amplitude of these fluctuations in vasomotor tone and thus the perturbation to sensed blood pressures. These larger perturbations, occurring at frequencies of about 0.04 Hz, are in turn translated into heart rate fluctuations at these frequencies through the mediation of the autonomic nervous system (*8*). Our data strongly suggest that the renin-angiotensin system indeed plays an important role in maintaining the short-term stability of the cardiovascular system under conditions of normal salt intake. Previously, blockade of the renin-angiotensin system could only be demonstrated to lead to physiologically significant changes in the salt-deprived, or otherwise stressed, animal (*3*).

Quantitative analysis of fluctuations in hemodynamic parameters is a powerful quantitative means of probing mechanisms of short-term cardiovascular control. We believe that this approach could provide a versatile, noninvasive clinical method for assessing the integrity of the cardiovascular control system in a variety of disease states.

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8. Lumbers *et al.* [E. R. Lumbers, D. I. McCloskey, E. K. Potter, *J. Physiol. (London)* **294**, 69 (1978)] have shown that angiotensin II inhibits vagal parasympathetic outflow through a central nervous mechanism. Thus blockade of the renin-angiotensin system might be expected to increase vagal activity, which should increase all peaks in the power spectrum. Our data show a dramatic selective effect on the low-frequency peak, which cannot be attributed therefore merely to an increase in overall vagal activity. We believe this frequency-specific effect is probably due to changes in the amplitude of the fluctuations of the peripheral resistance upon blockade of the renin-angiotensin systems.
9. This study was supported by a grant from the R. J. Reynolds Industries, NIH grants SO7 RR 07047 and HL 19467, NSF grant ECS-7922091, and ONR grant N000014-80-C-0520. S.A. is grateful for support from a Weizmann Institute Fellowship and R.J.C. is grateful for support from a Hartford Foundation Fellowship. We thank M. Bailin, E. Farhi, and J. Cant for their surgical assistance in the instrumentation of the experimental animals and for numerous helpful discussions; J. Fox and the staff of the MIT division of comparative medicine for their assistance in animal care; and P. Schluter, S. Burns, and D. Wade for assistance in the ECG signal analysis. We also thank R. Mark and G. Benedek for advice and encouragement.
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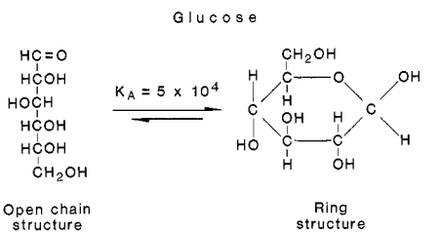
26 February 1981

Reaction of Monosaccharides with Proteins: Possible Evolutionary Significance

Abstract. *Measurements were made of the rate of condensation of various monosaccharides with amino groups of hemoglobin to form Schiff base linkages. The reactivity of each sugar was dependent on the extent to which it exists in the open (carbonyl) structure rather than in the ring (hemiacetal or hemiketal) structure. Among the 15 monosaccharides tested, aldoses showed higher reactivities than ketoses. Glucose was the least reactive of the aldohexoses. The emergence of glucose as the primary metabolic fuel may be due in part to the high stability of its ring structure which limits potentially deleterious nonenzymatic glycosylation of proteins.*

As a rule chemical reactions in living tissues are under strict enzymatic control and conform to a tightly regulated metabolic program. One of the processes implicit in biomolecular evolution is the minimizing of unwanted side reactions. Nevertheless, uncontrolled and potentially deleterious reactions occur, even under physiologic conditions. Examples include deamidation, transamidination, sulfhydryl oxidation, and lipid peroxidation. Recently, attention has focused on the nonenzymatic condensation of glucose with proteins to form stable covalent adducts. Under physiologic conditions, glucose in solution exists as a stable pyranose ring structure in equilibrium with the open chain aldehyde form:



The most abundant minor hemoglobin component in human red cells, HbA_{Ic}, is formed by the reaction of the aldehyde

