Involvement of His HC3(146)β in the Bohr Effect of Human Hemoglobin

STUDIES OF NATIVE AND N-ETHYLMALEIMIDE-TREATED HEMOGLOBIN A AND HEMOGLOBIN COWTOWN (β146 HIS→LEU)*

(Received for publication, March 14, 1983)

Tzu-bi Shiht and Richard T. Jones‡
From the Department of Biochemistry, School of Medicine, Oregon Health Sciences University, Portland, Oregon 97201
Joseph Bonaventura§ and Celia Bonaventura§
From the Department of Biochemistry, Duke University Marine Laboratory, Pivers Island, Beaufort, North Carolina 28516
Rose G. Schneider
From the Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas 77555

The involvement of the COOH-terminal histidines of the β chains of human hemoglobin in the allosteric mechanism of oxygen binding has been the topic of intensive discussion. Data presented here on the functional properties of native and chemically modified forms of Hb Cowtown (β146 His→Leu) suggest that approximately half of the alkaline Bohr effect is attributable to the imidazole of His HC3(146)β. The contribution of this residue to the alkaline Bohr effect has been estimated variably as 40–60% and 15% or less. Equilibrium and kinetic studies show that the amino acid substitution in Hb Cowtown decreases the stability of the low affinity conformation, resulting in an increased oxygen affinity and altered sensitivity to anionic effectors. Detailed analysis of Hill plots of oxygen binding according to the Adair scheme reveals that, under conditions of moderate ionic strength (chloride = 0.1 M), the K₂ and K₄ values for Hb A and Hb Cowtown differ, whereas the K₁ and K₄ values are closely similar over the physiological pH range. The decreased pH sensitivity of Hb Cowtown is associated with a decreased pH sensitivity of Kβ, the first Adair constant. In contrast to des-His(146)β hemoglobin, the cooperative interactions shown by Hb Cowtown under conditions of moderate ionic strength are not reduced in comparison to those of Hb A. This and the similarity of K₁ and K₄ values for Hb A and Hb Cowtown indicate that under these conditions the salt bridge formed by the COOH-terminal imidazole group does not significantly contribute to the free energy difference between "T-state" and "R-state" hemoglobin. It appears that the salt bridge formed by the COOH-terminal carboxyl group stabilizes the deoxy, T-state, conformation to a greater degree than previously appreciated. Chemical modification of the Cys(93β) residue of Hb Cowtown with N-ethylmaleimide causes a decrease in its oxygen affinity, in contrast to the increase in affinity exhibited by N-ethylmaleimide-modified Hb A. Hemoglobins A and Cowtown have remarkably similar oxygen binding properties after this modification and are shown to have K₁ and K₄ values distinctly different from those of unmodified Hb A. The properties of native and chemically modified forms of Hb Cowtown are indicative of a large contribution of the His HC3(146)β residue to the alkaline Bohr effect and also illustrate how chemical modifications or changes of strategic amino acid residues can result in pronounced differences in the conformational equilibrium of an allosteric protein.

The linkage between proton- and oxygen-binding by human hemoglobin and the involvement of His HC3(146)β in this linkage has been the subject of extensive experimentation and discussion (1–17). Conceptually, it is debated as to whether hemoglobin shows its characteristic pH sensitivity as a result of many small contributions from a large number of interacting groups or if a limited number of residues in strategic locations play dominant roles. A central question in this debate is the extent to which His HC3(146)β contributes to the alkaline Bohr effect. The results briefly presented here and described in detail in the accompanying Miniprint on native and N-ethylmaleimide-modified Hb A1 and Hb Cowtown are relevant to this question.

Hb Cowtown is an adult human hemoglobin variant in which the histidine residue normally found at the β chain COOH terminus is replaced by a leucine (18, 19). Fig. 1 of the Miniprint provides a schematic representation of the structural differences inferred to exist between Hb A and Hb Cowtown based on this sequence alteration. Following the results of x-ray crystallographic analysis (3–5, 13), the l-

* This study was supported in part by National Science Foundation Grant PCM 79-06462. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Supported in part by United States Public Health Service Research Grants HL20142 and National Institutes of Health Grant SM17869.
§ Supported in part by United States Office of Naval Research Contract N00014-83-K-0016.

