CONCISE REPORT

Glycosylated Hemoglobin A2 Components

By C. Tegos and E. Beutler

Partially purified hemoglobin A2 has been examined for the existence of glycosylated components by isoelectric focusing and by acid agar gel electrophoresis. Bands analogous to the glycohemoglobin derivatives of hemoglobin A, hemoglobin-A1ace, were readily detected. Evidence that these minor bands are in fact glycohemoglobins was obtained by showing that 14C-glucose bound to hemoglobin A2 moved with these minor bands. The amounts of the glycohemoglobin derivatives of hemoglobin A2 were increased in the blood of diabetic patients.

HETEROGENEITY of the major hemoglobin (HbA) of normal adult erythrocytes has been recognized for over 20 yr. The concentration of some of these fractions, particularly HbA1c, was found to be increased in diabetic patients, and these are now recognized to be glycohemoglobins. These hemoglobins have proven to be good markers for the control of therapy of diabetes mellitus. They may prove to be a useful biochemical model for studying the secondary complications of this disease. Hemoglobin A2, a minor component of normal adult erythrocytes, has never been described in a glycosylated form. We present data suggesting the existence of glycosylated hemoglobin A2 components analogous to those of hemoglobin A1c.

Five normal persons and five uncontrolled diabetic patients (blood sugar: 300–550 mg/100 ml) were studied. HbA and HbA2 were purified by DE-52 chromatography, and Hb-A1c was obtained by chromatography on Bio-Rex 70 cation exchange resin (Isolab). Hemoglobin samples were concentrated to contain 2–3 g/100 ml and were focused in 5% acrylamide slab gels, containing 2.2% pH 6–8 ampholine (LKB). Hemoglobin bands were quantitated by homogenizing cut portions of the gel and extracting repeatedly with 0.15 M NaCl. A blank was prepared in the same way from a piece of gel not containing hemoglobin. The concentration of hemoglobin was measured at 415 nm. The concentration of HbA1c and HbA2c was expressed as a percentage of HbA and HbA2, respectively. Glycosylated HbA components (HbA1ace) were also measured by chromatography on the Bio-rex cation exchange resin. Electrophoresis was also performed on acid agar gels, pH 6.2.

The appearance of HbA2 and HbA after isoelectric focusing is shown in Fig. 1. In addition to the major band present in the HbA preparation, three more anodal bands presumably representing the glycosylated components of HbA, (HbA1ace) were apparent. An essentially identical hemoglobin banding pattern was observed when HbA2 from diabetic patients was focused: three minor bands, presumably representing HbA2ace were present anodally to the main component of HbA2. In samples from normal subjects, only the major of these bands (HbA2c), with the highest isoelectric point, was visible. The quantity of HbA2c was found to be increased in diabetic samples and correlated well with the levels of HbA1c. The concentration of two other glycoslated components (HbA2b and HbA2b) seemed, by inspection, to be correlated with the concentration of HbA2c. The acid agar gel electrophoretic pattern of HbA2 was identical to that of HbA1c (Fig. 2).

Further evidence was obtained that the anodal minor bands found after isoelectric focusing of HbA1c were, in reality, glycohemoglobins. Two and one-half millimolar glucose containing approximately 2.4 mCi 14C/mmole was incubated for 24 hr at 37°C with 2.5 mM HbA2 (calculated as the dimer) in 0.01 M bis-Tris buffer, pH 7.2. Unbound glucose was removed by filtration on Sephadex-G-25. Under these conditions, globin prepared by acid-acetone precipitation was found to have incorporated approximately 0.04 mmole labeled glucose/m mole of hemoglobin dimer. On isoelectric focusing of the hemoglobin, 80% of the applied radioactivity was recovered from the gel, and 85% of this was found in the anodally moving hemoglobin bands.

These results demonstrate that glycosylation of HbA2 as well as that of HbA1c occurs in normal adult erythrocytes. The structural similarity between β-chain of HbA and δ-chain of HbA2, which have the same NH2-terminal amino acid, makes likely the operation of the same mechanism of glycosylation in these two hemoglobins. As in the case of HbA1c, glucose and/or one of its derivatives may react nonenzymati-

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Fig. 2. Electrophoresis of hemoglobin A and hemoglobin A2 preparations on agar gel electrophoresis (pH 6.2). The cathodal moving minor components are present not only in the case of hemoglobin A but also hemoglobin A2.

cally with NH2-terminal amino acid of the δ-chain of HbA2 by way of a ketoamine linkage, resulting in the formation of glycosylated HbA2 components.

REFERENCES
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