Structure of Carbohydrate of Hemoglobin $\text{A}_{1c}$

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Hemoglobin $\text{A}_{1c}$ is a minor component of normal adult erythrocytes whose concentration is elevated approximately 2-fold in patients with diabetes mellitus. Previous work suggested that the unique structural feature of hemoglobin $\text{A}_{1c}$ is the presence of a low molecular weight sugar moiety at the NH$_2$-terminal valine of the $\beta$ chain. In this study the structure of the carbohydrate moiety and the nature of its linkage to the $\beta$ chain were investigated. Enzymatic digestion of borohydride-reduced $\beta^1$-chains followed by ion exchange chromatography led to the isolation of two distinct NH$_2$-terminal glycovanlyhistidines. Comparison of these glycodipeptides with synthetic glycovanlyhistidines by thin layer chromatography, gas-liquid chromatography, and proton magnetic resonance spectroscopy gave direct evidence that the naturally derived materials correspond to glucitol and manniitol valylhistidines. Model reactions showed that glucose and mannose react with valine under mild conditions to form an adduct which upon sodium borohydride reduction yields in both cases glucitol and mannitol valylhistidines. These reactions proceed with high selectivity for the NH$_2$ terminus of the $\beta$ chain, 1-deoxy-1-(N-valyl)fructose, for both reactions. From these studies we conclude that hemoglobin $\text{A}_{1c}$ has, as the NH$_2$ terminus of the $\beta$ chain, 1-deoxy-1-(N-valyl)fructose. The possible biosynthetic pathways of hemoglobin $\text{A}_{1c}$ are discussed.

Ninety to ninety-five per cent of adult human hemoglobin consists of one major species, hemoglobin $\text{A}_{1c}$. The remaining 5 to 10% is a mixture of hemoglobin $\text{A}_{1a}$ (2.5%) (1), $\text{A}_{1b}$ (0.5%) (1), $\text{A}_{1a}$ (1 to 2%) (2), and $\text{A}_{1e}$ (3 to 6%) (2). Hemoglobins $\text{A}_{1a}$ and $\text{A}_{1c}$ are particularly interesting because their concentrations are elevated approximately 2-fold in patients (2) and animals (3) with diabetes mellitus. The formation of $\text{Hb A}_{1c}$ occurs in mice (3) and humans (1) at a constant slow rate as a postsynthetic modification of Hb $\text{A}_{1b}$ throughout the life of the red cell. The increased concentration of $\text{Hb A}_{1c}$ in diabetes has been shown by biosynthetic studies in the mouse (3) to be the result of an increased rate of synthesis. The rate of synthesis is dependent upon the physiological state of the animal. Erythrocytes from both nondiabetic and diabetic mice synthesize $\text{Hb A}_{1c}$ 2.7 times faster when circulating in a diabetic rather than nondiabetic mouse. The nature of the plasma factor controlling the rate of $\text{Hb A}_{1c}$ synthesis is undetermined, although in diabetic humans a significant correlation exists between the amount of $\text{Hb A}_{1c}$ and the degree of carbohydrate control (4). It appears that $\text{Hb A}_{1c}$ acts as an indicator molecule which monitors and, in effect, integrates the plasma blood glucose concentration of the patient over the previous weeks (5). The quantification of $\text{Hb A}_{1c}$ in the erythrocytes of patients with diabetes mellitus offers a new way to detect the diabetic state and monitor the effectiveness of therapy.

The nature of the modification of Hb $\text{A}_{1b}$ to form $\text{Hb A}_{1c}$ has not been fully elucidated. Initial studies (6) indicated that the amino acid sequences of Hbs $\text{A}_{1b}$ and $\text{A}_{1c}$ are identical. The only detectable difference was a lower molecular weight sodium borohydride-reducible moiety, presumably a Schiff base, at the NH$_2$ terminus of the $\beta$ chain of $\text{Hb A}_{1c}$. Subsequent work pointed to a carbohydrate as the modifying group on the $\beta^1$-chains (7). Recently, Bunn et al. (8) have proposed that glucose is the carbohydrate and that it undergoes an Amadori rearrangement subsequent to Schiff base formation. This proposal was based on the observation that acid hydrolysis of $\text{Hb A}_{1c}$ liberated 0.3 mol of hexose, consisting of glucose and mannose in a 3:1 ratio, per mol of Hb. In addition to the low recovery of hexose, another unknown component was liberated under these conditions. The present work was undertaken to definitively determine the nature of the carbohydrate on $\text{Hb A}_{1c}$ and the nature of its linkage to the $\beta$ chain.

