STUDIES OF AN UNUSUAL HEMOGLOBIN IN PATIENTS WITH DIABETES MELLITUS

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Summary:

The properties of an unusual hemoglobin found in patients with diabetes mellitus resembled those of hemoglobin A_{1c} prepared from normal subjects. A two-fold increase of hemoglobin A_{1c} was found in diabetic patients. Structural studies suggest the possibility that an amino sugar is bound to hemoglobin A_{1c} in diabetic patients.

In a previous communication one of us reported the observation of an unusual hemoglobin (Hb) in patients with diabetes mellitus (1). The present report is concerned with studies of the structure of this hemoglobin component designated "diabetic component" which has now been observed in hemolysates of 140 patients with diabetes mellitus. (Observations on the occurance of the diabetic component in families has been the subject of a separate report (2).) The best electrophoretic separation of the diabetic component of hemoglobin was achieved on electrophoresis in agar gels in 0.05M citrate buffer pH 6.2 (3). In this system hemoglobins migrate toward the cathode and the diabetic component moves between Hb A and Hb F.

Increased proportions of the diabetic component were seen in the hemolysates obtained from patients with diabetes, particularly from those

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on insulin, but smaller proportions of a component with the same electrophoretic mobility were frequently seen in hemolysates from apparently healthy non-diabetic subjects. The diabetic component did not appear to be normal hemoglobin bound to oxidized glutathione since hemoglobin A treated with oxidized glutathione by the method of Huisman and Dozy (4) did not exhibit the electrophoretic mobility of the diabetic component. The chromatography of normal hemoglobin on Bio-Rex 70 columns by the method of Holmquist and Schroeder (5) yielded a minor component with an electrophoretic mobility identical with that of the diabetic component.

In the chromatographic system utilized by Holmquist and Schroeder (5) for the preparation of normal Hb A_{1c}, five minor components are eluted from Bio-Rex 70 columns before the major portion of the hemoglobin (Hb A_{II}). These minor components are designated Hbs A_{1a}, A_{1b}, A_{1c}, A_{1d} and A_{1e}. Hemoglobin A_{1c}, the third component to be eluted, had the same electrophoretic mobility as did the diabetic component (Figure 1). On chromatography on IRC-50 resin, with sodium phosphate cyanide buffer, pH 6.75, hemoglobin A_{1c} is the largest of the several minor rapidly eluting components of hemoglobin.

When hemoglobin prepared from normal and from diabetic subjects was chromatographed on Bio-Rex 70 as described by Holmquist and Schroeder (5), an increased proportion of Hb A_{1c} was regularly observed in hemolysates of diabetics as compared with normals. In normal subjects, Hb A_{1c} comprised 4-6 per cent of the hemoglobin, in agreement with the results of previous workers (6,7). In patients with diabetes mellitus, Hb A_{1c} represented 7.5 to 10.6 per cent of the total hemoglobin. The proportions of other minor components eluted before the major component, Hb A_{II}, were within the same range in normal and in diabetic subjects.

When Hb A_{1c} isolated from diabetic patients was treated with paramercuriobenzoate (PMB) by a modification (8) of the method of Bucci and Fronticelli (9), the β chain of diabetic A_{1c}, like that of normal Hb A_{1c}