1 The abbreviations used are: Hb A, adult human hemoglobin; NEM, N-ethylmaleimide; NEM-modified Hb, Hb A after N-ethylmaleimide treatment; DPG, 2,3-diphosphoglycerate; IHP, inositol hexaphosphate; bis-Tris, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; Pₒₒ, oxygen partial pressure necessary for half-saturation; Y, the fractional saturation of hemoglobin with ligand; nₐₓ and nₒₒ, the maximum Hill coefficient and the Hill coefficient at half-saturation, T-state and R-state, low and high oxygen affinity conformations of hemoglobin, respectively, with intrinsic association constants Kₐ and Kᵣ, Lₐ, the allosteric equilibrium constant between T-state and R-state hemoglobin in the unliganded condition.
ganded R-structure representations shown in Fig. 1 lack COOH-terminal salt bridges, while the deoxy T-structure representations show salt bridges between the imidazole group of His HC3(146)β and the carboxyl group of Asp FG1(94)β in Hb A and between the COOH-terminal carboxyl group and the ε-amino group of Lys C5(40)α in both Hb A and Hb Cowtown.

A number of human hemoglobin variants have previously been reported with amino acid substitutions at the β146 position. Variability in the functional properties of these hemoglobin variants is shown in Table I. Also presented is data on des-His (146)β hemoglobin. This chemically modified form of Hb A has been used extensively in studies of the functional role of the COOH-terminal histidine residues (8–13).

Treatment of Hb A with N-ethylmaleimide results in blocking the —SH group of Cys F9(93)β and inhibits formation of the COOH-terminal salt bridge typically formed by the imidazole group of the COOH-terminal histidine (6). As will be shown, chemical modification of Hb Cowtown by NEM results in a decreased oxygen affinity, instead of an increased affinity as previously reported for NEM-modified Hb A.

MATERIALS AND METHODS

Standard methods were utilized to prepare and analyze Hb A and Hb Cowtown (18–32). Oxygen binding curves were measured with an automatic recording apparatus as described by Imai et al. (29, 33). Rapid-mixing experiments and flash photolysis experiments were performed as previously described (31). Details of the experimental methodology are presented in the Miniprint.

RESULTS

The Miniprint to this paper provides data on normal and NEM-modified forms of Hb A and Hb Cowtown. Figs. 2 through 7 show detailed oxygen binding curves. Table II summarizes the results of oxygen binding experiments carried out with Hb Cowtown and Hb A at neutral pH under various ionic conditions. Figs. 8 and 9 document differences between Hb A and Hb Cowtown in the kinetics of oxygen dissociation, while Figs. 10 and 11 illustrate differences between the two proteins in regard to the kinetics of ligand binding.

DISCUSSION

Figs. 1–11 and Table II clearly indicate that the functional properties of human hemoglobin are altered as a result of the substitution of leucine for histidine at the β chain COOH terminus. The allosteric property most notably altered in Hb Cowtown is the alkaline Bohr Effect, which is diminished to one-half that of Hb A. A salt bridge that would be absent in Hb Cowtown, between the imidazole of His HC3(146)β and Asp FG1(94)β, has been proposed as the factor responsible for about one-half of the alkaline Bohr effect (36, 37). Although its Bohr effect is decreased, we find at pH 7.4, and under conditions of moderate ionic strength (chloride = 0.1 M), that Hb Cowtown has K1 and n0 values similar to those of Hb A. Hemoglobin that is carbamylated at its α chain NH2 terminus similarly shows a decreased Bohr effect and increased oxygen affinity, without a decrease in cooperative interactions (38).

An extreme case, which similarly demonstrates a decreased heterotropic response to protons without diminution of homotropic cooperative interactions, is that of Trout I Hb which has a phenylalanyl group at its COOH terminus. This hemoglobin exhibits normal cooperativity with essentially no pH sensitivity (39). These and other examples bear out the conclusion that the conformational changes that are the basis for cooperative oxygen binding can occur independently of alterations in pKs values of ionizable groups at the NH2 and COOH terminus of the hemoglobin tetramer. This conclusion is in agreement with the interpretation of Wajcman et al. (40), who concluded from their studies of Hb Barcelona (β89 Asp→His) that the normal Asp β89-His β146 bridge is required for the full expression of heme-heme interaction.

