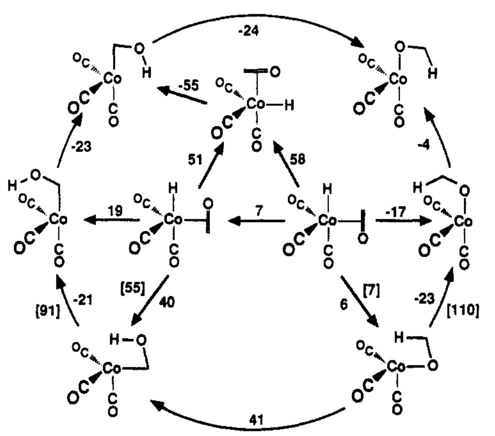

Figure 7. Energy requirements for the insertion of formaldehyde into the Co–H bond of η^2-HCo(CO)$_3$–H$_2$CO. All energies are in kJ/mol. The numbers in brackets represent the activation energies for the reaction in the direction of the arrows. The numbers without brackets stand for the energy difference between two isomers, where a positive value indicates that the resulting complex is higher in energy.

activation energy of only 55 kJ/mol. As indicated above this route might not produce much methanol, since the migratory shift of the hydroxymethyl group to a carbonyl ligand is, in analogy to the alkyl reaction, a very facile process.

VI. Summary and Conclusions

We have investigated here the geometries of the π-complexes that result from an interaction between formaldehyde and the coordinatively unsaturated cobalt complex HCo(CO)$_3$. Furthermore, the insertion of the coordinated formaldehyde group into the Co–H bond was also studied by determining the geometries of some of the insertion products as well as by evaluating the energy profile of the insertion mechanism by a linear transit procedure.

The migratory insertion of formaldehyde into the Co–H bond was shown to produce stable methoxy or hydroxymethyl intermediates. However, the methoxy complexes were 25 to 40 kJ/mol lower in energy. The energy difference was traced to the stronger Co–O bond of the methoxy ligand. Both types of compounds were stabilized by the formation of an agostic interaction between the shifted hydride and the cobalt center. The migration of the hydride ligand to the carbon atom of the complexes aldehyde molecule was found to have a reaction enthalpy, ΔE, of 6 kJ/mol and an activation barrier, ΔE^*, of less than 5 kJ/mol. These values parallel the results of the ethylene insertion and underline the similarity of the two molecular systems. The alternative process where the hydride ligand migrates to the oxygen atom of the H$_2$CO group was somewhat disfavored by an endothermicity of 40 kJ/mol and an activation barrier of 15 kJ/mol. However, the energy requirements of the later process are small enough such that both insertion modes are operative under catalytic conditions where the formation of the methoxy intermediate is favored over the hydroxymethyl analogue. The energetics for the insertion process is summarized in Figure 7.

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Theoretical Evidence for Destabilization of an α-Helix by Water Insertion: Molecular Dynamics of Hydrated Decaalanine

Frank M. DiCapua, S. Swaminathan, and D. L. Beveridge*

Contribution from the Department of Molecular Biology & Biochemistry and Department of Chemistry, Hall-Atwater Laboratories, Wesleyan University, Middletown, Connecticut 06457.
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Abstract: A molecular dynamics (MD) simulation was performed on the hydrated decapeptide Ala$_{10}$·550H$_2$O under periodic boundary conditions. The initial configuration of the peptide was a canonical right-handed α-helix. Over the course of the MD trajectory, the helix is overall intact and dynamically stable, except for one position in which incipient helix destabilization is observed. The helix hydrogen bond at this position was first destabilized transiently for ~10 ps. Some 35 ps later, a persistent destabilization occurred. Analysis reveals that the helix destabilizations are both accompanied by water insertion in a manner consistent with that recently proposed by Sundaralingan and Sekharudu[1] based on a survey of α-helices in protein crystal structures. The geometry of the inserted water was seen to be more favorable for a water bridge in the persistent destabilization than in the transient case. The water-inserted structures may be intermediates in protein-folding processes.