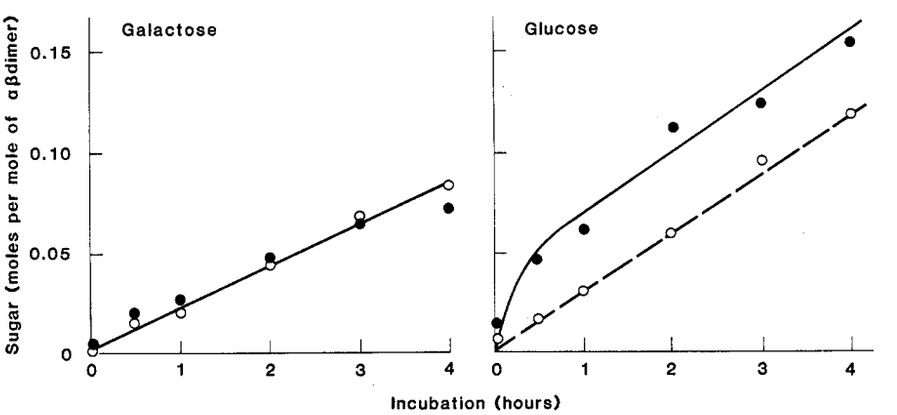


Fig. 1. Measurement of k_1, the rate of condensation of monosaccharide with hemoglobin, by two methods: (i) incubation with unlabeled sugar and reduction of aldimine linkage with ^3H-labeled cyanoborohydride (○); (ii) incubation with ^{14}C-labeled sugar and reduction with unlabeled cyanoborohydride (●); (left) 12 m*M* D-galactose; $k_1 = 1.9 \times 10^{-3}$ mM^{-1} per hour; (right) 42 m*M* D-glucose; $k_1 = 0.6 \times 10^{-3}$ mM^{-1} per hour. The initial rapid rate of incorporation of D-[^{14}C]glucose can be explained by the small amount of rapidly reacting impurity remaining in the preparation (*11*, *15*).

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function of glucose with the NH_2-terminal amino of the β chain to form a Schiff base (or aldimine) which can undergo an Amadori rearrangement to a more stable ketoamine linkage (*1*). This nonenzymatic reaction takes place slowly and continuously throughout the 120-day life-span of the human red cell (*2*). Ketoamine-linked glucose adducts can be found on other amino groups of the hemoglobin molecule (*3*) as well as on other proteins such as lens crystallin (*4, 5*), collagen (*6*), and proteins of the red cell membrane (*7*). Nonenzymatic glycosylation is increased significantly in the proteins of patients with diabetes; many investigators have speculated that this phenomenon may contribute to the long-term complications of the disease (*8*).

Because of the prevalence of nonenzymatic glycosylation of proteins and its potential pathophysiologic significance, we have examined the scope of this process by comparing the reactivities of various monosaccharides with human hemoglobin, which can be considered as a model protein. Our results identify certain properties of sugars that limit the extent of nonenzymatic glycosylation of protein and suggest the possible evolutionary significance of this phenomenon.

Human red cell hemolyzates were gassed with carbon monoxide and chromatographed on Bio-Rex 70 cation exchange resin (Bio-Rad) (*9*). The major hemoglobin component (HbA_o) was separated and concentrated by pressure filtration (Amicon PM 10 membrane) and dialyzed against Krebs Ringer phosphate buffer, *p*H 7.3. Sodium cyanoborohydride (Aldrich), was tritiated and purified (*10*); the sugars were obtained or prepared as described (*11*).

Purified HbA_o was incubated with each monosaccharide in the presence of 20 m*M* [^{3}H]$NaCNBH_3$ in a sterile solution of the Krebs Ringer buffer at 37°C. The Schiff base (aldimine) formed by the reaction of HbA_o with monosaccharide is reduced in the presence of [^{3}H]$NaCNBH_3$ and tritium is incorporated into the hemoglobin-sugar adduct. The rapid reduction by cyanoborohydride prevents the reverse rate reaction k_{-1} from taking place:

$$\text{Hb–NH}_2 + \text{O=C(H)–R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Hb–N=C(H)–R}$$

$$\xrightarrow{[^3\text{H}]\text{CNBH}_3} \text{Hb–N(H)–C(}^3\text{H)–R}$$

In contrast, cyanoborohydride does not react with the free monosaccharide (*11*). Concentration of the monosaccharides in the incubation ranged from 2 to 50 m*M*. Solutions of HbA_o in which there was no sugar were also incubated with 20 m*M* [^{3}H]$NaCNBH_3$ to determine the extent of nonspecific uptake of tritium by HbA_o. Samples were taken from the incubation solution (over a 4-hour period) at specific times, and unbound [^{3}H]$NaCNBH_3$ and sugar were removed by passage through Sephadex G-25 (Pharmacia). In parallel experiments, purified D-[^{14}C]glucose and D-[^{14}C]galactose were also incubated with HbA_o in the presence of unlabeled 20 m*M* $NaCNBH_3$. The specific activity of each sample was determined by measuring hemoglobin concentration and radioactivity. Counts were corrected by means of a standard quench curve. Nonspecific tritium uptake by HbA_o was subtracted from the total tritium uptake of the HbA_o monosaccharide incubations to determine that attributable to $NaCNBH_3$ reduction of the hemoglobin-sugar reaction product.

