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Oxygen Equilibrium and Kinetics of Isolated Subunits from Hemoglobin Kansas

(hemoglobin A/β chains)

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ABSTRACT **The isolated β subunit of hemoglobin Kansas has an oxygen affinity that is as low relative to the oxygen affinity of the β_A subunit as the affinity of hemoglobin Kansas is low relative to hemoglobin A. Thus the low affinity properties of hemoglobin Kansas are almost completely reflected in the properties of the isolated subunits. The kinetic results show that the equilibrium affinity difference results both from a much larger oxygen dissociation rate constant in β_{Kansas} (k = 37 sec^{-1} and 18 sec^{-1} for β_{Kansas} and β_A, respectively) and from a lower association reaction rate. The properties of the α chains from hemoglobins A and Kansas appear to be identical, as expected.**

Hemoglobin Kansas is characterized by an unusually low affinity for oxygen (1). It is natural to inquire whether the low affinity is reflected in the properties of the isolated β subunits in which the aminoacid substitution occurs (1). We have, therefore, isolated the α and β subunits from hemoglobins A and Kansas and compared their kinetic and equilibrium properties.

METHODS

The kinetic experiments were performed with apparatus and methods recently described or referenced by Olson and Gibson (2). The equilibrium determinations were performed as described by Bonaventura and Riggs (1) and Tomita and Riggs (3), except that the air to be injected into the tonometer was first diluted as described for the dilution of carbon monoxide by Imamura, Riggs, and Gibson (4). Sedimentation velocity measurements were performed as described (1).

MATERIALS

Hemoglobins A and Kansas were prepared as described (8). The α and β subunits were prepared as described by Geraci, Parkhurst, and Gibson (5). Since residual mercurial in the preparation might greatly alter the results, we determined the mercury content of the isolated subunit preparations by atomic absorption spectrophotometry with a flame less technique (6). This analysis showed the presence of residual mercury to the extent of about one atom of mercury per 227 chains, which is close to the level obtained by Geraci *et al.* (5). The subunits were prepared at 4° and stored at 0°, and kept saturated with carbon monoxide. In 50 mM Tris·HCl buffer (pH 7.5) with 0.1 M NaCl and 0.1 mM EDTA, the subunits remained stable for at least 10 days, with negligible oxidation to methemoglobin. After measurement of the oxygen equilibria, the maximum amount of methemoglobin did not exceed 2–3%.

RESULTS

The experiments shown in Fig. 1 at pH 7.5 show that β_{Kansas} chains have a much lower affinity for oxygen than do β_A chains. The log P_{50} values* differ by about 0.8, which is close to the difference found at this pH between the intact hemoglobins A and Kansas (1). Measurements at other pH values showed the absence of a Bohr effect in any of the subunits. None of the isolated subunits β_A, β_{Kansas}, α_A, or α_{Kansas} displayed any significant cooperativity in oxygen binding. The value of the Hill coefficient, n, was about 1.07, 1.10, and 1.05 for the β_A, α, and β_{Kansas} subunit equilibria, respectively. The α subunits from hemoglobins A and Kansas displayed identical behavior, as expected from the earlier finding of structural identity (1). It is noteworthy that the oxygen affinity of the isolated β_A subunits is significantly higher than that of the isolated α_A or α_{Kansas} subunits. The results cannot be rigorously compared with observations on the intact hemoglobins A and Kansas (1, 2, 7–10) because of the interactions between the subunits.

The combination of β_{Kansas} subunits with α subunits results in a substantial drop in oxygen affinity (Fig. 1) and a return to the original degree of cooperativity. In the upper part of the oxygenation curve (Fig. 1), n = 1.4, and the log P_{50} value is only slightly lower than that obtained in intact hemoglobin Kansas. The pronounced flattening of the bottom of the curve (0–15% oxygenation) presumably results from a small degree of heterogeneity arising from the presence of a small quantity (about 15%) of uncombined subunit. The characteristics of the upper part of the curve indicate that the recombined material is essentially identical to the original hemoglobin Kansas in oxygen binding.

The rate of deoxygenation of the subunits was measured in the presence of dithionite (Fig. 2). The time course of the α subunit deoxygenation appeared homogeneous and followed first-order kinetics (k = 21 sec^{-1}). The kinetics of the β_A and β_{Kansas} deoxygenation were clearly heterogeneous, but the initial rate of deoxygenation (37 sec^{-1}) of β_{Kansas} was about twice that of β_A (18 sec^{-1}). During the reaction the β_{Kansas} rate slowed to about the same as that of the β_A subunit. Spectral observation showed that these changes could not be attributed to methemoglobin formation.