In light of the fact that cooperative interactions are not impaired, the simplest explanation for the halved Bohr effect of Hb Cowtown is that this variant lacks an important Bohr group, specifically the imidazole of His HC3(146)β. This is of particular significance because of the current debate concerning the contribution of this residue to the alkaline Bohr effect.

The degree of stabilization of the deoxy T-state conformation that can be directly attributed to the formation of salt bridges by the COOH terminal histidine is also clarified by the data presented here on Hb Cowtown. A comparison of the Adair constants of Hb A and Hb Cowtown at pH 7.4 under conditions of moderate ionic strength shows their K1 values to be similar. The K1 values calculated for Hb Cowtown show a decreased pH sensitivity of K1, that is indicative of a somewhat reduced T-state Bohr effect, but the value of K1 at pH 7.4 is equal to that of Hb A within experimental error. This similarity in K1 and K2 values indicates that, under conditions of moderate ionic strength, the salt bridge normally formed by the imidazole group of His HC3(146)β does not contribute significantly to the total free energy difference between the T-state and R-state conformations. This situation can readily be described in terms of the two-state allosteric model of Monod et al. (2) where the T and R conformations of Hb A and Hb Cowtown are equivalent and the differences in their oxygen binding curves are due to a shift in the allosteric equilibrium constant L. The shift in L associated with the amino acid substitution in Hb Cowtown favors the high affinity conformation. This is evidenced by the higher oxygen affinity at half-saturation of Hb Cowtown, in comparison to Hb A, over most of the pH range studied, a phenomenon that is reflected in altered K1 and K2 constants of the Adair scheme.

The shift in the allosteric equilibrium constant is also apparent in three phenomena: altered time courses of oxygen dissociation (Fig. 8); more pronounced fast phases of CO binding to the deoxyhemoglobin in rapid-mixing experiments at high pH (Figs. 10 and 11); and a higher percentage of quickly reacting material in CO combination experiments after partial flash photolysis at a given flash intensity.

Both equilibrium and kinetic experiments indicate that Hb Cowtown is more sensitive to chloride than is Hb A, although chloride titrations (Fig. 2) suggest that neither the number nor the affinity of oxygen-linked anion binding sites changes. This can also be accounted for in terms of the loss of the imidazole salt bridge at the COOH terminus in Hb Cowtown, which makes attainment of the T-state more sensitive to the presence or absence of other stabilizing influences. When Hb Cowtown is studied under conditions of minimal chloride (~7 mM), the value of the first Adair constant, K1, is found to be significantly different than that for Hb A and cooperativity is also decreased (see Table II). Under these conditions, the COOH-terminal imidazole salt bridge does contribute significantly to the free energy difference between the conforma-
tions assumed by the liganded and unliganded protein. This effect necessitates an extension of the two-state model, since under these conditions the T-state assumed by Hb Cowtown differs from that of Hb A and the parameter $c$ which denotes the ratio of T-state and R-state affinities is not a constant. We note in this regard that Kilmartin et al. report from studies of des-His(146)$\beta$ hemoglobin that in the absence of phosphates the contribution of His HC3(146)$\beta$ to the alkaline Bohr effect increases from 40% in solutions with 0.1 M chloride to 60% in solutions with 0.01 M chloride (13). Paradoxically, it is specifically under conditions of low ionic strength, with chloride ranging from 0.005 to 0.06 M, and in the absence of phosphate, that other studies suggest that the $pK_c$ of the COOH-terminal histidine residue is only slightly altered upon ligation of the hemoglobin tetramer (10).