Introduction

Recently, Sundaralingam and Sekharudu[1] surveyed the crystal structures of a number of proteins containing hydrated α-helices. They found a number of instances in which a water molecule is inserted into the N—H···O═C hydrogen bond and correlated this observation with a local destabilization of the helix in the direction of reverse turns and open structures in (ψ,ϕ) space. They further suggested that the observed water-inserted structures may be intermediates in protein-folding processes. Similar observations have been reported in the crystallography of oligopeptide units by Karle and co-workers.[2,3] In view of the current interest in understanding the various mechanisms involved in protein-folding processes,[4] we have carried out a molecular dynamics (MD) simulation on the hydrated decapeptide Ala$_{10}$·550H$_2$O and ex-

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(2) Karle, I. L.; Flippen-Anderson, J L.; Uma, K.; Balaram, P. *Biopolymers* **1989**, *28*, 773.

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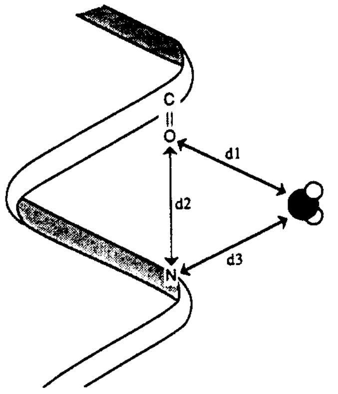

Figure 1. Schematic drawing illustratrating the diagnostic parameters *d*1, *d*2, and *d*3.

plored the extent to which water insertion and helix destabilization is observed in the simulation. MD simulation has been previously used to study diverse aspects of α-helix structure and dynamics,[5] but the nature of helix hydration has not, to our knowledge, been previously studied by MD.

Calculations

The system considered in the simulation is the polyalanine decamer, Ala_{10}, together with 550 water molecules in a hexagonal prism cell. The canonical α-helix for Ala_{10} was generated with use of QUANTA 2.0A[6] on a Silicon Graphics IRIS 4D workstation. The N-terminal group is taken to be NH_3 and the C-terminal group COO, but the molecule is taken to be electrically neutral overall. The configurational energies and forces were computed with GROMOS86 parameters[7] for the polypeptide and the SPC model[8] for water. Switching functions were used to make the long-range nonbonded interactions go smoothly to zero between 7.5 and 8.5 Å. In order to avoid artificially splitting dipoles, the switching function was applied on a group by group basis. The groups correspond to sets of atoms comprising well-defined chemical subunits that, on the basis of structural chemistry, are assumed to be electrically neutral.

The model polypeptide was refined in vacuo by 25 steps of conjugate gradient minimization to a structure bearing on a root mean square difference of 0.136 Å with respect to the canonical form. The canonical ψ, φ, and ω values were −44°, −65°, and 180°, respectively. The refined structure was placed in a simulation cell of adequate dimensions to provide a water bath in excess of two hydration shells deep around the solutes, ca. 8.75 Å. Sufficient water molecules were added to produce a total density of 1 g/cm^3 in the cell, hence the choice of 550. The solvent water in the presence of the polypeptide was equilibrated via a preliminary Metropolis Monte Carlo (MC) calculation of 30K passes (16.5 M configurations). Unfavorable van der Waals contacts and geometric strain in the entire system were removed by an additional 25 steps of conjugate gradient minimization.

