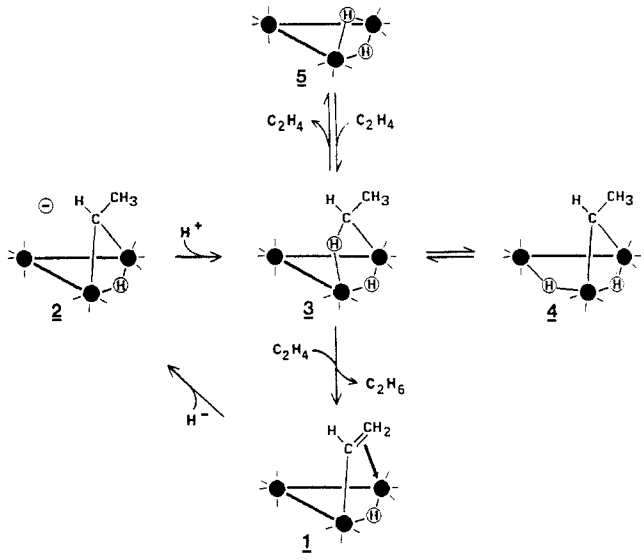
Scheme I



When the temperature of a solution of **3** is raised above −20 °C, the ^{1}H NMR signals due to **3** decrease in intensity while four new signals appear and increase in intensity (δ 6.76 (q, 1 H), 2.49 (d, 3 H, J = 7.0 Hz), −14.26 (s, 1 H), −20.43 (s, 1 H). These signals arise from the ethylidene tautomer **4**, formed by elimination of a C_α–H bond of **3**. This process is first order in **3** with a rate constant at −10 °C of 1.6 ± 0.1 × 10^{-4} s^{-1}. The equilibrium constant K = [**4**]/[**3**] = 7.3 ± 0.7 is temperature independent over the range −10 to 16 °C. This value compares with K = 3.5 ± 0.1 (CD_2Cl_2, 32 °C) for the analogous methyl/methylene system.[8b]

Upon raising the sample temperature to ca. 19 °C, a C_β–H elimination process becomes observable as ethylene and **5** form. The appearance of **5** is first order with a rate constant of 5.9 ± 0.3 × 10^{-4} s^{-1} at 19 °C. At this temperature the rate of α-elimination is approximately 100-fold greater.[10] The overall β-elimination process is actually reversible,[11] since upon treatment of **5** with a large excess of ethylene (ca. 5 atm) approximately 10% of **3** + **4** is observable after ca. 3 h. However, over the same time period formation of **1** together with an equivalent of ethane also occurs.[12] Thus, ethylene coordination to **3** induces reductive elimination of ethane at a rate which is comparable with the formation of **3** from **5**. Reductive elimination of ethane from **3** is induced also by excess styrene (with formation of $HOs_3(CO)_{10}(\mu$-CH=CHPh)) or more readily by stronger nucleophiles L = PPh_2Me or t-BuNC (with formation of $Os_3(CO)_{10}L_2$). This ligand dependence controls the competition between ethane and ethylene elimination. For example, with L = t-BuNC added to the **3** + **4** mixture at 25 °C, a L:Os_3 ratio of 2:1 leads to a C_2H_6:C_2H_4 ratio of 3:1 in the gas produced, but if the L:Os_3 ratio is increased to 10:1, the C_2H_6:C_2H_4 ratio becomes 18:1.

Although the methylene hydrogens in **3** should be diasterotopic, they appear equivalent by ^{1}H NMR even at −90 °C. Examination of the ^{13}C NMR spectrum of **3** down to −90 °C shows averaged spectra indicative of a time-averaged plane of symmetry perpendicular to the osmium triangle.[13] We propose that equilibration of the methylene hydrogens occurs by breaking the C··H··Os bond on one side of the cluster and then forming a new C··H··Os bond on the other side.[14] This process,[15] which apparently has $\Delta G^* \leq 5$ kcal/mol, presumably proceeds via a symmetric unsaturated intermediate, electronically analogous to the dihydride complex **5**. This symmetric species, in which *neither* C–H bond interacts with the osmium, may be highly significant for the β-elimination process. Although the ground-state structure of **3** involves an α-C–H bond in a donor interaction with the metal center, interaction of a β-C–H bond must develop in order for β elimination to occur.[16] We are unable to say whether this involves two metal centers or just one.[2a,b]