An important point that is brought out by the results obtained on Hb Cowtown is that a single amino acid residue may contribute in several ways to the functional properties of hemoglobin. This generalization may seem obvious, but it appears that discussions of the role of the COOH-terminal histidine in the literature have focused on its imidazole group. The reduction in cooperativity in des-His(146)$\beta$ hemoglobin (26), under conditions where Hb Cowtown shows normal levels of cooperative interactions, suggests that the COOH-terminal carboxyl group of His HC3(146)$\beta$ plays a more important role in T-state stabilization than previously appreciated. The degree to which the carboxyl group of the COOH-terminal residue can form a stabilizing salt bridge would clearly depend on the nature of the residue occupying the COOH-terminal position. This fact may be a substantial contributor to the variability of function that is shown in Table I for hemoglobin forms with alterations at HC3(146)$\beta$. Alternatively, as pointed out by Perutz, we cannot rule out the possibility that the hydrophobic nature of the leucine residue that replaces histidine in Hb Cowtown allows for a different type of T-state stabilization, one that is possibly operational in other cooperative hemoglobins, such as the pH-sensitive Trout I Hb, where a hydrophobic residue (phenylalanine) occupies the $\beta$ chain COOH terminus (39).

A number of x-ray crystallographic studies (3-7) indicate that the salt bridges formed by the COOH-terminal histidine residue in deoxyhemoglobin are broken in fully liganded hemoglobin, as shown schematically in Fig. 1. Human hemoglobin variants with differences at the HC3(146)$\beta$ might therefore be expected to be functionally equivalent in the liganded R-state. This is an oversimplification, in that the COOH-terminal residue appears to play a significant role in modulating the oxygen affinity of the R-state. This is evidenced by the fact that the $K_i$ values calculated for Hb Cowtown and Hb A are equivalent within experimental error and show only a slight pH sensitivity, while des-His(146)$\beta$ hemoglobin and Hb Cochin-Port Royal (H146$\beta$Arg→Arg) were found by Kwiatkowski and Noble to have diminished R-state Bohr effects relative to Hb A (41). Additional evidence for R-state inequivalence of COOH-terminal Hb variants is that Hb Cowtown appears to have a reduced tendency to dissociate into dimers relative to Hb A and does not show diminished cooperativity or altered $K_i$ values in the presence of inositol hexaphosphate as is the case for Hb A. The latter appears to be relevant to the chloride-binding data of Chiancone et al. (42) which suggests that the COOH-terminal histidine can act as an anion binding site.

Modification of R-state hemoglobin is clearly brought about by treatment of Hb A or Hb Cowtown with NEM. As previously reported, NEM-treated Hb A has increased oxygen affinity, decreased cooperativity, and a Bohr effect that is one-half that of untreated Hb A (6, 44). The same treatment decreases the oxygen affinity of Hb Cowtown without appreciably altering its already reduced Bohr effect (Fig. 5). After NEM-treatment, Hb A and Hb Cowtown are shown to be functionally equivalent (Fig. 6). The chemical modification appears to restrict the conformational mobility of hemoglobin and to decrease the free energy difference between its liganded and unliganded conformations. Our data suggest that neither the liganded nor the unliganded structures are functionally equivalent to those of native hemoglobin. These results are consistent with the data of Imai (44) on NEM-modified Hb A and of Simon et al. (43) on N-substituted maleimide derivatives of human hemoglobin, but must be reconciled with the fact that the three-dimensional structures of native and NEM-modified forms of liganded hemoglobin were found to be similar in comparative x-ray crystallographic studies (3). High resolution crystallographic studies offer a possible structural explanation for the significant functional differences between normal and NEM-treated Hb A. Specifically, as illustrated in Fig. 1, the $\beta$83 cysteine residue can occupy either "internal" or "external" positions, and the latter, found in the T structure, favors the high spin state of methemoglobin (45). As pointed out by Perutz, the stabilization of the "external" position brought about by NEM treatment may be responsible for the alterations in affinity and conformational flexibility that we observe. The data presented here on native and NEM-modified Hb Cowtown clearly indicate that alterations in amino acid sequence in strategic locations can lead to pronounced alterations in function. The data also show that the measured contribution of a specific salt bridge toward T-state stabilization is a function of the presence or absence of additional stabilizing influences.

Acknowledgments—We express our appreciation to Dr. K. Imai for supplying the computer programs which were used for the Adair analysis calculation and to Dr. M. F. Perutz for helpful discussions.

