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Fructosylvaline. A Simple Model of the *N*-Terminal Residue of Human Haemoglobin A_{1c}

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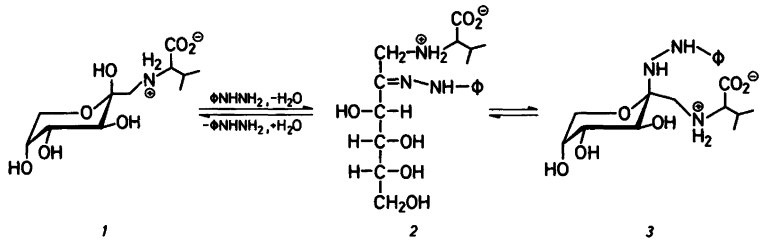
The phenylhydrazone of *N*-[D-fructosyl-(1)]-L-valine (1-deoxy-L-valine-D-fructose) was synthesized. The hydrazone was shown to exist in open form in basic solution and in closed form in acidic solution. The findings have bearings upon the discussion of the reaction of human haemoglobin A_{1c} with phenylhydrazine.

Recently much effort has been devoted to the study of the reaction between glucose and the *N*-terminal valine amino group of the β-chain of human haemoglobin A (HbA). The reaction proceeds with the formation of a labile Schiff's base (aldimine) which in an Amadori rearrangement forms the corresponding fructosyl derivative (ketoamine, HbA_{1c}).[1] We have argued, based on comparisons with literature studies of simple systems, that the glucose moiety of the aldimine is present in the β-D-glucopyranose form and that HbA_{1c} has the β-D-pyranose configuration.[2] The pyranoid structure of HbA_{1c} has been advocated by Fisher and Winterhalter.[3] We agree with the conclusion of these authors although we do not agree with the interpretation of their experimental evidence.

In order to clarify this point a simple model compound, *N*-[D-fructosyl-(1)]-L-valine (1-deoxy-L-valino-D-fructose, *1*) and the corresponding phenylhydrazone (*2*,*3*) have been synthesized (see Scheme 1). Valine derivatives have been used as a model of the *N*-terminal valine in haemoglobin.[4]



1 *2* *3*

Scheme 1.

0302-4369/85 $2.50

RESULTS AND DISCUSSION

The evidence presented by Fischer and Wintherhalter[3] for the ring form of the fructose moiety of HbA, is mainly based on the absence of UV-absorption around 350 nm in phenylhydrazine treated globin A_{1c}. The absence of this absorption is taken as evidence that no reaction has occurred and consequently that globin A_{1c} contains no free keto group and then must exist as a hemiketal. This deduction is questionable since *e.g.* glucose easily forms a phenylhydrazone even if the aqueous solution contains only about 0.001 % of the open form.[6]

We have shown that HbA_{1c} reacts with phenylhydrazine[2] and wish to point out that lack of the 350 nm absorption can only be taken as evidence that the product lacks the C=N chromophore. If the hydrazone exists mainly in a ring form this chromophore is not visible. Comparison of phenylhydrazine-treated HbA_{1c} and glyceraldehyde-treated haemoglobin A[7] with respect to the 350 nm absorption band clearly is not valid since the latter substance has no possibility of intramolecular association.

A recent study, based on 220 MHz ^{1}H NMR analysis, concludes that at pH≥7 *N*-[D-fructosyl-(1)]-L-valine *1* adopts exclusively the $^2C_5$ conformation of the β-pyranose form.[8]

As revealed by ^{13}C NMR, the phenylhydrazone of *N*-[D-fructosyl-(1)]-L-valine *1* exists almost exclusively in the open form *2* in basic (D_2O plus NaOH) and DMSO-d_6 solution (⟩C=N: δ 147.8), while only the closed form *3* could be detected in acid (D_2O plus D_2SO_4) solution (⟩C⟨$^{O}_{N}$: δ 97.5). Accordingly the phenylhydrazone at pH 9.5 exhibits mutarotation signifying that an equilibrium is established. The apparent first order rate constant was estimated as 1.1×10^4 s^{-1}, virtually the same value (1.2×10^4 s^{-1}) as was found for the disappearance of a 275 nm UV absorption band. The UV absorption curves have clearly defined isosbestic points.

The exact stereochemical constitution of the closed form of the hydrazone is tentatively assigned as *3* in analogy with the postulated configuration of similar compounds.[9]

These results demonstrate that an initially formed phenylhydrazone may very well lack the C=N chromophore due to intramolecular cyclization, and that at least in this respect fructosylvaline *1* may be regarded as a model of HbA_{1c}.

EXPERIMENTAL

Preparation of N-*[D-fructosyl-(1)]-L-valine* (1): A mixture of pyridine (800 ml), glacial acetic acid (800 ml) and L-valine (37.4 g, 0.320 mol) was stirred for 20 min followed by addition of D-glucose (80 g, 0.444 mol). After stirring for 4 d at room temperature the reaction mixture was taken to dryness *in vacuum* and the residue was taken up in methanol (1 l), filtered, and the volume reduced to 500 ml in vacuum. Cooling to 0 °C produced a precipitate, which after recrystallization from methanol (1 l) gave 27.4 g (30.7 %) pure *N*-[D-fructosyl-(1)[-L-valine (*1*), m.p. 154 °C (lit. 156 °C dec.)[5] This method of preparation avoids the time-consuming ion exchange chromatographic step.

Preparation of the phenylhydrazone of N-*[D-fructosyl(1)]-L-valine* (2,3): Phenylhydrazine (1.08 g, 0.01 mol) was added at room temperature to a solution prepared by refluxing (1 h) *N*-[D-fructosyl-(1)]-L-valine (*1*) (2.79 g, 0.01 mol) in methanol (100 ml). The reaction mixture was left at 4 °C for one week Isolation and drying *in vacuum* over sulfuric acid (conc.) gave 2.6 g of product, m.p. 154–155 °C. Recrystallization from methanol yielded 1.88 g (50.7 %) pure material, m.p. 182–183 °C. Anal. $C_{17}H_{27}N_3O$: C, H, N. ^{13}C NMR spectra were recorded in basic (D_2O+NaOH), acidic (D_2O+D_2SO_4), and DMSO-d_6 solutions and are reported in that order: 20.8, 21.9, 18.5, 20.6, 18.1, 19.3 (two $-CH_3$'s);

33.4, 31.1, 30.4 (Me_2CH); 65.5, 66.1, 63.3, ($-O_2C-CH-NH-$); 184.5, 172.3, 174.7 ($-CO_2-$); 47.4, 55.1, 63.3 ($N-CH_2-C-N$); 72.3–80.0, 68.5–72.3, 66.1–73.7 (carbons of fructose); 151.5, 145.6, 147.1 (phenyl C-1); 115.9, 118.0, 112.1 (phenyl C-2); 122.7, 126.2, 118.4 (phenyl C-3); 131.9, 132.0, 128.9 (phenyl C-4); 147.8, absent, 145.8 ($C=N$); absent, 97.5, absent (C–N).

UV spectra were recorded of aqueous solutions at pH 3.0; λ_{max}, nm (log ε) 200(3.92), 220(3.74), 275(3.26); at pH 9.5: 200(3.98), 275(3.97), 350(2.95); and at pH 5–6: 200(3.98), 230(3.70), 275(3.80), 350(3.88). The solutions were kept at room temperature until equilibrium was established. The optical rotation of an aqueous solution (1.0 g/100 ml, pH 9.5) was determined as a function of time. These data gave the apparent first order rate constant for the mutarotation.

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