For all the sugars tested, incorporation of tritium into protein was linear with time and therefore provided reliable measurement of the rate of reaction (k_1) between sugar and HbA_o. A direct comparison between measurement of this rate by incorporation of tritium from [^{3}H]$NaCNBH_3$ and the incorporation of ^{14}C-labeled sugars is shown in Fig. 1. The two methods showed excellent agreement for both glucose and galactose. Thus, the incorporation of tritium from [^{3}H]$NaCNBH_3$ appears to be an acceptable way to measure the forward reaction rate (k_1), applicable to other monosaccharides that may not be available in isotopically labeled form.

A list of the monosaccharides tested and their respective values for k_1 with HbA_o is presented in Table 1, along with values of the percent carbonyl form of each sugar, determined by circular dichroism (*12*). We examined seven of the eight D-aldohexose stereoisomers and three of the four D-ketohexoses. The rate of reaction of various monosaccharides with HbA_o varied over a 300-fold range and correlates strongly with the extent to which the sugar exists in the open form (Fig. 2). We have shown that specific amino groups on the hemoglobin molecule undergo nonenzymatic glycosylation in vivo and in vitro (*3*) by making a nucleophilic attack on the carbonyl group of the sugar resulting in the formation of a Schiff base, which then can slowly rearrange to the more stable ketoamine linkage.

In addition to the equilibrium between the open and ring structures, two other factors influence the rate of interaction of monosaccharides with protein. (i) Aldose sugars react more rapidly than do ketose sugars (Fig. 2), as would be expected in that aldehyde carbonyl groups are relatively more electrophilic than ketone carbonyl groups. (ii) The interaction of monosaccharides with hemoglobin can be affected by the presence of charged groups on the sugar. Glucose-6-

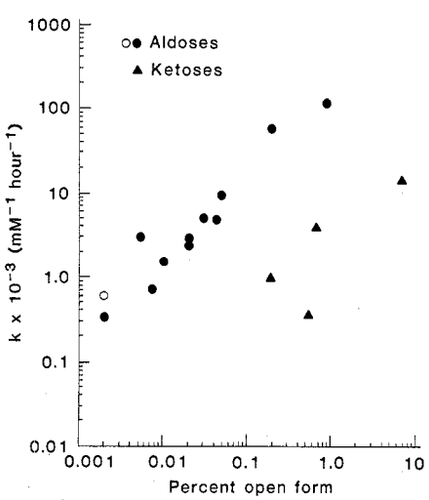

Fig. 2. Relation between the rate of condensation of monosaccharide with hemoglobin (k_1) and the equilibrium between the open and ring structures of the monosaccharide. The equilibrium data were taken from (*12*). Symbols: ○, glucose; ●, other aldoses; and ▲, ketoses.

Table 1. Rates of reactivity of monosaccharides with hemoglobin.

Monosaccharide	k_1 ($\times 10^{-3}$ mM^{-1} hour^{-1})	Carbonyl* (%)
	Aldoses	
6-Deoxy-D-glucose (rhamnose)	0.3	0.002
D-Glucose	0.60 ± 0.07†	0.002
D-Mannose	3.2	0.005
6-Deoxy-L-mannose (fucose)	0.7	0.007
D-Allose	1.4	0.01
D-Galactose	2.8 ± 0.3†	0.02
D-Xylose	2.9	0.02
D-Talose	5.2	0.03
D-Altrose	5.0	0.04
D-Ribose	10.0	0.05
D-Idose	55	0.2
5,6-Di-*O*-methyl-D-glucose	104	1.0
	Ketoses	
L-Sorbose	1.0	0.2
D-Tagatose	0.3	0.6
D-Fructose	4.5	0.7
D-Threo-2-pentulose (xylulose)	15.8	8.0

*From (*12*). †Mean ± S.E.M.; 11 determinations.

phosphate behaves as an affinity label and binds with deoxyhemoglobin specifically at the 2,3-DPG (diphosphoglycerate) binding site, allowing the aldehyde function to form a Schiff base with the β NH_2-terminal amino groups (*13*). In order to circumvent the added effects due to electrostatic interactions, only uncharged monosaccharides were included in our experiments.

Our observations may be considered in terms of biomolecular evolution. During the sequential development of anaerobic glycolysis, photosynthesis, and aerobic glycolysis, glucose rather than other stereoisomers has emerged as the universal metabolic fuel. This selectivity could be attributed to the stability of its ring structure, owing to the equatorial orientation of its hydroxyl groups. This stable ring structure greatly retards the rate at which glucose condenses with hemoglobin and, presumably, other proteins and amino-containing compounds (*14*). Conversely, if organisms contained a comparatively high concentration of a metabolic fuel having a relatively unstable ring structure, it is likely that extensive covalent modification of proteins would occur along with potential impairment of function. This prediction is supported by our observations on rats fed a high galactose diet (*5*). (The stability of the ring structure of galactose is one-tenth that of glucose.) The galactosemic rats had more extensive nonenzymatic glycosylation of lens crystallins than did normal rats or even diabetic rats (having elevated blood glucose). Thus, the relatively high stability of its ring structure allows high concentrations of glucose and proteins to coexist in various tissues with a minimal risk of irreversible covalent modification.