REFERENCES


M. F. Perutz, personal communication.
Functional Studies of Hb Cowtown

44. Imai, K. (1973) Biochemistry 12, 799–808
Functional Studies of Hb Cowtown

| TABLE I. Oxygen equilibrium parameters for human hemoglobin with alteration at H(2)159Ala.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>tEA</td>
<td>t0.5</td>
<td>K02 (mM)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>Cowtown</td>
<td>2.3</td>
<td>0.5</td>
<td>200</td>
</tr>
<tr>
<td>Cyclodi-Port-Rayl</td>
<td>2.1</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>Mihmogen</td>
<td>2.2</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>True (100% O₂)</td>
<td>2.2</td>
<td>0.3</td>
<td>200</td>
</tr>
<tr>
<td>True (2% CO₂)</td>
<td>2.3</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>True (2% CO₂) (2% CO₂) Phosphate</td>
<td>2.3</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>True (2% CO₂) (2% CO₂) Phosphate</td>
<td>2.3</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.1</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>2.2</td>
<td>0.3</td>
<td>200</td>
</tr>
<tr>
<td>histidin</td>
<td>2.2</td>
<td>0.3</td>
<td>200</td>
</tr>
</tbody>
</table>

After the discovery of hemoglobin variants that modify the pH effect on oxygen affinity, several studies have focused on the relationship between these variants and the pH effect. The pH effect is a phenomenon where the oxygen affinity of hemoglobin increases as the pH decreases. This effect is due to the interaction of the pH value with the oxygenated form of the cysteine residue in the heme group, leading to a decrease in the oxygen affinity.

Several studies have investigated the effect of various amino acid substitutions on the pH effect, and some studies have found that certain substitutions can either increase or decrease the pH effect. For example, the substitution of alanine for histidine at position 159 of human hemoglobin (HbA) results in a decrease in the pH effect, while the substitution of aspartate for histidine at the same position results in an increase in the pH effect.

The pH effect is also influenced by other factors, such as the presence of certain ions or ligands. For instance, the presence of phosphate ions can increase the pH effect, while the presence of carbon dioxide can decrease it.

In conclusion, the pH effect is a complex phenomenon that is influenced by many factors, and further studies are needed to fully understand its mechanisms and implications for human health and disease.
Functional Studies of Hb Cowtown

Figure 2. Effects of pH on oxygen binding to liganded Hb (panel A) and Hb (panel B) at 0.85 M Hb concentration. At all points of oxygen binding given from left to right are at pH 8.4, 8.6, 8.9, and 7.6, respectively. No solid lines are drawn over the graph, and all solid curves are placed above the data points. The solid lines for Hb Cowtown and the dotted line for Hb are theoretical curves based on the estimated values of Hill coefficients. B, Hb affinity plots showing the dependence of log P vs. pH for the data described in Fig. 2A. C: Evaluation of the first and fourth order constant.

Oxygen Equilibrium Studies. The effect of pH on oxygen binding curve for Hb Cowtown and Hb at moderate salt strengths (0.85 M KCl) is shown in Fig. 2A and B. The Curves in Fig. 2A and B are theoretical curves based on the estimated values of Hill coefficients. The Hill coefficients for Hb Cowtown are significantly different from those of Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are equal to the values of Hill coefficients for Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are similar to those of Hb at high pH, but show less pH sensitivity.

TABLE II. Hill coefficients for Hb Cowtown. Comparison of data for Hb A and B are presented below in brackets [ ]. The results were confirmed in 0.5 M KCl in 7.4 [6].

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ta (M)</th>
<th>Kd (M)</th>
<th>pH</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>0.15</td>
<td>0.02</td>
<td>7.4</td>
<td>1.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.15</td>
<td>0.02</td>
<td>7.4</td>
<td>1.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The Hill coefficients for Hb Cowtown are significantly different from those of Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are equal to the values of Hill coefficients for Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are similar to those of Hb at high pH, but show less pH sensitivity.

Figure 3. The effect of chloride concentration on the 50 mmol/liter solutions of Hb Cowtown (a) and Hb (b) in 0.015 M NaCl-NaCl buffer, at 2.4 [6]. A: Comparison of Hill coefficients. B: Hill coefficients vs. pH. C: Evaluation of the first and fourth order constant.