In summary, for this cluster–alkyl system the familiar β-elimination process is thermodynamically favored, at least when driven by ethylene dissociation. However, the kinetically favored α-elimination process generates a relatively high concentration of the alkylidene form. This serves to "protect" the alkyl by inhibiting destructive pathways. This property could be important also for alkyl reactions on metals, if, for example, migration onto CH_2 or CO moieties must compete with elimination of alkene or alkane. Furthermore, an analogous ethyl → ethylidene conversion is likely to be involved in the conversion of ethylene to ethylidyne observed on many metal surfaces.[17]

Acknowledgment. This work was supported by National Science Foundation Grant CHE 84-07233. Instruments supported by the Grants NSF CHE 79-16100 and NIH GM-27029 were utilized for NMR and mass spectra, respectively.

(10) The fact that the D atom in **3-*d*** stays in the α position rules out rapid equilibration with an ethylene complex, assuming facile rotation in the latter.

(11) This is consistent with previous observations that $H_2Os_3(CO)_{10}$ is an olefin isomerization catalyst: (a) Keister, J. B.; Shapley, J. R. *J. Am. Chem. Soc.* **1976**, *98*, 1056; (b) Deeming, A. J.; Hasso, S. *J. Organomet. Chem.* **1976**, *114*, 313.

(12) Keister, J. B.; Shapley, J. R. *J. Organomet. Chem.* **1975**, *85*, C29.

(13) The ^{13}C NMR spectrum (90 MHz, CD_2Cl_2, −90 °C) (from ca. 50% ^{13}CO-enriched $Os_3(CO)_{12}$) shows carbonyl signals at δ 183.6 (s, 0.5 C; d, 0.5 C, ^2J(C–C) = 35 Hz), 182.3 (s, 0.5 C; d, 0.5 C, ^2J(C–C) = 35 Hz), 176.6 (s, 2 C), 176.0 (s, 2 C), 172.3 (s, 2 C), 170.7 (d, 2 C, ^2J(C–H) = 11 Hz).

(14) The complexes [ReW(μ-CH_2R)(μ-$Me_2PCH_2PMe_2$)$(CO)_7$] (R = C_6H_4Me, Me) have a C_α··H··W bridge that is fixed; the two methylene hydrogens do not exchange: Jeffery, J. C.; Orpen, A. G.; Robinson, W. T.; Stone, F. G. A.; Went, M. J. *J. Chem. Soc., Chem. Commun.* **1984**, 396.

(15) This process is related conceptually to the σ,π-interconversion of the bridging vinyl group in $HOs_3(CO)_{10}(\mu$-CH=CH_2): Shapley, J. R.; Richter, S. I.; Tachikawa, M.; Keister, J. B. *J. Organomet. Chem.* **1975**, *94*, C43.

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Does Lysozyme Follow the Lysozyme Pathway? An Alternative Based on Dynamic, Structural, and Stereoelectronic Considerations†

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In this paper we present a pathway (Scheme I) for the hydrolysis of oligoglycosides by the enzyme lysozyme that differs from the accepted mechanism (Scheme II).[1-4] The new pathway, suggested by results of a 55-ps molecular dynamics simulation of a lysozyme complex with hexakis(*N*-acetylglucosamine), $(GlcNAc)_6$, is consistent with the available experimental data and with stereoelectronic considerations. A fundamental feature of the modified pathway is that an *endo*cyclic bond is broken in the initial step, in contrast to the *exo*cyclic bond cleavage in the accepted mechanism.

The molecular dynamics simulation employed an initial structure with $(GlcNAc)_6$ built into the active site by use of the coordinates of a lysozyme–$(GlcNAc)_3$ complex and of the native enzyme; details are given separately.[5] Minor reorientation of