The kinetics of the combination of the subunits with carbon monoxide is shown in Fig. 3. The data for the α subunits

* P_{50} is the O_2 pressure at 50% oxygenation.

showed only very slight heterogeneity (rates for α_A and α_{Kansas} were 5.3×10^6 M^{-1} sec^{-1} and $5.1^* \times 10^6$ M^{-1} sec^{-1}, respectively), but the β-chain kinetics showed a large degree of heterogeneity. The β_A kinetics could be dissected into two parts: fast (67%) and slow (33%) components with apparent rate constants of 4.2×10^6 M^{-1} sec^{-1} and 1.7×10^6 M^{-1} sec^{-1}, respectively. The rates of reaction of the β_{Kansas} subunits were also heterogeneous, but much slower. The fast β_{Kansas} component (70%) had an apparent rate constant of 1.4×10^6 M^{-1} sec^{-1}, whereas the slower β_{Kansas} component (30%) gave an apparent rate constant of 1×10^6 M^{-1} sec^{-1}.

The kinetics of the dissociation of carbon monoxide were measured at 420 nm by replacement with nitric oxide as described (8). The nitric oxide concentration was 1 mM after mixing. As expected, the results with α_A and α_{Kansas} were identical, with apparent rate constants of 0.0132 sec^{-1} and 0.0134 sec^{-1}, respectively. The kinetics for the β_A subunits were closely first order with a constant of 0.0063 sec^{-1}. The β_{Kansas} subunit gave an initial apparent rate of 0.0105 sec^{-1}, which decreased to 0.008 sec^{-1} at 75% completion of the reaction.

The measurement of the rate of oxygen binding by α_A and β_{Kansas} subunits failed to give results of high precision, but the kinetics for β_{Kansas} were characterized by a very rapid phase (completed within the stopped flow dead-time) followed by a substantial slow phase with characteristics much like that of intact hemoglobin Kansas. The estimated initial rate for α_{Kansas} and β_{Kansas} subunits were 5×10^7 M^{-1} sec^{-1} and 2.5×10^7 M^{-1} sec^{-1}, respectively. However, the data for β_{Kansas} showed considerable heterogeneity.

The sedimentation velocity was measured for the β_A and β_{Kansas} subunits both in the unliganded and in the carbon monoxide forms under the same conditions as used for the oxygen equilibria (Fig. 1). The hemoglobin concentration was 0.1% and schlieren optics were used. Although the results were not of high precision, they showed clearly that the β_{Kansas} subunits were more highly dissociated than the β_A subunits in both the liganded and unliganded state. The values found for the unliganded subunits were: $s_{20,w} = 3.2$ (β_{Kansas}) and $s_{20,w} = 4.4$ (β_A). The same difference was found for the carbon monoxide forms but the $s_{20,w}$ values were 0.2–0.3 higher. The reason for this small difference is unknown.

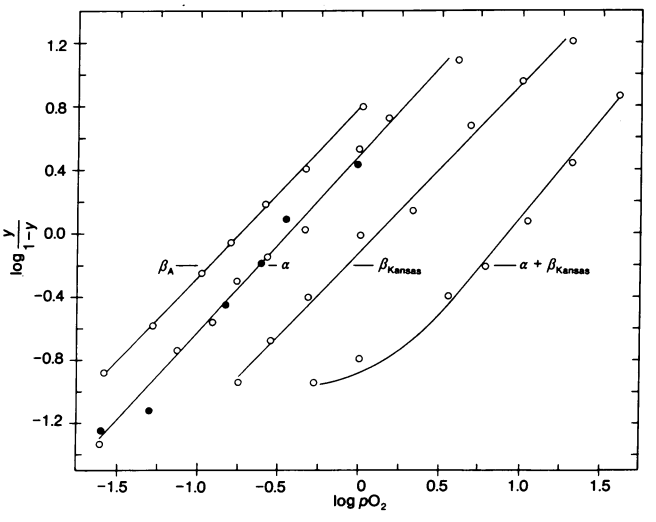

FIG. 1. Hill plot of the oxygen equilibrium data for the subunits of hemoglobins A and Kansas. y is the fractional degree of oxygenation; pO_2 is the oxygen pressure expressed in mm Hg. α-subunit line: *open circles*, α_{Kansas}; *closed circles*, α_A.