**MATERIALS AND METHODS**

Reagents — All chemicals and solvents were reagent grade. Trypsin, papain, protease VI, and Sil-A (silylating reagent) were obtained from Sigma Chemical Co., St. Louis, Mo. The ion exchange resins Bio-Rex 70, AG50W-X2, AG50W-X8, and AG1-X8 were purchased from Bio-Rad Laboratories, Richmond, Calif.; and cellulose phosphates P1 and P11 from Whatman, Inc., Clifton, N. J. Silicic acid G-10 was obtained from Pharmacia Fine Chemicals, Piscataway, N. J. Silica Gel GF thin layer chromatography plates were purchased from Analtech, Inc., Newark, Del. Valylhistidine was purchased from Calbiochem, Los Angeles, Calif., NaB$^4$H$^4$, and [3H]valine from Amersham/Searle, Arlington Heights, Ill., and 100% d-deuterium oxide, 99.5% pyridine d$_5$, 99.9% acetic acid d$_4$, and sodium-3-trimethylsilylpropionate-2,3,5,4-d$_4$ from Wilmad Glass Co., Buena, N. J.

**Isolation of $\text{Hb A}_{1c}$**

Blood was drawn from normal human volunteers into heparinized flasks. The red cells were separated from the plasma by centrifugation, washed twice with 2 volumes of 0.14 N NaCl, lyzed by the addition of 2 volumes of distilled water, and the hemoglobin solution saturated with carbon monoxide. The carboxyhemoglobin solution was dialyzed against Developer 6 (0.05 M sodium phosphate and 0.01 M KCN, pH 6.75) saturated with CO. From 50 to 75 g of hemoglobin (50 to 75 mg/ml) were loaded onto a column of Bio-Rex 70 (15 x 50 cm) previously equilibrated with Developer 6 (2). Hemoglobins $\text{A}_{1a}, \text{A}_{1b}, \text{A}_{1c}$ were eluted with Developer 0 at a rate of 3 liters/h. 1.5-liter fractions were collected. The absorbance of the eluent at 415 nm was monitored to determine the elution profile. Two to three grams of $\text{Hb A}_{1c}$ were isolated. The pH of the pooled $\text{Hb A}_{1c}$ fractions (7 liters) was lowered to 6.4 with H$_2$PO$_4$, and the solution was applied to a column (5 x 70 cm) of cellulose phosphate P1, previously equilibrated with 0.05 M sodium phosphate buffer, pH 6.4, at 4°. $\text{Hb A}_{1c}$ adsorbed to the resin while contaminating $\text{Hb A}_{1a}, \text{A}_{1b}$ did not. The column was then brought to room temperature and the
Hb Aβ, eluted with 0.25 M sodium phosphate-0.01 M KCl, pH 7, buffer. This procedure resulted in further purification and a 10-fold concentration of the Hb Aβ.

Isolation of Glycopeptides from Hb Aβ. The Hb Aβ solution (15 mg/ml) was dialyzed against 0.1 M sodium phosphate buffer, pH 7, and subsequently reacted with a 200 molar excess of NaBH₄ (1.4 × 10⁶ dpm/pmol) at room temperature for 1 h. After removing the unreduced sodium borohydride by dialysis, the hemoglobin was extracted in acetone-2% HCl at 20°C (9), and the α and β chains separated (10).

The β chains were dissolved at 1 mg/ml in 1% NH₄HCO₃ and digested for 4 h at 37°C with trypsin (50 μg/mg of globin). The tryptic peptides were separated by ion exchange chromatography on AG50W-X2 (11) and further purified by chromatography on cellulose phosphate P12 (12). The fractions containing the triated NH₂-terminal glyco-octapeptide were pooled and lyophilized. The purity of the NH₂-terminal glyco-octapeptide (β-NH₂) was assessed by hydrolysis of a portion in 6 N HCl at 105°C in vacuo for 16 h followed by amino acid analysis (Beckman 19C amino acid analyzer).