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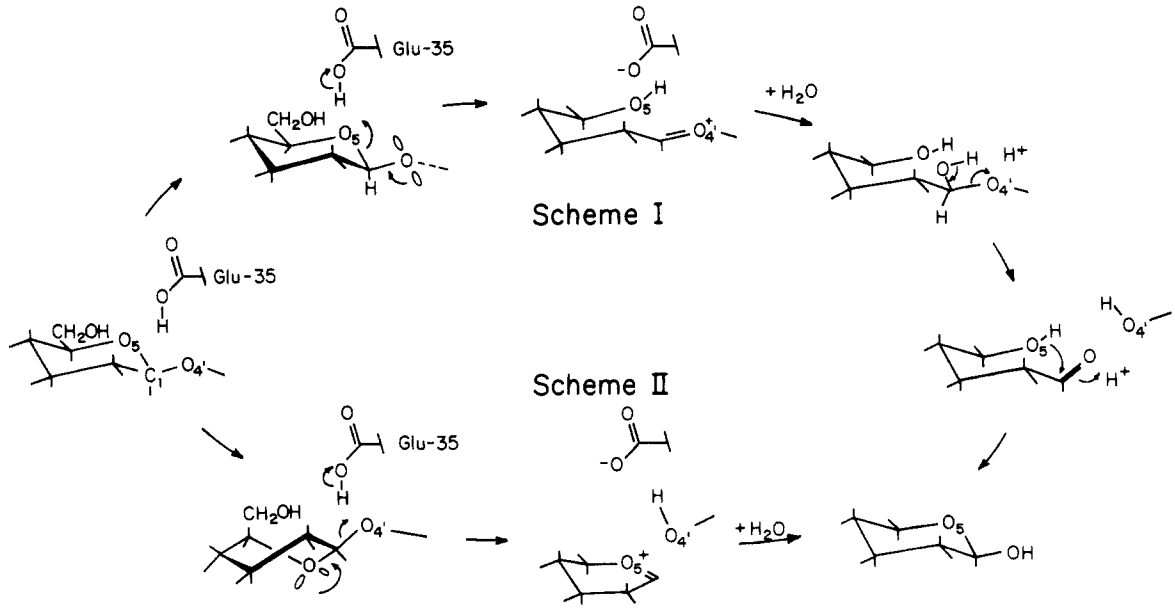
Schemes I and II



certain amino acid side chains of native lysozyme led to favorable binding of the *N*-acetylglucosamine residue to site D of the enzyme active site in an undistorted chair conformation. During the molecular dynamics simulation, the chair form of the pyranose ring in site D remained unperturbed and the glycosidic dihedral angle O_5–C_1–$O_{4'}$–$C_{4'}$ between rings D and E, which was −54° in the initial structure, stabilized at −62°. The motions of the carboxyl group of Glu 35 led to hydrogen bonds with the endocyclic D ring oxygen O_5 and the hydroxymethyl oxygen O_6 but *not* with the exocyclic oxygen $O_{4'}$; the closest approach of Glu 35 to $O_{4'}$ was greater than 4 Å with an improper orientation for hydrogen bond formation.

The accepted mechanism for lysozyme (Scheme II) was proposed by Phillips and co-workers[2-4] on the basis of model building and data for the nonenzymatic hydrolysis of glycosides. An essential element of this proposal is the distortion of the *N*-acetylglucosamine residue in site D. The resulting twist-boat conformation makes it possible to take advantage of stereoelectronic assistance[6-8] from ring oxygen O_5 in the transition state leading to cleavage of the exocyclic C_1–$O_{4'}$ bond. Scheme II also involves protonation of $O_{4'}$ by Glu 35 and yields the cyclic oxycarbonium ion which can be stabilized by the carboxylate group of Asp 52. Much of Scheme II is supported by experimental data.[1-4] However, the required ring distortion is not,[9-12] nor is it in accord with independent energy minimization results[13,14] and the present simulation.

The present molecular dynamics results suggest an alternative hydrolysis mechanism that does not require substrate distortion. The initial step in the reaction is protonation of the ring O_5 by Glu 35 (Scheme I). Cleavage of the endocyclic C_1–O_5 bond forms the acyclic oxocarbonium ion intermediate,[15] which is stabilized by Asp 52. Attack by water, cleavage of the C_1–$O_{4'}$ bond, and ring closure then lead to the observed products. Existing experimental data on lysozyme hydrolysis are consistent with Scheme I.[16-25] Moreover, distortion of the ring in site D is not required and the antiperiplanar orientation of an exocyclic $O_{4'}$ lone pair