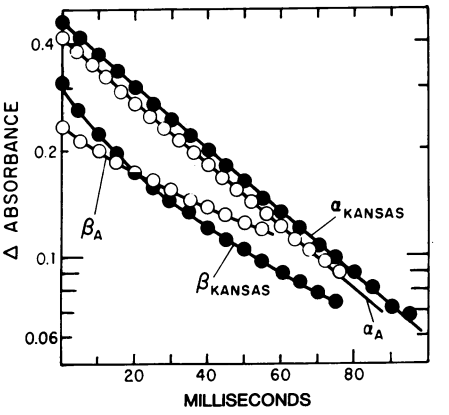

FIG. 2. Kinetics of deoxygenation of the subunits of hemoglobins A and Kansas in the presence of dithionite. The air-equilibrated stock solutions of the chains were diluted with O_2-free buffer (50 mM phosphate, pH 7) to 6 μM (heme) before mixing and reacted with 0.2% sodium dithionite in the same buffer. The reaction was followed at 432 nm with a 2-cm light path at 20°.

DISCUSSION

The equilibrium results (Fig. 1) show that the low affinity of intact hemoglobin Kansas is reflected in the properties of its variant β chain. The kinetic results indicate that the isolated β_{Kansas} subunit reacts with carbon monoxide at an initial rate only one-third as great as normal. The initial rate of dissociation of oxygen from β_{Kansas} is twice that observed for β_A. The initial kinetic rates for carbon monoxide are completely consistent with the equilibrium oxygen-binding data, and provide equilibrium carbon monoxide association constants of 3.79 ×

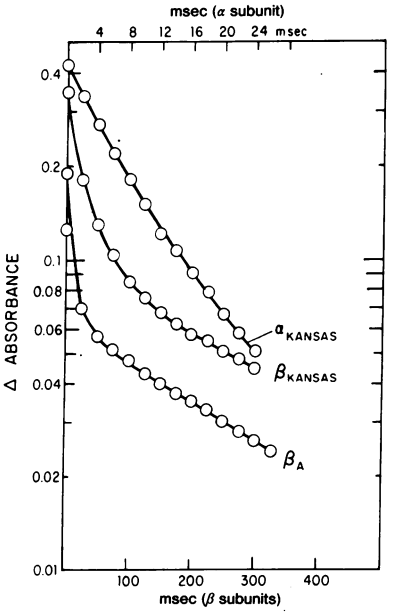

FIG. 3. Kinetics of the combination with carbon monoxide of α_{Kansas}, β_{Kansas}, and β_A subunits from hemoglobins A and Kansas. Buffer conditions: as in Fig. 2. Initial carbon monoxide concentration after mixing, 23 μM.

$10^8\ M^{-1}$, $6.67 \times 10^8\ M^{-1}$, and $1.33 \times 10^8\ M^{-1}$ for the α, β_A, and β_{Kansas} subunits, respectively. Thus the kinetic results predict a $\Delta \log P_{50}$ value between β_A and α subunits of 0.25 compared with 0.3 found in the oxygen equilibrium measurements, and a $\Delta \log P_{50}$ between β_A and β_{Kansas} of 0.7, which is close to the value of 0.8 found in the oxygen equilibria.

Greer (11, 12) has proposed, on the basis of x-ray diffraction studies, an explanation of the low oxygen affinity of hemoglobin Kansas. In normal human oxyhemoglobin only one polar bond is present across the $\alpha_1\beta_2$ interface. This bond is between asparagine G4 (β 102) and aspartic acid G1 (α 94). Since hemoglobin Kansas has a threonine substitution replacing asparagine at position β 102 (1), this bond does not form; so Greer has suggested that the oxy form is "destabilized relative to the deoxy form" (12). However, Greer (11, 12) also found that the heme group and the F_β, E_β, and B_β helices were all shifted slightly in the deoxy crystal of hemoglobin Kansas. Perutz and Lehmann (13) have predicted that the α-methyl group of the threonine at β 102 in hemoglobin Kansas would make contacts with the vinyl and methyl side chains of the heme. Our finding that the low affinity of hemoglobin Kansas is a property also of the isolated β subunits indicates that the destabilization of the $\alpha_1\beta_2$ interface, suggested by Greer, cannot be the sole explanation of the low affinity, but rather that the observed effects of the substitution in altering the tertiary structure of the β subunit itself and the shift in the position of the heme must be responsible.

Compelling kinetic evidence now exists that the α and β subunits within tetrameric hemoglobin differ in their ligand-binding properties (2, 7, 9). Mutations resulting in an altered α or β subunit might result in changes in conformation largely confined to the aberrant subunit or they might also affect the neighboring normal subunit. Although small differences were found by Bunn (14) in the oxygen affinity of α chains isolated from hemoglobin Chesapeake by the method of Bucci and Fronticelli (15), these differences could conceivably have been due to small quantities of residual mercurial remaining after the separation procedure. The present results appear to be the first demonstration of large differences between the properties of isolated β_A and mutant β subunits. The differences we have found cannot be attributed either to residual mercurial or to the formation of methemoglobin. At the least they reinforce the requirement that models of hemoglobin behavior must include provision for differences between the subunits such as that proposed recently by Ogata and McConnell (16).

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