The peptide β-NH₂ was dissolved (1 mM) in a solution of 0.05 M NaCN, 0.002 M EDTA, and 0.1 M ammonium acetate buffer, pH 5.5, and digested for 16 h at 39°C by the addition of pepsin (45 units/mg of peptide). The NH₂-terminal glycopeptide (glycovalylhistidine) was isolated by column chromatography on AG 50W-X8 according to the procedure of Holmquist and Schroeder (6), except that the glycopeptide was eluted at room temperature using a linear gradient (250 ml, total volume) of pyridine acetate, pH 5.28, where the pyridine concentration increased from 0.175 to 0.250 M. The purity of the glycovalylhistidine was determined by hydrolysis in 6 N HCl in vacuo for 16 h, followed by amino acid analysis. Digestion of the glycovalylhistidine with protease VI was not successful, although under similar conditions complete digestion of valylhistidine was observed.

Preparation of Adducts of Valine and Valylhistidine with Hexoses—Adducts of various hexoses (D-glucose, D-mannose, and D-galactose) with L-valine were synthesized according to the method of Dixon (13). D-[14C] Valine (7 mmol) (specific activity, 1.4 μCi/mmole) and hexose (9 mmol) were stirred for 3 days at room temperature in 150 ml of 0.1 M sodium phosphate buffer, pH 7, with 0.5% KMnO₄ in 1 M NaOH. The Rf value of the unreduced adducts was 0.58 and the residues dissolved in 180 ml of 1 M morpholine. A 25 molar excess of sodium borohydride was added to each reaction mixture and the mixtures stirred for 3 h at room temperature. The progress of the reaction was monitored by thin layer chromatography on Silica Gel GF in methyl ethyl ketone:isobutyl alcohol:water:diethylamine (80:80:40:9). The compounds were visualized by spraying the plate with 0.5% KMnO₄, in 1 M NaOH. The Rf value of the unreduced adducts was 0.35 and those of the reduced materials are listed in Table II. The reaction mixtures were lyophilized, dissolved in 10 ml of 0.1 M sodium phosphate buffer and applied to columns (2.5 × 50 cm) of Sephadex G-10. The reduced adducts were eluted with 0.5 M morpholine and thus separated from the bulk of the salts and unreacted reagents. Fractions containing the glycolic valine (reduced adducts) were lyophilized and the oily residues were dissolved in 150 ml of distilled water. The solutions were made basic (pH 11 to 12) by the addition of piperidine and then applied to columns of AG1-X8 (4 × 50 cm) previously equilibrated with morpholine acetate buffer, pH 8.6 (0.25 M morpholine). The adducts were eluted with this buffer in approximately 5 column volumes and lyophilized. The residual morpholine acetate was removed by repeated evaporations from pyridine on a rotary evaporator until constant weight was achieved. The products were recrystallized from ethanol/water (2:1) with an overall yield of 30%.

The (275 mm) was reacted with L-valyl-L-histidine (180 μmol) in 7 ml of pyridine-acetic acid (3:1) according to Dixon (13). The reaction mixture was lyophilized and the residue dissolved in 1.5 ml of 1 M morpholine containing 6.9 mmol of NaBH₄ (2 × 10⁶ dpm/pmol). After 6 h at room temperature the material was lyophilized and the unreduced NaBH₄, was quenched with 2 M acetic acid. The material was dried by rotary evaporation, dissolved in 7 ml of 1 M acetic acid, and the pH adjusted to 2 with 6 M HCl. The sample was applied to a column of AG 50W-X8 (0.9 × 100 cm). The column was developed with Buffers A and B according to Holmquist and Schroeder (6), followed by a linear gradient (400 ml total volume) of pyridine acetate buffer, pH 5.28, 0.175 M to 0.260 M pyridine. Fractions of 2.7 ml were collected at a flow rate of 10 ml/h.

Analysis All synthetic and natural glycopeptides were analyzed by thin layer chromatography on Silica Gel GF using the solvent systems propanol:water (85:15) and methyl ethyl ketone:isobutyl alcohol:water:diethylamine (80:80:40:9). The plates were developed twice and sprayed with either 0.5% KmnO₄ in 1 N NaOH, Pauly reagent (14), or ninhydrin (14). Gas-liquid chromatography, performed on the trimethylsilyl derivatives (15) of both the glycovalylhistidine and glycovalylhistidine methyl esters formed in methanolic-1.5 N HCl. Analysis was performed using a OV-17 column (4 mm × 6 foot) (Supelco Company, Bellefonte, Pa.) in a Packard 417 gas chromatograph. The gas flow rates were carrier nitrogen, 45 ml/min; hydrogen flow to detector, 30 ml/min; air flow to detector, 230 ml/min. For analysis of the glycolyl valine derivatives the instrument was programmed from an initial temperature of 175°C with a temperature increase of 0.1°C/min. The glycovalylhistidine derivatives were analyzed from an initial temperature of 145°C with a temperature increase of 0.1°C/min.