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(8) Formation of the cyclic oxycarbonium ion is greatly facilitated by a lone pair of O_5 antiperiplanar to the C_1–$O_{4'}$ bond. Thus, there is a kinetic requirement for distortion of the ring into a twist boat with the hydroxymethyl group in an axial position, although a half-chair conformation approximates the correct orbital orientation. Since the potential barrier for such a ring distortion is approximately 6–10 kcal/mol (Anet, F. A. L.; Brown, J. R. *J. Am. Chem. Soc.* **1967**, *89*, 760. See also ref 4, p 861), a proposed role of the enzyme in catalyzing the reaction is to induce the conformational change in the ring.
(9) Schindler, M.; Aesaf, Y.; Sharon, N.; Chipman, D. M. *Biochemistry* **1977**, *16*, 423.
(10) The interpretation of the data concerning the energetics of binding to different sites[9] is complicated by the presence of contributions from stabilizing interactions between the substrate and the enzyme and from the release of bound water molecules. The molecular dynamics results suggest that the two quantities are of the same order. In particular, there are several strongly bound water molecules in site D that are expected to be displaced on substrate binding.
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(16) Secondary isotope effects[17] for deuterium or tritium substitution on C_1 lead to the conclusion that there is a sp^3 to sp^2 transition in the rate-limiting step, consistent with both I and II. Glu 35 serves as a generalized acid and Asp 52 acts to provide electrostatic stabilization in both pathways; that both residues are essential to catalysis has been demonstrated by chemical modification.[18] The stereochemistry at the anomeric carbon (C_1) can be retained[19] in the enzymatic process via either pathway.
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(20) It might be argued that the available measurements on the hydrolysis of aryl glycosides in the presence of lysozyme,[21-25] for which the introduction of electron-withdrawing groups causes a rate increase and for which there is a normal ^{18}O isotope effect[24] at O_4, provide evidence against Scheme I. However, the literature makes clear that these synthetic substrates all have k_{cat}/K_m values orders of magnitude lower than that of the natural model substrate $(GlcNAc)_6$ and that in the one case subjected to detailed analysis, $(GlcNAc)_2$ PNP, the bond cleaved is that between the two (GlcNAc) units.[23] Thus, it is not clear that any of these model studies are relevant to the lysozyme mechanism. Moreover, the introduction of a strongly electronegative group is expected to enhance the exocyclic pathway (Scheme II) at the expense of the endocyclic pathway (Scheme I).
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orbital relative to the cleaved C_1–O_5 bond found in the simulation is in accord with stereoelectronic requirements.[6,7]

In our proposed Scheme I, a role of the enzyme is to catalyze the reaction by means of orientational (entropic) contributions, instead of the distortional (enthalpic) stabilization assumed in the classic mechanism (Scheme II). In particular, the molecular dynamics simulation indicates that the exocyclic dihedral angle (O_5–C_1–$O_{4'}$–$C_{4'}$) oscillates in the neighborhood of the value required for optimum stereoelectronic assistance in Scheme I. Further, there is the possibility that interactions with the enzyme aid in maintaining the proper geometry for reclosing the ring in site D and are involved in the retention of configuration at C_1. The role of the catalytic residues Glu 35 and Asp 52 in the enzymatic reaction is analogous in the two pathways.

Although the molecular dynamics results are only suggestive (e.g., it is possible that hydrogen bonding of Glu 35 to the substrate O_4 is a rare event not sampled by the simulation), it is hoped that the formulation of an alternative mechanism will lead to renewed interest in the catalysis of polyglycoside hydrolysis and transglycosylation by lysozyme; nothing in the present analysis would require that the same mechanism is found in all β-glycosidases. Experiments that aim to establish whether the hydrolytic pathway proceeds according to Scheme I or II in lysozyme are in progress.[26]

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Stereoelectronic Effects in Intramolecular Long-Distance Electron Transfer in Radical Anions as Predicted by ab Initio MO Calculations

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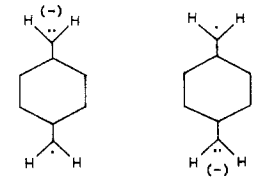
Recent experimental work has been directed toward the study of intramolecular electron transfer (ET) rates in radical anions of the type

$$A_1^-\text{–Sp–}A_2 \rightarrow A_1\text{–Sp–}A_2^-$$

where A_1 and A_2 are electron acceptors and Sp is a rigid hydrocarbon spacer with no electron affinity of its own.[1] Specifically, for the case of A_1 = 4-biphenylyl, A_2 = 2-naphthyl, and Sp = 1,4-cyclohexadiyl it was found that the cis isomer reacts slower than the trans although the distance between acceptors is shorter for the cis isomer.[2] With other spacers, such as decalins, it also appears that distance between acceptors is not the only factor in determining the rate.[2] It is most probable that electronic coupling between donor and acceptor is influenced by the σ-orbitals of the spacer, making it a function of the position as well as the geometry of attachment. While the problem of through-bond coupling has been addressed before,[3] here we want to pay attention to stereoelectronic effects.[4] To test this hypothesis we have carried out ab initio calculations for the simplified model **1** with trans-



1a **1b**

equatorial-equatorial (*t*-(e,e)-**1**), trans-axial-axial (*t*-(a,a)-**1**), and cis-equatorial-axial (*c*-(e,a)-**1**) geometries.

