although the monoligand complexes of the group 4 metals, **2**, are quite reactive in this regard. The phenyl complex **4** and the corresponding benzyl derivative **5** display analogous solution behavior as the starting chloride complex **3**, in that, at room temperature one observes a doublet in the $^{31}P\{^1H\}$ NMR spectrum which broadens at low temperature to an AA'BB'X spin system.

Now that the door to phosphine complexes of yttrium has been opened, we will endeavor to extend this methodology to the lanthanoid metals.[3] This is already in progress.[12]

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Supplementary Material Available: Experimental, microanalytical, and ^1H, ^{13}C, and ^{31}P NMR data for all new compounds (4 pages). Ordering information is given on any current masthead page.

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Proton Transfer Is Not Rate-Limiting in Buffer-Induced Nonenzymic Glucation of Hemoglobin

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The reaction of glucose with amino groups in proteins occurs nonenzymically[1] in vivo. This intrinsically very slow process appears to be critical in the pathogenesis of various secondary complications associated with diabetes mellitis[1,2] and in the process of aging,[3] so that its mechanistic features are important to understand. The paradigmatic reaction of glucose with hemoglobin has been extensively studied and is known[4] to occur most rapidly at the N-terminal valine of the β-subunit. An initial, rapid imine formation is succeeded by slower Amadori rearrangement to the final aminoketone[5] (Figure 1).

The reaction is accelerated by several buffers,[6] including phosphate buffer (Figure 2). This catalysis, or similar effects by other species present in the biological environment, may lead to as much as a doubling of the rate of protein damage at the aggregate concentrations of several millimolar expected for such species. In fact, general acid-base catalysis by buffer is expected for the proton-abstraction and proton-donation steps at the Amadori rearrangement. However, we find that the phosphate-dependent reaction occurs with identical rate constants in protium oxide, deuterium oxide, and with either glucose-*2-h* or glucose-*2-d* as reactant (Figure 2). This absence of either solvent or substrate isotope effect *excludes as a rate-limiting step BOTH proton-transfer steps of the general acid-base-catalyzed Amadori rearrangement* (i.e., proton abstraction by buffer, which would show a substrate isotope effect, and proton donation by buffer, which would show a solvent isotope effect). Thus the buffer acceleration of the rate is not classical, protolytic general acid-base catalysis. Instead, some other process (conceivably a buffer-induced change in the conformation of the hemoglobin-glucose imine, although since it is not certain that this process would lack a solvent isotope effect, such a conclusion cannot be made with

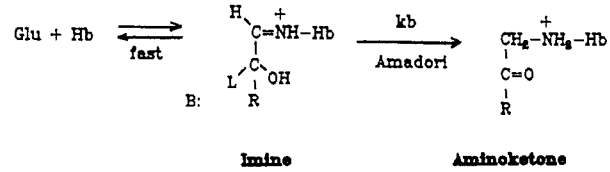

Figure 1. Mechanistic scheme for the nonenzymic glucation of proteins. L represents the substrate label (H or D) in D-glucose. When B abstracts H to form BH^+ in DOD as solvent, rapid exchange should produce BD^+. Thus the proton-donation step in HOH will involve BH^+; in DOD it will involve BD^+.