All compounds were analyzed at 220 MHz by proton magnetic resonance spectroscopy employing a Varian HR/NTC-220 proton magnetic resonance spectrometer having pulse Fourier transform and decoupling facilities. For the naturally derived glycopeptides, 256 acquisitions were taken using a 90° pulse. Solvent for the valycarbohydrates was deuterovaline; for the glycovalylhistidines, deuterated pyridine acetate (12 mm pyridine, pH 6.04) was used. Chemical shifts of the glycolyl valine derivatives are relative to Me₄Si-propionate.1 Acetate was used as internal reference for the glycolyl valylhistidine. The chemical shift of acetate (1.92 ppm, in the d3 range employed) was determined using Me₃Si-propionate as an internal standard and the chemical shifts of the glycolyl dipeptides are related to Me₃Si-propionate in this manner.

RESULTS

Three grams of Hb Aβ were isolated, reduced with NaBH₄, and the β chains then isolated as described under “Materials and Methods.” After digestion with trypsin and purification by column chromatography, the NH₂-terminal glyco-octapeptide (β-NH₂) had the amino acid composition listed in Table I. This agrees well with the expected composition except that a valine peak was not observed in the analysis. A triptid-containing peak which was ninhydrin-negative eluted prior to the position of aspartic acid and presumably was a substituted valine which is stable to acid hydrolysis.

Purified β-NH₂ was digested with papain and subjected to chromatography on AG 50W-X8. The elution profile is shown in Fig. 1. Two radioactive components, in approximately a 2:1 ratio, were resolved. Amino acid analysis of acid-hydrolyzed portions of both of these gave histidine as the only amino acid, in agreement with the structure glycovalylhistidine for both naturally derived products (R₁-Val-His and R₂-Val-His). Four micromoles of R₁-Val-His (Fractions 161 to 168) and 2 μmol of R₂-Val-His (Fractions 172 to 178) were isolated from 62 pmol of β-NH₂ chains for an overall yield of 10%.

When glucose was reacted with valine and the reaction mixture subsequently reduced with NaBH₄, (see “Materials and Methods” for details), two chromatographically distinct 1-

1 The abbreviations used are: Me₃Si-propionate, sodium-3-trimethylsilylpropionate-2,3,3,4-d₄; Me₂Si, trimethylsilyl; pmr, proton magnetic resonance.
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**FIG. 1.** (left). AG 50W-X8 elution profile of the papain-digested, NaB₃H₄-reduced β⁺-chain NH₂-terminal glyco-octapeptide (β⁺-T1). Five micromoles of peptide digest in 2 ml of pyridine acetate buffer, pH 3.29, were applied to a column (0.8 × 46 cm) and eluted as described under "Materials and Methods." Fractions of 1.5 ml were collected at a flow rate of 15 ml/h. The glycopeptides, R₋-Val-His (Fractions 161 to 168) and R₋-Val-His (Fractions 172 to 178), from two columns were pooled yielding 4 µmol of R₋-Val-His and 2 µmol of R₋-Val-His.

**FIG. 2 (right).** The 220 MHz pmr spectrum of the major product obtained from the reaction of glucose with valine, followed by NaBH₄ reduction. The spectrum is recorded in D₂O using Me₃Si-propionate as an internal standard. Assignment of chemical shifts is based upon pmr spectra of valine and glucitol and spin decoupling studies described under "Results."

Deoxy-1-(N-valyl)hexitol products were obtained. Similarly, the reactions of mannose and galactose with valine each gave two products. Rᵥ values of all products and gas chromatographic retention times of their respective Me₃Si derivatives are listed in Table II. In all cases the major product was obtained in crystalline form. The pmr spectrum of the major product obtained from the reaction of glucose with valine and the assignment of chemical shifts is shown in Fig. 2. Irradiation of the H₉ multiplet of valine at 2.26 ppm caused the doublet centered at 3.56 ppm to merge into a singlet, whereas none of the sugar resonances were effected, thereby establishing the chemical shift of the proton attached to the valine α carbon (H₆). Similarly, irradiation of the multiplet at 4.1 ppm (H₉) converted the two-proton octet (AB portion of ABX system) centered at 3.22 ppm into a quartet, establishing the chemical shifts of the H₆, H₉, and H₈ protons. The major product (75%) obtained from the reaction of mannosyl-Val-His had the identical pmr spectrum (not shown). The minor products of both the glucose and mannose reactions were not isolated free of the major product, but both mixtures had identical pmr spectra. The pmr spectra of the two galactosyl-valine adducts differed significantly from each other and from the glucosyl valine (and mannosyl valine) spectra. These data are consistent with an initial hexose-valine Schiff base or aldosylamine linkage followed by an Amadori rearrangement (16) prior to borohydride reduction (Fig. 3).