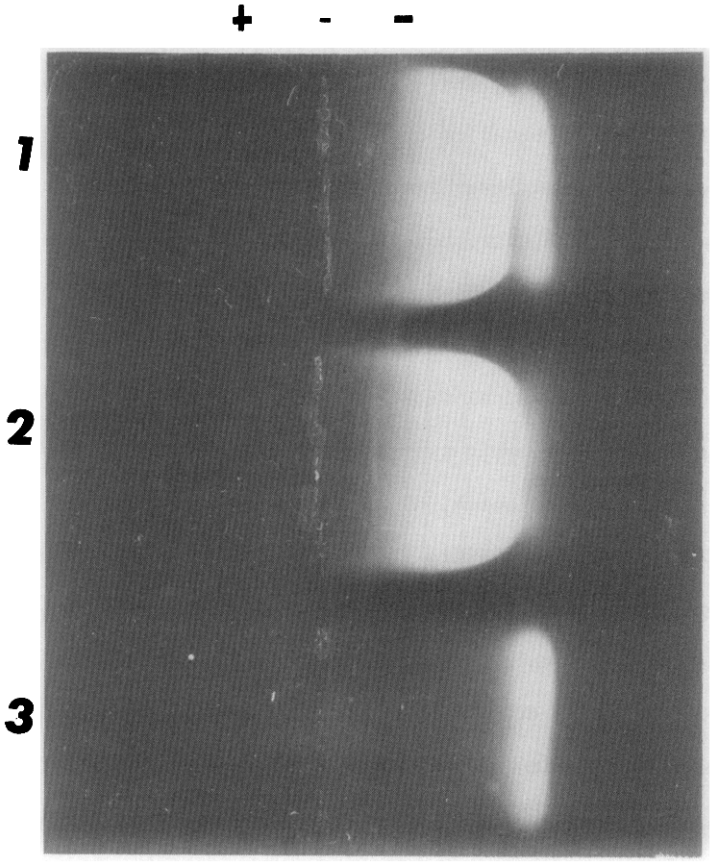

Fig. 1

Agar gel electrophoresis, pH 6.2, 0.05M citrate buffer. (1) Hemolysate from diabetic. (2) Hemolysate from normal. (3) Purified Hb A_{1c}.

(10), migrated faster than the normal β chain of Hb A on starch gel electrophoresis pH 8.6. The polypeptide chains of Hb A_{1c} from diabetic and from normal subjects were isolated by chromatography of PMB-treated hemoglobin on carboxymethylcellulose (CMC) by a modification of the original method of Bucci and Fronticelli (9) using 0.01M phosphate buffer, pH 5.8 for equilibration of CMC and 0.01M phosphate buffer, pH 6.7 for elution.

Alpha and beta chains of Hb A_{1c} were also prepared by column chromatography of diabetic and normal Hb A_{1c} globins on carboxymethylcellulose in 8M urea by the method of Clegg, Naughton and Weatherall (11). Maps of

tryptic peptides of the β chains of diabetic Hb A_{1c} were indistiguishable from β chain of normal Hb A_{1c} as described by Bookchin and Gallop (10). The βTp I peptide was missing from its normal position and two new peptides migrating anodal to normal βTp I were present (Figure 2); both of the new peptides had the same amino acid composition as that of normal βTp I.

Studies of Holmquist and Schroeder (5) indicated that the N-terminal group of one of the β chains of Hb A_{1c} was bound through a borohydride reducible linkage to an unknown compound; recent studies of Bookchin and Gallop (10) suggested that this compound is a hexose, probably bound to both β chains of Hb A_{1c}.

Unfractioned hemolysate from diabetic subjects (1.5 grams hemoglobin per 100 ml in 0.1M sodium phosphate buffer, pH 7) was reacted with a 200 molar excess sodium borohydride ($NaBH_4$) for 10 minutes at room temperature, 50 minutes at 10°C and dialyzed to remove excess $NaBH_4$. On agar gel electro-