The molecular dynamics phase of the calculation involved a period of 1.5 ps of slow heating to 300 K, followed by 3.5 ps of Gaussian equilibration. Non-Gaussian equilibration, with a temperature window of 5 K, was continued at 300 K for an additional 25 ps. The temperature window was then increased to 20 K, and an additional 100 ps of trajectory dynamics was gathered. The total energy of the simulation was found to be well-conserved with no rescaling of velocities, and the temperature remained stable at 300 ± 10 K. All dynamics calculations were performed with use of WESDYN 1.0, a local Monte Carlo and molecular dynamics computer program that uses the GROMOS86

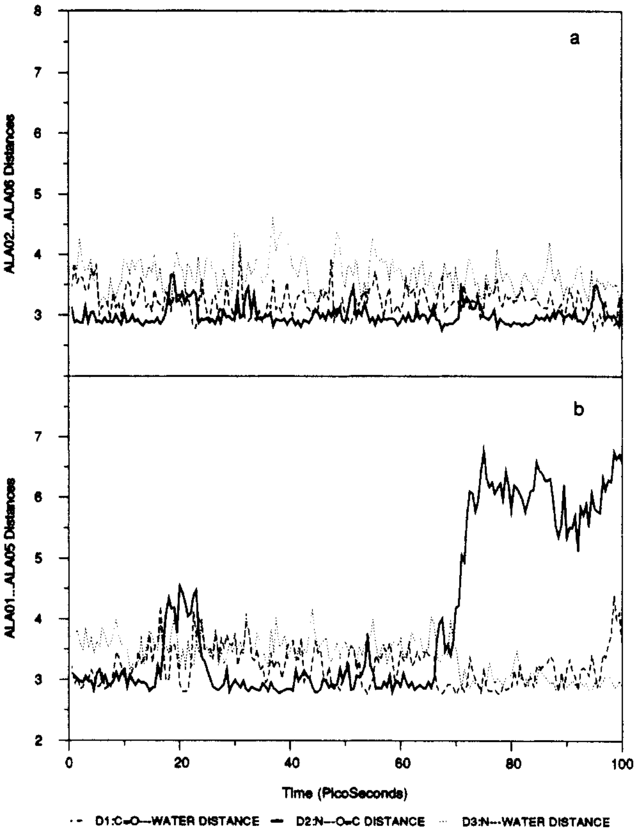

Figure 2. Dynamical behavior of *d*1, *d*2, and *d*3 distances for both the Ala02···Ala06 and Ala01···Ala05.

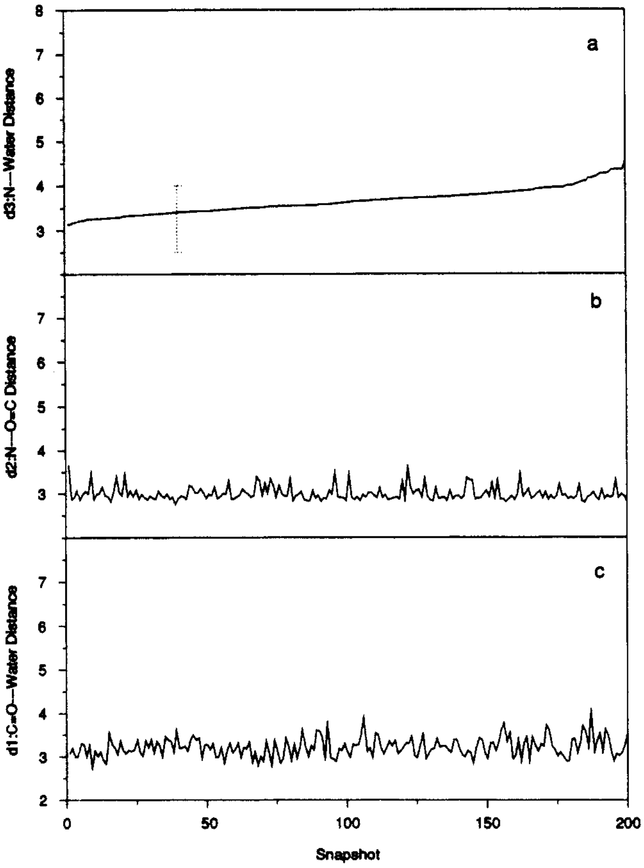

Figure 3. Structural behavior of *d*1, *d*2, and *d*3 for the Ala02···Ala06 hydrogen bond. A total of 4000 snapshots were taken over the 100 ps of trajectory; in each snapshot, the closest water was used in the calculation of distances. Distances were sorted in ascending order of the *d*3 parameter, and every 20 points were averaged; the location of 3.4 Å is noted by the dotted line.