Glucose was reacted with valylhistidine, reduced with NaB₃H₄, and the product applied to an AG 50W-X8 column. The elution profile is shown in Fig. 4. Peak 1 (Fractions 110 to 119) was eluted with Buffer D of Holmquist and Schroeder.

**TABLE II**

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
<th>System 1*</th>
<th>System 2b</th>
<th>Gas chromatographic retention time c (min)</th>
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</thead>
<tbody>
<tr>
<td>Glucose + valine</td>
<td>Major product</td>
<td>0.20</td>
<td>0.29</td>
<td>33.5</td>
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<tr>
<td></td>
<td>Minor product</td>
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<td>0.30</td>
<td>31.6</td>
</tr>
<tr>
<td>Mannose + valine</td>
<td>Major product</td>
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<td>0.29</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>Minor product</td>
<td>0.20</td>
<td>0.30</td>
<td>31.6</td>
</tr>
<tr>
<td>Galactose + valine</td>
<td>Major product</td>
<td>0.24</td>
<td>0.32</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>Minor product</td>
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<td>0.40</td>
<td>33.9</td>
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<tr>
<td>Glucose + Val-His</td>
<td>Peak 1*</td>
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<td>0.23</td>
<td></td>
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<tr>
<td></td>
<td>Peak 2*</td>
<td>0.24</td>
<td>0.21</td>
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</tr>
<tr>
<td></td>
<td>Peak 3*</td>
<td>0.21</td>
<td>0.17</td>
<td>24.1</td>
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<td></td>
<td>R₋-Val-His⁺⁺⁺*</td>
<td>0.24</td>
<td>0.21</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>R₋-Val-His⁻⁻⁻*</td>
<td>0.21</td>
<td>0.17</td>
<td>24.1</td>
</tr>
</tbody>
</table>

* Methyl ethyl ketone:isobutyl alcohol:water:diethylamine (80:80:40:9); two developments.

b Propanol:water (85:15); two developments.

c See "Materials and Methods" for program conditions.

d Gas chromatography was performed on Me₃Si derivatives of the methyl esters of these compounds.

e Naturally derived products isolated from hemoglobin Aᵦ.

Peaks 2 (Fractions 258 to 267) and 3 (Fractions 275 to 281) were subsequently eluted with the pyridine acetate gradient. Analysis by thin layer chromatography and gas-liquid chromatography indicated glycitol valyhistidine peaks 2 and 3 were...
The Schiff base adduct between valine and glucose undergoes an Amadori rearrangement to form 1-deoxy-1-(N-valeryl)fructose. Borohydride reduction of this compound gives a mixture of the C2 isomeric glycitol valines.

The pmr spectra of the naturally derived Rv-Val-His and Rv-Val-His are compared in Fig. 5. Assignment of resonances is based on the chemical shift data of valylhistidine and glycitol valine (see above). Small but distinct differences in the splitting patterns of the sugar protons and the two AB portions of the spectra were observed. Upon expansion of these regions, these differences became clearer (Fig. 6). Comparison of the expanded spectra of the naturally derived Rv-Val-His and Rv-Val-His to those of the synthetically derived glycitol Val-His peaks 2 and 3, respectively, shows them to be identical (Fig. 6).

The adduct of glucose plus valylhistidine found in peak 1 gave a pmr spectrum qualitatively similar to the above derivatives; the major difference being that by integration the substance contained 2 mol of hexose/mol of valylhistidine. The material is tentatively assigned as containing two hexoses attached at the NH2 group of valine (16).

