MECHANICAL STABILITY OF HEMOGLOBIN SUBUNITS: AN ABNORMALITY IN β^S-SUBUNITS OF SICKLE HEMOGLOBIN

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Summary: In order to study the mechanism of the ease of precipitation of oxyhemoglobin S by mechanical shaking, the rates of precipitation of α- and β-subunits of oxyhemoglobin A and oxyhemoglobin S were compared. At pH 8.0, the α^A-subunits precipitated rapidly, while the β^A-subunits were very stable, although a part of β^A-subunits converted to the hemichrome form. At pH 6.0, the β^A-subunits precipitated rapidly while the α^A-subunits were stable. Similar studies with β^S-subunits showed that β^S-subunits precipitated rapidly both at acidic and alkaline pHs. The abnormal precipitation of tetrameric oxyhemoglobin S during mechanical shaking may be due to this instability of the β^S-subunits.

The oxy- and carboxy-forms of sickle hemoglobin in solution precipitate rapidly during mechanical shaking (1,2). Deoxyhemoglobin S, on the other hand, is extremely stable compared to the other forms of hemoglobin (2). The rates of precipitation are also affected by 2,3-diphosphoglycerate and inositol hexaphosphate (3). These results suggest that the rates of precipitation of hemoglobin by mechanical shaking are related to the protein conformation in solution and that the marked difference between the rates of precipitation of oxyhemoglobin A and oxyhemoglobin S is due to a conformational difference between the hemoglobins in the dissolved state (2). Since the gross structure of oxyhemoglobin A is reported to be similar to that of oxyhemoglobin S (4), such a conformational change, if present, must be located near the abnormal substitution site in the β-subunits.

On the basis of this assumption, we have separated normal and sickle hemoglobin into subunits and compared the stability of β^S-subunits with those of normal α^A- and β^A-subunits. The results clearly show that the oxy-form of β^S-subunits is far more unstable than that of β^A-subunits, suggesting that the ease of precipitation of oxyhemoglobin S is due to the unstable structure of the β^S-subunits. In addition, the comparison of the stability of α^A and β^A-subunits under different pH conditions showed that the α^A- and β^A-subunits have completely opposite stabilities from each other in acidic and basic buffers. The hemoglobin tetrameter is a complex of such subunits having complementary conformational properties. The pH-dependent conformational change of hemoglobin subunits may be related to the mechanism of the Bohr effect in oxygen binding.

MATERIALS AND METHODS

Solutions of normal and sickle hemoglobin were prepared from red cells as described elsewhere (2). Separation of carbon monoxide hemoglobin into its subunits was carried out by the method of Bucci and Fronticelli (5). p-Chloromercuribenzoate was removed from the subunits according to DeRenzo et al. (6). Solutions of hemoglobin or its subunits were passed through a Sephadex G-25 column and diluted to 10-15 μM with appropriate buffer. Two ml of oxyhemoglobin or subunit solution were shaken in a 15 x 40 mm vial with a TCS shaker (Model 150, Southampton, Pa. 18966) at a frequency of 28 c/s at room temperature. After shaking, the vial was centrifuged at 4,000 rpm for 5 min in order to remove the precipitated protein. The concentration of hemoglobin remaining in the supernatant solution was measured spectrophotometrically as described previously (2).

RESULTS AND DISCUSSION

Figure 1 compares the rates of precipitation of the oxyform of hemoglobin subunits obtained from both normal and sickle hemo-

globins in 0.1 M Bis-Tris-HCl buffer, pH 7.4. The α^A- and α^S-subunits, which have an identical amino acid sequence, precipitate at the same rate, suggesting that the tertiary structures of these subunits are also similar. The rates of precipitation of α-subunits are approximately 173-fold faster than that of tetrameric sickle hemoglobin in 0.1 M Bis-Tris buffer, pH 8.0. The absorption spectrum of the remaining α-subunits in the supernatant is typical of oxyhemoglobin, indicating that no oxidation takes place during the mechanical shaking. The β^A-subunits, on the other hand, are relatively stable at pH 7.4 or pH 8.0, and precipitate at a rate slower

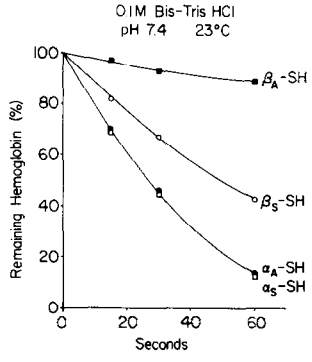
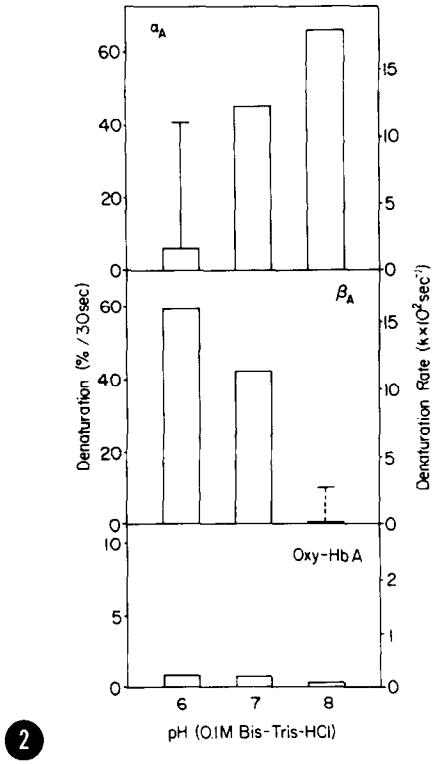