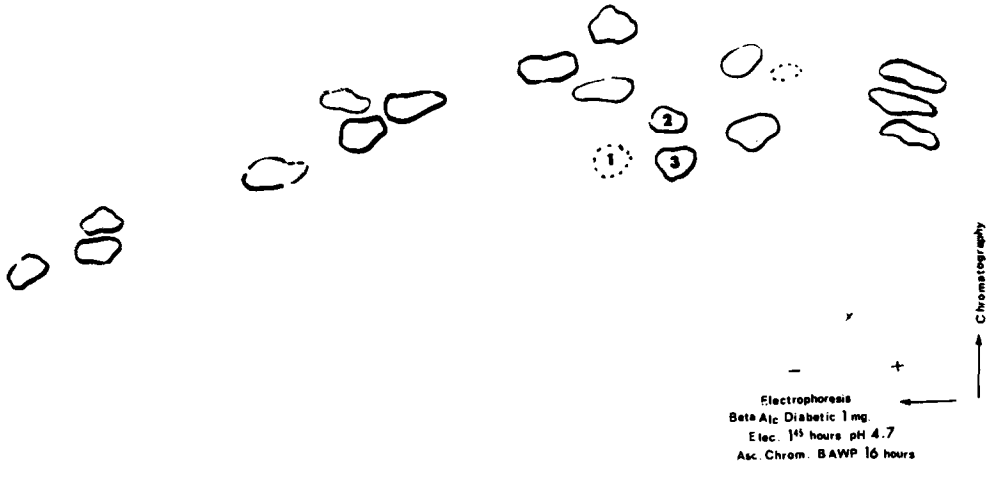

Fig. 2

Tracing of peptide maps of βA_{1c} prepared from diabetic subject. Normal βTp I is indicated by 1 on map; 2 and 3 are two 'new' peptides each with the amino acid composition of βTp I.

phoresis of this hemolysate, the diabetic component was no longer seen.

Preparations of Hb A_{1c} and of Hb A_{II} from diabetic patients were separately reacted with ^3H-labelled $NaBH_4$ of effective specific activity 8 x 10^6 dpm per μ mole, as defined and calibrated by Gallop and co-workers (12), using the method described above. The reduction was followed by extensive dialysis, and globins were prepared by acid-acetone precipitation at -20°. The diabetic A_{1c} globin had a specific activity of 14.7 x 10^6 dpm per μ mole of tetramer as compared to 5.2 x 10^6 dpm per μ mole tetramer in Hb A_{II} globin.

The α and β chains of reduced, ^3H-labelled Hb A_{1c} from a diabetic subject were isolated on a carboxymethylcellulose column as described above; the specific activity of the α chain was 1.3 x 10^6 dpm per μ mole monomer and that of the β chain, 6.7 x 10^6 dpm per μ mole monomer suggesting a single one-step reduction of each β chain in which one non-exchangeable $-\overset{|}{\underset{|}{C}}-\ ^3H$ was formed. Peptide maps of soluble tryptic peptides of reduced ^3H-labelled diabetic β A_{1c} globin showed two peptides with mobilities slightly different from the two new peptides of βTp I seen in Hb A_{1c} before reduction. The radioactivity of peptides from the maps of ^3H-labelled β A_{1c} was determined in Bray's solution; most of the eluted radioactivity was found in the two new peptides (βTp I), as Bookchin and Gallop (10) found in Hb A_{1c} from normal subjects.

The similarities of electrophoretic and chromatographic behavior and peptide maps of diabetic component and Hb A_{1c} suggest that the presence of Hb A_{1c} in increased amounts accounts for the unusual Hb component observed in diabetics. However further studies suggested possible differences between normal and diabetic Hb A_{1c}: when whole β chains of Hb A_{1c} from diabetic and from normal subjects were separately hydrolyzed in 6N HCl at 105°C for 18 hours and chromatographed on a Beckman 120B amino acid analyser, a peak emerging before lysine on the short column (pH 5.28) was noted in Hb A_{1c} hydrolysates from diabetic subjects; this pre-lysine peak was absent in hydrolysate of Hb A_{1c} prepared from normal subjects. The pre-lysine peak

disappeared following reduction with $NaBH_4$ of Hb A_{1c} from diabetic subjects suggesting the reduction of the unidentified component. This peak disappeared when periodate oxidation was performed on an acid hydrolysate of Hb A_{1c} from diabetic subjects.

The position of the peak in the amino acid analyser, its susceptibility to borohydride reduction and to periodate oxidation suggest the possibility that the unknown component of Hb A_{1c} prepared from diabetic subjects is an amino sugar. Work on the structure of this unknown component is in progress.

Acknowledgement

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