, respectively. They assigned these values to histidine-97β, which resides along the α^1-β^2 interface. The pK value of 6.3 obtained with COHb in our study (instead of 7 obtained by Briehl and Hobbs, 1970) serves to exclude histidine-97β as a possible candidate for pH-dependent spectral changes.

Polar groups, such as those of aspartate, arginine, and lysine which are involved in the formation of salt bridges, are known to have pK values outside the range examined in this study (Tanford, 1962) and can therefore be excluded from our considerations altogether.

The pK value of hemic proton dissociation in aquomet Mb remains unaltered upon modification of exposed histidine groups by iodoacetamide (Hartzell et al., 1968). It seems therefore unlikely that ionizable groups at the surface of the hemoglobin molecule may cause a quaternary conformational change. X-ray studies (Perutz, 1970) also indicate that most of the interactions except those of C-terminal amino acids at the surface remain undisturbed by the reaction of the hemes with ligands.

Following this process of elimination, we therefore conclude that the major part of the Soret spectral changes in deoxy-Hb appears to stem from histidine-146β and valine-1α.

Acknowledgments

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Heavy-Chain Variable-Region Sequence from an Inulin-Binding Myeloma Protein†

Mark Vrana,* Stuart Rudikoff, and Michael Potter

ABSTRACT: The entire variable-region sequence of the heavy chain from ABE-47N, a BALB/c inulin-binding myeloma protein, has been determined. This protein is unusual in that the third complementarity region (H3) is extremely short, consisting of at the most three and probably only one amino acid. A comparison of the heavy-chain hypervariable regions from mouse, human, and rabbit proteins shows that the variability in length of H3 is greater than that seen in the first or second hypervariable regions. This variability in H3 length suggests a specialized function for this region.

Hapten-binding mouse myeloma proteins have been used for several years as models to study the structural relationships among proteins with similar binding specificities and the three-dimensional structure of the antibody combining site. Extensive primary structural data have been obtained on the heavy chains from seven phosphorylcholine-binding proteins (Rudikoff and Potter, 1974; Hood et al., 1975; Rudikoff and Potter, 1976; Rudikoff et al., 1977 (submitted for publication)).

We are currently analyzing a second group of heavy chains from myeloma proteins with specificity for inulin[1] (Vrana et al., 1976; Potter et al., 1976b,c) to determine if the pattern of variation observed is similar to that of the heavy chains of phosphorylcholine-binding proteins. The eleven anti-inulin proteins characterized to date have been shown to possess individual, as well as cross-reacting, variable-region antigenic determinants (idiotypes) (Lieberman et al., 1975) and are thus a potential model for attempting to analyze the structural basis of idiotypy. We are currently performing structural determinations on five of these inulin-binding proteins (Lieberman et al., 1975; Potter et al., 1976c), and report here the complete heavy-chain variable-region sequence from the ABE-47N protein, which we intend to use as a prototype for this group.

Materials and Methods

Protein Purification. ABE-47N was purified by affinity chromatography on Sepharose–inulin columns as previously described (Vrana et al., 1976).

† From the Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received October 5, 1976.*

[1] Inulin is a short linear polyfructan of β2-1 linked fructofuranose terminating with a nonreducing D-glucopyranose residue. Eleven proteins have been isolated that bind inulin, which thus provide a large group of functionally related homogeneous immunoglobins.