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16. Supported by the Howard Hughes Medical Institute and NIH grant AM-18223. We thank Drs. George Cahill, Edmund Lin, and Henry Paulus for helpful discussions.

12 November 1980; revised 27 March 1981

Calcium Dependence of the Inactivation of Calcium Currents in Skeletal Muscle Fibers of an Insect

Abstract. *Calcium currents in skeletal muscle fibers of an insect,* Carausius morosus, *inactivate under depolarization. This inactivation depends on the current being carried across the membrane by calcium ions, rather than strontium or barium ions.*

In many excitable cells, electrical activity is associated with an increase in the permeability of the membrane to calcium ions (*1*). The entry of Ca^{2+}, which occurs as a consequence of membrane depolarization, is important, in excitation-contraction coupling in some muscle fibers (*2*), in excitation-secretion coupling in nerve and secretory cells (*3*), and in the activation of membrane potassium conductance (*4*).

Because Ca^{2+} is involved in the regulation of cellular processes, it is important to know whether entry of Ca^{2+} is maintained during depolarization, as it is in internally perfused muscle fibers (*5*) and certain molluscan neurons (*6*), or whether inactivation occurs and, if so,

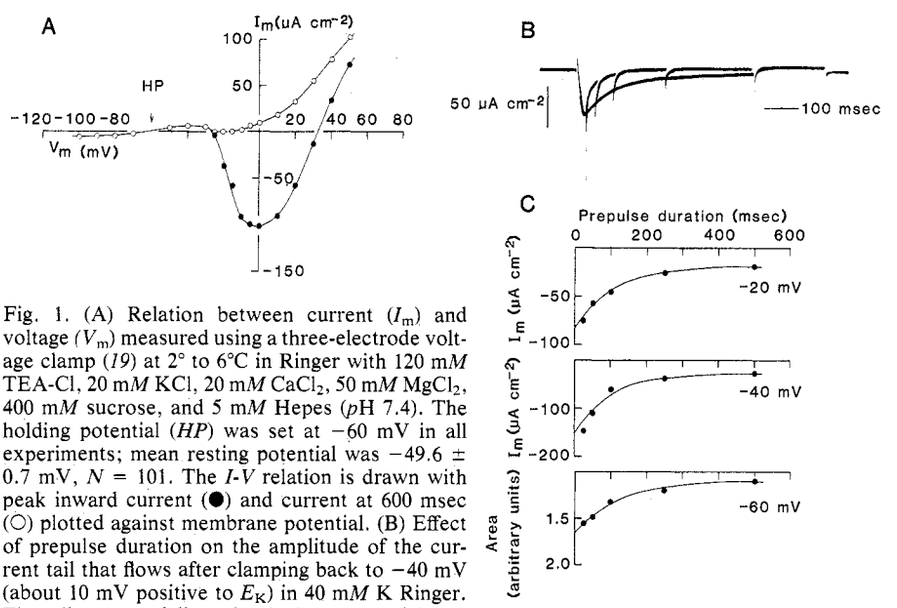

Fig. 1. (A) Relation between current (I_m) and voltage (V_m) measured using a three-electrode voltage clamp (*19*) at 2° to 6°C in Ringer with 120 m*M* TEA-Cl, 20 m*M* KCl, 20 m*M* $CaCl_2$, 50 m*M* $MgCl_2$, 400 m*M* sucrose, and 5 m*M* Hepes (*p*H 7.4). The holding potential (*HP*) was set at −60 mV in all experiments; mean resting potential was −49.6 ± 0.7 mV, N = 101. The *I-V* relation is drawn with peak inward current (●) and current at 600 msec (○) plotted against membrane potential. (B) Effect of prepulse duration on the amplitude of the current tail that flows after clamping back to −40 mV (about 10 mV positive to E_K) in 40 m*M* K Ringer. The tail currents followed a single exponential (τ = 2.64 msec) except during the first few milliseconds, when they were contaminated by capacity transients. The records were superimposed and traced by hand. Resting potential, −33 mV; holding potential, −60 mV; and temperature 4.0°C. (C) Relation between the peak amplitude of the current tail elicited on clamping back to −20, −40 [shown in (B)], or to −60 mV, and the duration of a prepulse to 0 mV. We plotted the area of the transient current at −60 mV, since the current turns off very rapidly at this potential. The curve is drawn to a time constant of 110 msec at −20 mV, 105 msec at −40 mV, and 123 msec at −60 mV. Essentially the same time constant was obtained at −40 mV (τ = 112 msec) by measuring the area of the transient. The Ca current in (B) decayed with a time constant of 105 msec at 0 mV.

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