THE SOLUBILITY OF HEMOGLOBIN β_4^S, THE MUTANT SUBUNITS OF SICKLE CELL HEMOGLOBIN

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Summary The single subunit hemoglobin β_4^S was found to have a solubility comparable to that of oxygenated rather than deoxygenated Hb S, although it contains twice as many mutant chains as the parent hemoglobin and probably has a quarternary structure similar to deoxy-hemoglobin A. This finding supports the assumption that receptor sites in the α chains of sickle hemoglobin are essential for sickling.

The hemoglobins of higher animals normally are tetramers, formed by pairs of two different polypeptide chains. Such heterotetramers can be split into the constituent subunits with retention of the hemes (1). When this is done, monomeric α chains are formed, but the other subunits (β in the case of Hb A) form very stable tetramers (2 - 5). β_4^A, the homotetramer corresponding to Hb A, occurs naturally in α thalassemia as Hb H (6, 7). Although it is structurally a tetramer, it behaves functionally like the monomer myoglobin, since it lacks all the allosteric properties of normal hemoglobin, such as the Bohr Effect, cooperative oxygen binding and a ligand-dependent affinity for DPG (8 - 10). The situation is analogous in other single subunit tetramers, such as γ_4^F (Hb Bart's), derived from fetal hemoglobin, $\alpha_2^A\gamma_2^F$ (11).

β_4^S, the homotetramer corresponding to sickle cell hemoglobin

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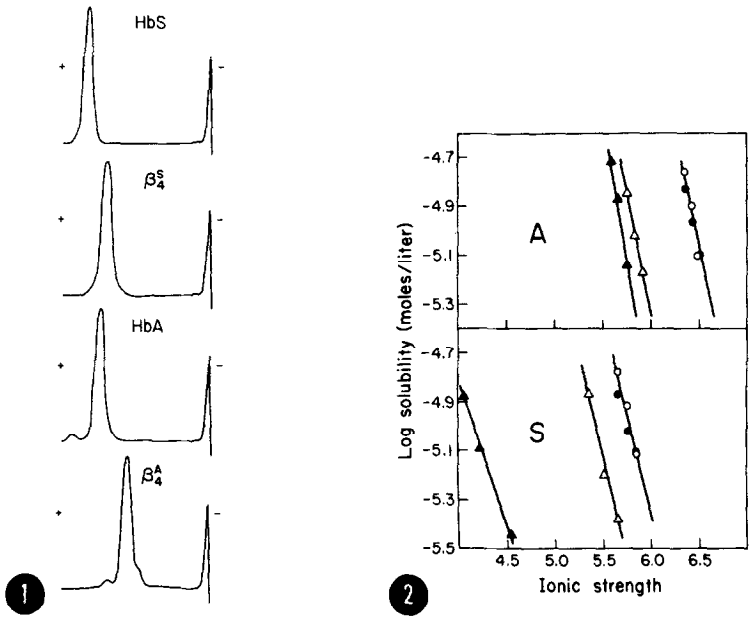

Fig. 1 Electrophoretic Mobility of Normal and Sickle Hemoglobin and their Homotetramers.

The gels were scanned without staining at 540 nm.

Fig. 2 Solubility of Hemoglobins

The experiments were carried out as described previously (16, 17), using 4 mg of hemoglobin in a final volume of 2.0 ml. The stock phosphate buffer solution had an ionic strength of 8.1 at pH 6.8.

At certain buffer concentrations β_4^A tends to crystallize which can be avoided by working rapidly. In the case of Hb S, where precipitation is slow, it was necessary to wait about 30 minutes before filtration.

A	Triangles	$\alpha_2^A \beta_2^A$
	Circles	β_4^A
	open symbols	oxy
	filled symbols	deoxy
S	Triangles	$\alpha_2^A \beta_2^S$
	Circles	β_4^S
	open symbols	oxy
	filled symbols	deoxy

($\alpha_2^A \beta_2^S$) is of special interest in relation to the molecular mechanism of sickling and also because of its possible occurrence in sickle cell disease associated with α thalassemia (12 - 14). It was first prepared

from Hb S and crystallized in this laboratory by Professor I. Tyuma in 1966. Like β_4^A, it was found to have a high oxygen affinity, no heme-heme interaction and to be very resistant to dissociation (15).

We have now investigated the solubility of β_4^S to compare it with that of the parent molecule $\alpha_2^A\beta_2^S$ (Hb S) where the insolubility of the deoxy form is responsible for the pathology of sickle cell anemia.

The homotetramers were prepared from Hb A and Hb S by the procedure of Bucci & Fronticelli (1). Mercury was removed by dialysis against N-acetylpenicillamine as described previously (10). The purity and identity of the products was confirmed by acrylamide gel electrophoresis (Fig. 1).

The solubility was measured as a function of ionic strength in phosphate buffer by a modification of the method of Itano (16, 17).

The data in Fig. 2 show that:

(1) The large decrease in solubility on deoxygenation characteristic of Hb S does not occur in β_4^S, although it contains twice the number of "abnormal" chains.

(2) The solubility of both homotetramers is unaffected by oxygenation.

(3) The specific substitution in the β^S chains (β6 Glu→Val) evidently decreases the solubility of hemoglobin even in the oxy state. Thus, oxy Hb S is somewhat less soluble than oxy Hb A and this difference is more pronounced in the case of the homotetramers.

Discussion Since Hb S sickles only in the deoxy conformation, a comparison with the β_4^S homotetramer would be particularly relevant if this

molecule were also in that conformation. There is ample evidence that hemoglobins composed of only one kind of subunit exist in only one conformational state irrespective of ligand binding (8, 9, 11, 18). Perutz & Mazzarella were the first to show that in crystals of β_4^A this fixed conformation closely resembles that of deoxy Hb A (19). This conclusion is strongly supported by the behavior of the cofactor 2,3-diphosphoglycerate toward β_4^A, to which it binds with the same stoichiometry and a similar affinity as to deoxy Hb A (10). Finally, β_4^A and β_4^S show the typical resistance to dissociation in strong salt or in dilute solution exhibited by deoxy but not by oxy Hb A (2, 15, 20).

It is clear that the presence of β^S chains in a hemoglobin tetramer in the deoxy conformation is not enough to induce the solubility changes associated with sickling. Receptor sites on the α chains can therefore be regarded as likely participants in the polymerization of Hb S and this is borne out by the decreased sickling tendency in $HbS_{Memphis}$, where the β6 Glu→Val substitution is accompanied by another one in the α chain, i.e. α23 Glu→Gln.

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