The results of oxygen-binding experiments carried out with Hb Cowtown and Hb at a range of oxygen partial pressure are summarized in Table II. The data show that Hb Cowtown has a higher oxygen affinity compared to a shift in the corresponding oxygen equilibrium curve toward that observed for Hb. The Hill coefficients for Hb Cowtown are significantly higher than those of Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are equal to the values of Hill coefficients for Hb, as shown in Fig. 2A. The Hill coefficients for Hb Cowtown are similar to those of Hb at high pH, but show less pH sensitivity.

Titrations of Hb Cowtown and Hb with 3,3'-diphenylhexatriene and 2,3-diphenylfluorescein are given in Figs. 4 and 5, respectively. The initial change in oxygen affinity is brought about by half of these effects. In less for Hb Cowtown than for Hb (see insets). The values of log P for Hb Cowtown remain high even in the presence of high concentrations of inorganic phosphates, whereas for Hb, the log P value is decreased under similar conditions. The phosphoproteins are completely unlabelled, whereas for Hb, the log P value is decreased under similar conditions. The phosphoproteins, Hb Cowtown, Hb, and Hb Cowtown, are not affected by the presence of inorganic phosphates, whereas for Hb, the log P value is decreased under similar conditions. The phosphoproteins are completely unlabelled, whereas for Hb, the log P value is decreased under similar conditions. The phosphoproteins, Hb Cowtown, Hb, and Hb Cowtown, are not affected by the presence of inorganic phosphates, whereas for Hb, the log P value is decreased under similar conditions.
Functional Studies of Hb Cowtown

Oxygen binding by NEM-modified Hb Cowtown and HbA. The pH dependence of oxygen binding by NEM-modified Hb Cowtown and HbA, expressed as a change in the dissociation constant (Kd) from pH 7.4 to pH 6.0, is shown in Fig. 6. The Kd plots for the two chemicallymodified forms are plotted at superimposed, and the A-values calculated from these curves are similar (see inset). The binding curves of normal and NEM-modified forms of Hb Cowtown and HbA at pH 7.4 are superimposed in Fig. 7. Analysis according to the linearized plot is increased by Kd is decreased for both proteins as a result of the chemical modification (see inset). It is consistent with the fact that the Kd of Hb Cowtown is increased by the addition of the NO group, while that of HbA is decreased, but that both functionally equivalent hemoglobin forms.

Kinetcs of Oxygen Dissociation. The kinetics of oxygen dissociation from Hb Cowtown and the effects of NEM modification were followed by measuring the oxygen tension that required to rebind of air-stored hemoglobin solution with equal volume of degassed buffer containing 0.4% ATP. The oxygen curves for Hb Cowtown and HbA under conditions of moderate basic strength (pH 7.4) showed decreased sensitivity to pH in comparison to those of HbA. The results of the initial stages of the oxygen dissociation process (calculated for log Kd, pH = 7.4 to 6.0) are also shown in Fig. 7. It is clear that there is a relatively slow initial phase during the reaction and the apparent rate of oxygen dissociation from HbA is greater than that of HbA, since the normoxic hemoglobin is better able to maintain a high-affinity state with a characteristic time of oxygen dissociation.

Fig. 8. Effects of 0.1M chloride on the time course of oxygen dissociation from 50% (100 g) hemoglobin solutions in 0.05 M Tris buffers buffers at pH 7.4, 25°C. Wavelengths of oxygenation were 537 cm, 537 nm. The upper (broken) traces correspond to conditions of sodium chloride (NaCl).
Functional Studies of Hb Cowtown
Involvement of His HC3 (146) beta in the Bohr effect of human hemoglobin. Studies of native and N-ethylmaleimide-treated hemoglobin A and hemoglobin Cawtown (beta 146 His replaced by Leu).
T Shih, R T Jones, J Bonaventura, C Bonaventura and R G Schneider


Access the most updated version of this article at http://www.jbc.org/content/259/2/967

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/2/967.full.html#ref-list-1