Figure 1. The rates of precipitation of hemoglobin subunits. The oxy-form of hemoglobin subunits were dissolved in 0.1 M Bis-tris buffer, pH 7.4, at a heme concentration of approximately 40 μM. Aliquots (2 ml) were shaken mechanically with a TCS-shaker at a frequency of 28 c/s at room temperature. After shaking, the solutions were centrifuged at 4,000 x g for 5 min. in order to remove the precipitated hemoglobin. The concentration of hemoglobin subunits remaining in the supernatant fluid was determined spectrophotometrically as described elsewhere (2).

than that of tetrameric oxyhemoglobin S at pH 8.0. In addition, a small fraction of the subunits in the supernatant solution converts to a hemichrome compound characterized by the appearance of absorption peaks at 535 and 620 nm. The β^S-subunits precipitate at a rate 10 times faster than β^A-subunits. Hemichrome does not form in the super natant of the β^S-subunit solution. The differences between β^A-



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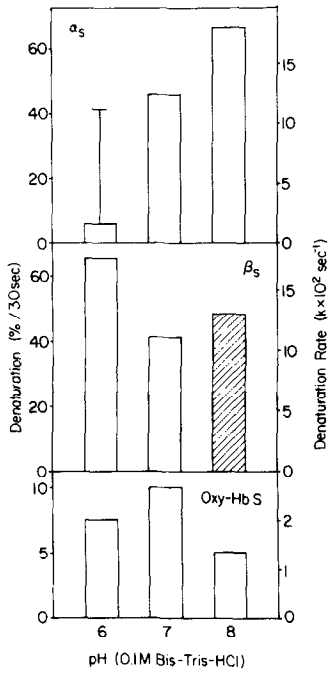

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Figure 2. Effect of pH on the rates of precipitation of α^A- and β^A-subunits. The experimental conditions were the same as shown in Figure 1 except for pH. The straight line for the α^A-chain and the dotted line for the β^A-chain on the top of the columns, indicate the percentage of oxidized hemoglobin formed in solution during mechanical shaking. The rate of precipitation for tetrameric hemoglobin A is shown at the bottom of the figure for comparison.

Figure 3. Effect of pH on the rates of precipitation of α^S- and β^S-subunits. The experimental conditions were the same as shown in Figure 2.

and β^S-subunits are especially clear at pH 8. As shown in Figures 2 and 3, approximately 50% of the β^S-subunits precipitate during shaking for 30 seconds, while almost no precipitate forms from β^A-subunits. Thus the oxyform of β^S-subunits has an abnormally unstable structure. This instability of the subunits may be the cause of the unusual instability of oxy-Hb S which we have previously reported (1,2).

The differences in stability of hemoglobin subunits under different pH conditions are striking. As shown in Figure 2, the rates of precipitation of both α^A- and β^A-subunits are drastically affected by changing the pH of the medium. At pH 8, the α^A-subunits are extremely unstable and precipitate rapidly, but the molecules are very stable at pH 6. Although approximately 35% of the α^A-subunits in the supernatant are oxidized to the met-form in acidic solutions (pH 6), the rate of precipitation is only 10% of that in alkaline solution (pH 8). On the other hand, the β^A-subunits are very stable at pH 8, but are markedly destabilized at pH 6. In addition, no oxidation in the supernatant β^A-subunits were observed at this pH during the shaking. Thus, the pH profiles of the ease of precipitation and the ease of oxidation are completely opposite in the α^A- and β^A-subunits. A tetrameric hemoglobin A is a complementary complex of such subunits which have opposite properties and is relatively stable over the pH range studied, as shown at the bottom of Figure 2.

The effect of pH on the rates of precipitation of oxyhemoglobin S and its subunits is shown in Figure 3. The pH effects of β^S-subunits are similar to those of β^A-subunits at pH 6 and 7. At pH 8, in contrast to the behavior of β^A-subunits, the β^S-subunits are not stabilized and still precipitate rapidly. Upon formation of a tetramer, the oxyhemoglobin S becomes stable as shown at the bottom of Figure 3, but the degree of this stabilization is smaller than that noted in oxyhemoglobin A (Fig 2). In addition, the pH profile of the stability of tetramer oxyhemoglobin S in Bis-tris-HCl buffer is different from that estimated from the pH effect on each type of subunit, suggesting that the conformational changes of the subunits which take place upon formation of a tetramer alters their stability.

The pH-dependent changes in the stability of α- and β-sub-

units of hemoglobin shown in the present study may be the largest difference ever noted between the properties of α- and β-subunits. If the stability of hemoglobin reflects the protein conformation in solution, pH-dependent conformational changes of hemoglobin subunits may be related to pH-dependent functional changes, such as the Bohr effect. Preliminary experiments have shown that the stability of the deoxy-form of α- and β-subunits has no pH-dependency. Therefore, the pH-dependent functional difference may depend on the interaction between the oxy- and deoxy-subunits, as proposed elsewhere (7).

These observations may be of importance in explaining some of the pathophysiology of the thalassemia syndromes. In homozygous β-thalassemia, excess α-chain precipitates rapidly in nucleated red cells soon after its synthesis. In contrast, in the α-thalassemia syndrome, hemoglobin H disease, the excess β-chains are relatively stable in comparison to free α-chain. In hemoglobin H disease precipitation of excess β-chains occurs much later in the life of the red cell, causing mainly peripheral blood hemolysis rather than destruction of young red cells in the marrow (8). The greater ease of precipitation of free α-chains compared to free β-chains at physiologic pH (Fig. 1) may be responsible in part for these abnormalities of the thalassemia syndromes.

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