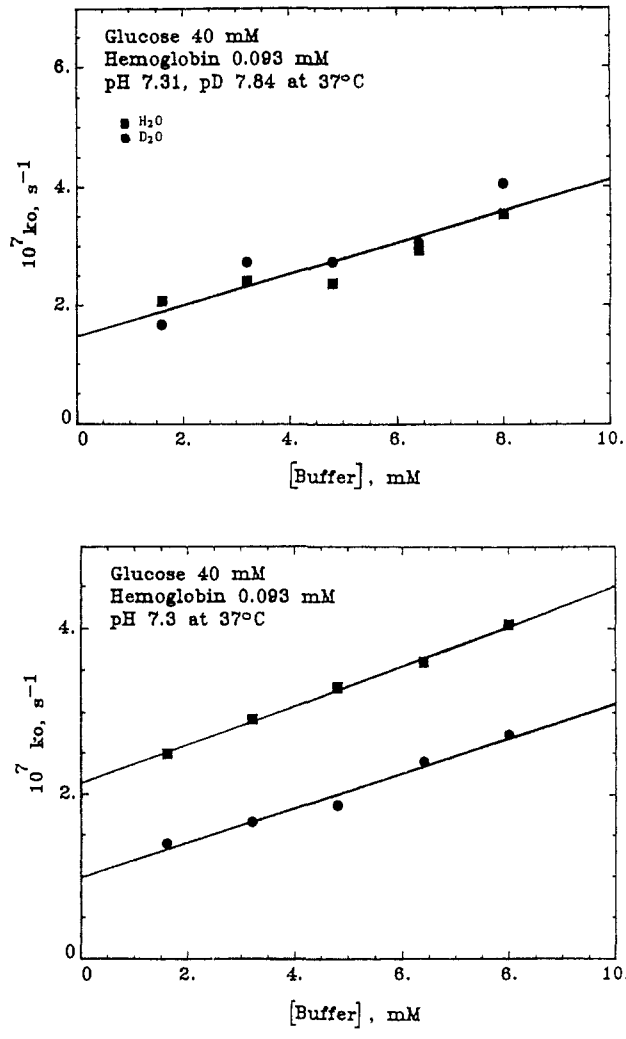

Figure 2. Top: First-order rate constants for the reaction of 40 mM D-glucose with hemoglobin in protium and deuterium oxides at pH 7.3, pD 7.8, 37°C, in sodium phosphate buffers at the indicated total buffer concentrations. The line shown fits the data for both HOH and DOD, corresponding to $k_{HOH}/k_{DOD} = 1.0$ for both buffer-independent and buffer-dependent rates. When the two data sets are fitted independently, 10^7k_{HOH}, $s^{-1} = (1.63 \pm 0.23) + (0.215 \pm 0.034)$ [buffer, mM], and 10^7k_{DOD}, $s^{-1} = (1.32 \pm 0.34) + (0.317 \pm 0.064)$ [buffer, mM]. Bottom: First-order rate constants for the reaction of 40 mM D-glucose-*2-h* (upper line, 10^7k_o, $s^{-1} = (2.13 \pm 0.04) + (0.238 \pm 0.008)$ [buffer, mM]) and D-glucose-*2-d* (lower line, 10^7k_o, $s^{-1} = (0.98 \pm 0.10) + (0.214 \pm 0.019)$ [buffer, mM]) with hemoglobin at pH 7.3, 37 °C. The rate constants in the bottom plot were determined from data over a substantial portion of the reaction and are thus of higher quality than those in the top plot, which were evaluated from only two time points; this probably accounts for the differences between the data for D-glucose-*2-h* in HOH in the top and bottom plots.

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confidence) must be rate-limiting in the buffer-catalyzed reaction.

Rates of glucation of hemoglobin under an atmosphere of air were measured by following the appearance of glycated product.[7] Glucohemoglobin HbA_{1c} was eluted from a cation-exchange column and measured spectrophotometrically at 415 nm (Sigma Kit[7] no. 440). Hemoblogin concentrations of 0.03–0.04 mM were employed with glucose at 40 mM. Reaction media were 0.15 M in sodium chloride, buffered at 37 °C with mixtures of NaH_2PO_4 and Na_2HPO_4 at pH 7.3. Reactions in deuterium oxide were conducted at the "corresponding pD" of 7.8. D-Glucose-*2-d* (97 atom %) was obtained from Sigma Chemical Co. The kinetics exhibited both a buffer-independent term (reflecting reaction assisted by water, lyons (hydroxide or hydronium ions), or protein functional groups) and a first-order term in phosphate buffer.

For the buffer-independent term, the substrate isotope effect of 2.2 ± 0.1 shows the proton-abstraction step of the Amadori rearrangement at least partially to determine the rate. The proton-abstracting base could in principle be (a) water, (b) hydroxide ion, or (c) a basic functional group of the protein. The solvent isotope effect can be predicted for each case. Water as abstracting base should give k_{HOH}/k_{DOD} = 1.0–2.1, depending on transition-state structure;[9] hydroxide ion as abstracting base should give k_{HOH}/k_{DOD} = 0.5–1.0, depending on transition-state structure;[9] a protein functional group should give k_{HOH}/k_{DOD} = 1.0, since the use of "corresponding pD" will cause the fractional ionization to be the same in the two isotopic solvents.[8] The data shown in Figure 2 yield k_{HOH}/k_{DOD} = 1.1 ± 0.1. While not decisive because consistent with any base if the transition-state structure is "early," the data are most readily reconciled with a protein functional group as the proton-abstracting base.