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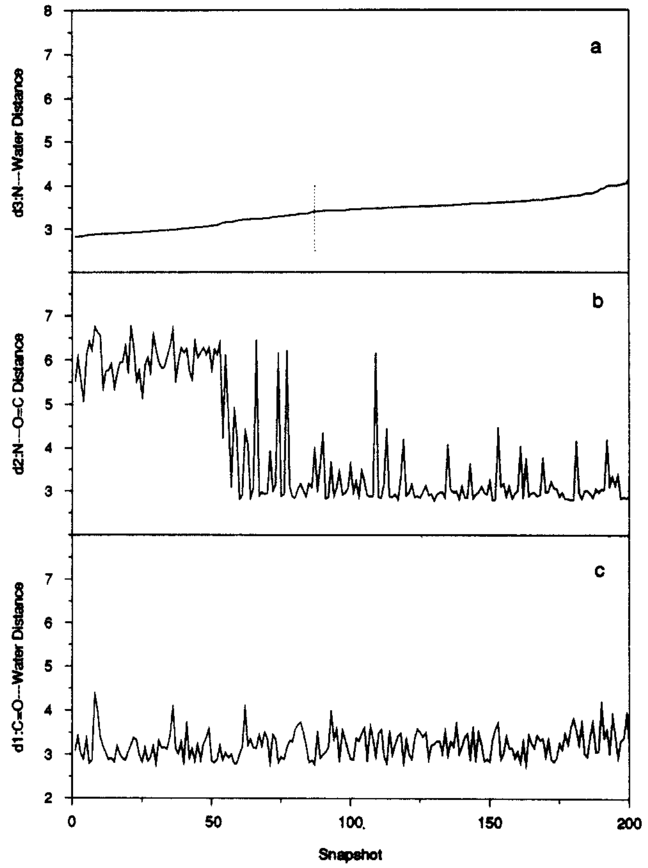

Figure 4. Structural behavior of $d1$, $d2$, and $d3$ for the Ala01···Ala05 hydrogen bond. All distances were calculated as in Figure 3.

force field (topology and parameter files).

Results and Discussion

The results of our calculations were analyzed in a form that corresponds as closely as possible to that adopted by Sundaralingam and Sekharudu, using the geometrical conventions defined in Figure 1 for the distances $d1$, $d2$, and $d3$. In this analysis, the primary diagnostic index of α-helix formation is the distance $d2$, the separation between the carbonyl oxygen and the amide nitrogen in the $(O{=}C)_i \cdots (N{-}H)_{i+4}$ hydrogen bond. The value of $d2$ in the canonical α-helix is 3.2 Å.

Figure 2a illustrates the dynamical behavior of $d1$, $d2$, and $d3$ for the helix hydrogen bond between residues 2 and 6. All distances, including $d2$, are seen to be oscillatory and stable. Occasional displacements of ca. 0.5 Å are seen, but these are short-lived. The nature of the situation at 2···6 is further revealed in Figure 3, which is constructed in a manner analogous to Figure 2B of Sundaralingan and Sekharudu. Here, the structures of the MD trajectory are reordered according to monotonically increasing $d3$, the $N \cdots O_W$ separation. The distance $d3$ vs the "increasing $d3$ structural index" ($d3$SI) is shown in Figure 3a. The corresponding behaviors of $d2$ and $d1$ are shown in Figure 3, parts b and c, respectively. Here, $d2$ and $d1$, the $C{=}O \cdots O_W$ distance, are seen to be stable and oscillatory, indicating the helix is dynamically stable in this region. The range of values seen for $d1$ indicates that the acceptor site for hydrogen bonding on the carbonyl oxygen is always saturated.