The calculations consist of finding the UHF broken-symmetry solutions as diabatic wavefunctions[5] and their energies corresponding to the localized electronic structures **1a** and **1b** as function of the torsional angles of the CH_2-groups which were restricted to be planar. The interaction matrix element between nonorthogonal UHF wavefunctions

$$V_{a,b} = (1 - S_{a,b}^2)^{-1}|\langle a|H|b\rangle - S_{a,b}(\langle a|H|a\rangle + \langle b|H|b\rangle)/2|$$

was evaluated along with the seam of their crossing, with $|a\rangle$ and $|b\rangle$ being the diabatic wavefunctions for **1a** and **1b**, $S_{a,b}$ the overlap integral, and H the electronic Hamiltonian.[6] Since in the isolated molecule model the ET promoting mode is restricted to the torsional motion of the CH_2 groups, the seam of the energy surface where **1a** and **1b** are isoenergetic is considered to describe the conformations where ET can take place.[7] In the trans isomer symmetry determines the seam for both *t*-(e,e)-**1** and *t*-(a,a)-**1** conformations. The rotational conformations are defined by the dihedral angle θ between the tertiary cyclohexane hydrogens and one of the CH_2 hydrogens (Figure 1 insert).

In all, six rotational conformations were tested in each of the *t*-(e,e)-**1** and *t*-(a,a)-**1** geometries by using a STO-3G basis set. The results are shown in Figure 1. It is found that the energy along the seam of crossing in *t*-(e,e)-**1** has a minimum for the 0,0-conformation in which the CH_2 planes bisect the cyclohexane ring. This is also the conformation with the largest $V_{a,b}$. The energetically least favored 90,90-conformation has an interaction element 12 times smaller corresponding to a rate difference of 144. In contrast, *t*-(a,a)-**1** shows an energy maximum for the 0,0-conformation and a maximum for $V_{a,b}$. Since the energies of the rotamers are largely determined by nonbonded interactions, CH_2 groups may be considered poor models for the 2-naphthyl

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[‡] University of Chicago.
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(6) If the UHF broken space symmetry solutions, $|a\rangle$ and $|b\rangle$, are strongly, charge-localized, they are appropriate for use as the zero-order initialand final electronic states in the time-dependent perturbation theory treatment. By the symmetric orthogonalization, they become $|a_0\rangle = C_1|a\rangle - C_2|b\rangle$ and $|b_0\rangle = -C_2|a\rangle + C_1|b\rangle$, where $C_1 = [(1 - S_{a,b})^{-1/2} + (1 + S_{a,b})^{-1/2}]/2$ and $C_2 = [(1 - S_{a,b})^{-1/2} - (1 + S_{a,b})^{-1/2}]/2$. Here we assume the existence of the zero-order Hamiltonian, of which $|a_0\rangle$ and $|b_0\rangle$ are eigenfunctions: $H^0|a_0\rangle = E_a^0|a_0\rangle$; $H^0|b_0\rangle = E_b^0|b_0\rangle$. Then the perturbation H' which causes the electron transfer is defined as the difference of the total electronic Hamiltonian H and this zero-order Hamiltonian H^0 as $H' = H - H^0$. The matrix element for the electron transfer between $|a_0\rangle$ and $|b_0\rangle$ defined as $V_{a,b} = |\langle a_0|H'|b_0\rangle|$ is calculated to be the expression given in the text. The crossing seam between $|a_0\rangle$ and $|b_0\rangle$ is exactly the same as the seam between $|a\rangle$ and $|b\rangle$ in the high-symmetry conformation. Even in the low-symmetry case, this is a reasonable approximation for small overlap $S_{a,b}$.

(7) Under the conditions of the experiment of the seam covers a much larger area of the surface because of the effect of solvation. However, here we are only interested in the electronic part contributing to the rate and we neglect the relative Franck–Condon factors including other vibrational modes.

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