For the buffer-dependent term, the absence of a statistically meaningful solvent isotope effect (the data of Figure 2 yield k_{HOH}/k_{DOD} = 0.7 ± 0.2) excludes proton donation from the buffer as the rate-limiting step; the absence of a substrate isotope effect (1.1 ± 0.1) means that proton abstraction is not rate-limiting. Thus a kinetic event other than proton transfer must limit the rate. The most straightforward hypothesis is that a protein structural change before or during the Amadori rearrangement is rate-limiting for the phosphate-accelerated process. It cannot be said with any general reliability whether a protein conformational change would exhibit a solvent isotope effect; this will be determined by whether changes in binding at exhangeable protonic sites occur in the course of the conformational change.[8] It is conceivable that phosphate binding induces such a conformational change, and then the ensuing proton-transfer step or steps are more rapid than in the absence of phosphate. It is known[6] that non-phosphate buffers such as MOPS or TAPSO are less effective in promoting glucation and that 2,3-diphosphoglycerate, an effector of hemoglobin action, accelerates glucation. Therefore a specific binding site, such as the 2,3-diphosphoglycerate site, may be involved.

Although nonenzymic protein glycation is commonly regarded as an adventitious feature of in vivo protein chemistry, the possible involvement of protein functional groups and specific binding sites for hemoglobin raises the possibility that some programmatic biological significance may attach to the process.

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Bismethyleneborane, $[B(CH_2)_2]^-$, and Trismethyleneborane, $[B(CH_2)_3]^-$, Anions. Do They Exist in the Gas Phase?

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Recent reports[1,2] of families of anions related to $[P(CH_2)_2]^-$, $[CH_2PO]^-$, $[P(CH_2)_3]^-$, and $[(CH_2)_2PO]^-$ have led us to consider whether the cognate boron species are stable. Such boron ions are of considerable theoretical interest since conventional valence bond theory would predict that $[B(CH_2)_2]^-$ and $[B(CH_2)_3]^-$ should have allene and trimethylenemethane[3] type structures, respectively, with the latter ion being a diradical. These predictions need to be tested since (i) boron structures sometimes do not conform to classical valence bond schemes,[4] (ii) the ionic species may have some carbanion character (nonplanar CH_2 groups), and (iii) if the CH_2 groups are planar, some out of plane twisting may occur, cf. $[P(CH_2)_3]^-$.[1,2]

Ions with atomic compositions consistent with structures $[B(CH_2)_2]^-$, $[CH_2BO]^-$, $[B(CH_2)_3]^-$, and $[(CH_2)_2BO]^-$ were formed in a VG ZAB 2HF mass spectrometer as follows. The reaction between HO^- and trimethylborane in the chemical ionization source yielded the precursor ions shown in eq 1 and 2.[5,6] These

$$HO^- + Me_3B \rightarrow Me_2\bar{B}{=}CH_2 + H_2O \quad (1)$$

$$HO^- + Me_3B \rightarrow Me_2\bar{B}{=}O + CH_4 \quad (2)$$

$$Me_2\bar{B}{=}CH_2 \rightarrow [B(CH_2)_3]^- + H_2 \quad (3)$$

$$Me_2\bar{B}{=}CH_2 \rightarrow [B(CH_2)_2]^- + CH_4 \quad (4)$$

$$Me_2\bar{B}{=}O \rightarrow [(CH_2)_2BO]^- + H_2 \quad (5)$$

$$Me_2\bar{B}{=}O \rightarrow [CH_2BO]^- + CH_4 \quad (6)$$

reactions are of a standard type.[2,7] Collisional activation[8] of the precursor ions yield a number of product ions including the required species shown in eq 3–6.[9] The spectra of the corresponding

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