All of the α-helix hydrogen bonds in the Ala decapeptide behave dynamically in this manner in the calculation except one, ($N_5{-}H \cdots O{-}C_1$). The behavior of $d1$, $d2$, and $d3$ vs time for this case is shown in Figure 2b. The behavior of $d2$ indicates that, at ca. 15 ps, the α-helix hydrogen bond separates for 10 ps but then re-forms, and then at ca. 70 ps separates and remains that way for the remainder of the trajectory.

We subsequently carried out an analysis with regard to the participation of water insertion in this process. The behavior of $d1$, $d2$, and $d3$ vs. $d3$SI is shown in Figure 4. The value of $d3$ = 3.4 Å was chosen by Sundaralingam as the threshold value for a water entering the first hydration shell.[1] Below this value of $d3$, we have "water-inserted" structures. We observe that when $d3 < 3.4$ Å (actually, ~3.0–3.2 Å as seen from Figure 4), the corresponding distance $d2$ moves into a dynamical regime of large values, indicating helix destabilization. This destabilization of the α-helix in a single MD trajectory is thus marked by similarities to structures observed as "water-inserted" in the ensemble of crystallographic structures by Sundaralingam and Sekharudu; cf. Figure 2B of ref 1.

The behavior of $d2$ above 3.4 Å in Figure 4b shows that some structures in this range also correspond to destabilized helices. This could arise from either a transient destabilization correlated with approaching water molecules or a destabilization persisting after an inserted water has departed. Figure 2b shows no evidence for the former, and thus the persistent destabilization is the likely explanation.

The next point of interest is the nature of the helix destabilization, with respect to both the structure of water and the polypeptide. Sundaralingam and Sekharudu proposed that helix destabilization occurs only when a water is inserted such that $d1$, $d2$, and $d3$ achieve values necessary for forming a "three-centered case". The results in Figure 2b support this proposal, as it is only when $d1$, $d2$, and $d3$ intersect that either the transient (~15 ps) or the persistent (~70 ps) destabilization is observed. Examination of the orientation of the water during the transient destabilization reveals that although a water is "inserted", a bridge structure is not actually formed; i.e., the orientation of the water is not correct for supporting a bridge between the C=O and N—H. In the 70-ps destabilization, the orientation of the inserted water is such that a bridge of the form $C{=}O \cdots H{-}OW \cdots H{-}N$ is clearly present. An illustrative structure corresponding to 69.2 ps into

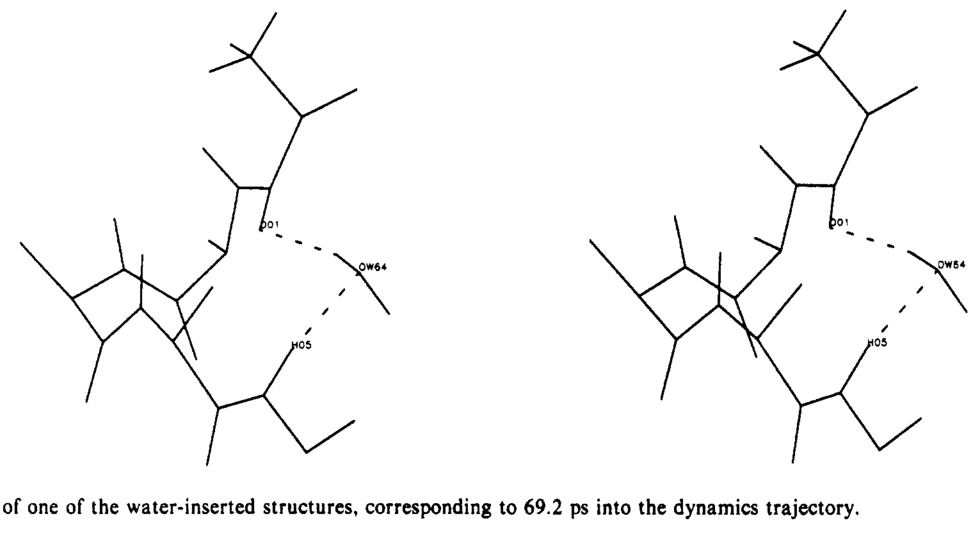

Figure 5. Example of one of the water-inserted structures, corresponding to 69.2 ps into the dynamics trajectory.

