Chemical Modifications That Inhibit Gelation of Sickle Hemoglobin

(α and β N-terminal amino groups/pyridoxal phosphate/pyridoxal sulfate/hemoglobin solubility)

REINHOLD BENESCH, RUTH E. BENESCH, AND SUZANNA YUNG

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032

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ABSTRACT Substitution of the N-terminal amino groups with pyridoxal compounds inhibits gelation and increases the solubility of deoxy sickle hemoglobin (Hb S). Pyridoxylation of the α chains has considerably more effect than that of the β chains. The increase in minimum gelling concentration of Hb S that results from modification of the α N-termini is the same as that produced by dilution of Hb S with an equal amount of Hb A.

Among abnormal hemoglobins, hemoglobin S is of special interest since the single substitution of valine for glutamic acid in the sixth position from the N-terminal end of the β chains of this variant forms the molecular basis for the pathology of sickle cell anemia (1–3). The deoxygenated form of Hb S has a low solubility (4, 5) and, in concentrated solution, aggregates to form helical gel fibers (6, 7).

Although the mechanism of this polymerization is still not fully understood, it is clear that several sites other than the β' position are involved. Thus substitutions at β2 (Hb C_Harlem and Hb Korle-Bu), β11 (Hb O_Arab and Hb D_Punjab), and α13 (Hb S_Memphis) have been shown to influence the sickling process decisively (8–11).

Interest in the role of the terminal amino groups in the sickling phenomenon first arose from the finding that cyanate inhibits sickling (12). While it was shown that the reaction of cyanate with hemoglobin leads predominantly to carboxymylyation of the N-terminal amino groups, the interpretation of its effect on sickling is complicated. First of all, it is not clear how much of the inhibitory effect of carboxymylation can be ascribed to stabilization of the oxy conformation as reflected by the increased oxygen affinity of the carboxymethylated hemoglobin (13–17). In addition, since selective carboxymylation of either the α or β N-terminal groups has not been achieved, the relative role of the two sets of amino groups has not been evaluated.

Since we have previously shown (18, 19) that normal adult human hemoglobin (Hb A) can be specifically substituted with pyridoxal compounds at the N-terminal amino groups of either the α or the β chain, we have now applied these modifications to Hb S.

EXPERIMENTAL

Hb S was isolated from the blood of homozygous or heterozygous donors by chromatography on CM-Sephadex using a linear gradient from 0.05 M phosphate, pH 6.7, to 0.05 M phosphate, pH 6.7, plus 0.1 M NaCl. β-Substituted Hb S was prepared by reaction of deoxyhemoglobin S with pyridoxal phosphate (PLP) and the α-substituted derivative by coupling CO Hb S with pyridoxal sulfate (PLS). The compounds used and their sources were the same as before and the reaction conditions were identical to those described for Hb A (18, 19). The products, i.e., αA(βPPLP)2 and (αAPLS)βP, as well as unmodified Hb S (αAβP) were isolated as their CO derivatives by isoelectric focusing in polyacrylamide gels as described previously (20). They were then converted to the oxy form by oxidation to ferrihemoglobin followed by reduction either enzymatically (20) or by the method of Dixon and McIntosh (21).

Since the gelation experiments require relatively large amounts of material, they were carried out with pooled samples from several separate preparations. The identity and purity of the final products was established by electrophoresis after resolution into subunits as described before (19).

The solubility was measured as a function of ionic strength in phosphate buffer by a modification of the method of Itano (22, 23). The minimum concentration for gel formation was determined by the method of Singer and Singer (24) as modified by Bookchin, et al. (25).

RESULTS

The solubilities of the α- and β-substituted Hb S derivatives are shown along with that of normal Hb S in Fig. 1. It is clear that neither modification alters the solubility of Hb S in the oxy form. Pyridoxylation does, however, significantly decrease the characteristic drop in solubility of Hb S upon de-
Pyridoxylation at the $\alpha$ N-termini will break this bridge. In addition, repulsion between the grafted N-terminal pyridoxyl sulfates might further favor dissociation into half molecules and thus make polymerization more difficult.

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