**DISCUSSION**

Hemoglobin A1c is the most abundant of the minor Hbs found in adult human red cells (1). The discovery that the concentration of Hb A1c is elevated 2-fold in diabetics (2) has aroused special interest in the structure and the synthesis of this molecule. The conversion of Hb A to Hb A1c has been proposed as a model for protein modification in diabetes that may explain many of the secondary sequelae of this disease (5). The concentration of Hb A1c in the peripheral blood of diabetics has been shown to accurately reflect the patient's mean blood sugar concentration of the previous several weeks (5). The structure of Hb A1c is identical with that of Hb A except for a low molecular weight borohydride-reducible moiety on the NH2-terminal valine of the $\beta^\text{A1c}$ chain (6). It has been proposed (8) that a glucose molecule is attached to the valine via a Schiff base which subsequently undergoes an Amadori rearrangement.
In the current study Hb $\alpha_{6}$ was isolated free (<1%) of hemoglobins $\alpha_{2}$ and $\alpha_{6}$ (which is also glycosylated (17)) by cellulose phosphate ion exchange chromatography. The Hb $\alpha_{6}$ was then reduced with NaBH$_4$ and the $\alpha$ and $\beta$ chains separated. The $\beta$ chains were digested with trypsin, and the NH$_2$-terminal glyco-octapeptide isolated was then further digested with papain. Two chromatographically distinct NH$_2$-terminal glycovalylhistidine peptides ($R_1$-Val-His and $R_2$-Val-His) were then isolated. The mild conditions used in the isolation of $R_1$-Val-His and $R_2$-Val-His assure that the $R$ groups were not modified (except for borohydride reduction) prior to identification. Purity of the NH$_2$-terminal glycopeptides isolated was assessed after each enzymatic digest. The NH$_2$-terminal glyco-octapeptide was shown by amino acid analysis to be derived solely from Hb (Table I). Similarly, the amino acid sequence of the glycodipeptides isolated was valylhistidine—the sequence at the NH$_2$ terminus of Hb $\beta$ chains. Thus, nonheme proteins that are known to contaminate Hb $\alpha_{6}$ isolated by ion exchange chromatography on Bio-Rex 70 (1) could not have been the source of $R_1$-Val-His or $R_2$-Val-His.

The structures of the naturally derived, pure $R_1$-Val-His and $R_2$-Val-His were compared with model compounds. Adducts of glucose, mannose, and galactose with valine were synthesized, reduced with NaBH$_4$, and purified. Each hexose resulted in the formation of two reduced adducts. The fact that two reduced adducts were obtained and that the glucose and mannose adducts are identical is consistent with initial formation of a hexose-valine Schiff base or aldosylamine linkage which undergoes an Amadori rearrangement (10) prior to reduction. The Amadori rearrangement product formed from the adducts of both glucose and mannose with valine is 1-deoxy-1-(N-valyl)fructose. Subsequent reduction with NaBH$_4$ is not stereospecific and therefore generates 2 $C_2$ isomeric products (Fig. 3). In a similar fashion the reaction of glucose with valylhistidine was found to yield, after borohydride reduction, both glucitol and mannitol valylhistidines. These products were separated by ion exchange chromatography and shown to be identical by gas-liquid chromatography, thin layer chromatography (Table II), and pmr spectroscopy (Fig. 6) to $R_1$-Val-His and $R_2$-Val-His obtained from Hb $\alpha_{6}$. Thus we conclude that Hb $\alpha_{6}$ has, as the NH$_2$ terminus of the $\beta$ chain, 1-deoxy-1-(N-valyl)fructose.

Assignment of the configuration at $C_2$ of $R_1$-Val-III and $R_2$-Val-His has not been attempted although it would be of general interest. However, in situ or in vivo, the material exists in a keto or hemiacetal form and only subsequent to borohydride reduction is the asymmetric $C_2$ center generated. Of greater interest would be the configuration of the carbohydrate in situ, i.e. an open chain or furanose or pyranose ring.

Although glucose or mannose could react in vitro with Hb A to form hemoglobin $\alpha_{6}$, it has not been possible to demonstrate this reaction in vitro (17). However, it has been possible to nonenzymatically glycosylate hemoglobin A in vitro with phosphorylated carbohydrates containing a free carbonyl group, resulting in Hb $\alpha_{6}$-like molecules (17). The phosphate appears necessary to orient the carbohydrate in the 2,3-diphosphoglycerate pocket of the Hb molecule to facilitate addition. It is thus possible that glucose 6-phosphate reacts with Hb A to form Hb $\alpha_{6}$, which is then dephosphorylated in vivo to yield Hb $\alpha_{6}$.

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REFERENCES

5. Koenig, R. J., Peterson, C. M., Jones, R. L., Saudoh, C., Lehr-
Structure of carbohydrate of hemoglobin A1c.
R J Koenig, S H Blobstein and A Cerami


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