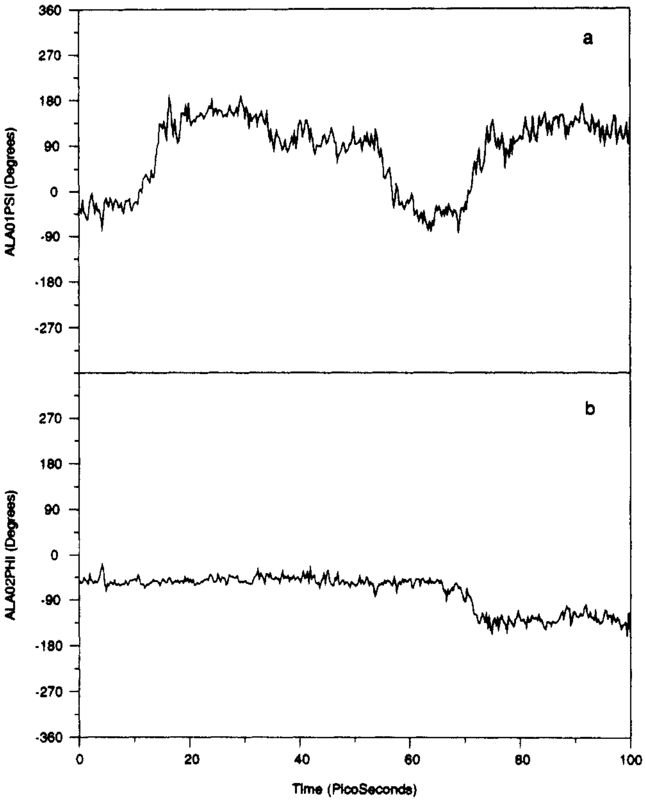

Figure 6. Dynamical behavior of (ψ,ϕ) angles in the vicinity of the helix destabilization, ψ_1,ϕ_2

the trajectory is shown in Figure 5.

The dynamical behavior of the (ψ,ϕ) angles in the vicinity of the helix destabilization, ψ_1 and ϕ_2, is shown in Figure 6 and reveals the nature of the structural changes involved in the polypeptide on destabilization. Here ψ_1 vs time (Figure 6a) reveals that at the point of the transient destabilization (15–25 ps) the dihedral shifts from −47° to ca. +180°, the trans-extended form. While Figure 2b shows the helix re-forms after ~10 ps, Ψ_1 only gradually reverts to the canonical value and arrives there only after a period of ~36 ps following the break. After the destabilization at 70 ps into the run, ψ_1 remains at ~180°. The next dihedral in sequence, ϕ_2 (Figure 6B), is unperturbed by the 15–25-ps destabilization but transits sharply to ca. −180° at the point of the 70-ps destabilization. The calculation indicates that the destabilized form of the helix is the all-trans extended form, $\psi_1 = -180°$ and $\phi_2 = -180°$.

The calculations presented here, while extensive by current MD standards, can only be considered an exploratory step in treating this problem. Since a 230-ps control study of the α-helix in vacuo reflects no destabilization, the effects observed are clearly due to water. The Ala residue is known to favor α-helix formation,[9] and the fact that we see a destabilization of the form observed by Sundaralingam and Sekharudu is thus of special significance and generally corroborates independently their ideas about water insertion. We are confident that the destabilization behavior is due to a water insertion and is not influenced by end effects. A destabilization due to end effects gives rise to significantly different water distance plots, which do not show that the C=O···N—N distance increases cleanly with approaching water molecules. In addition to issues of sequence dependence, we are also currently performing studies of longer helix structures that illustrate this point, and will be described in a subesequent paper.

Summary and Conclusions

Molecular dynamics calculations on an α-helix in water, represented by the decapeptide Ala_{10} and 550 water molecules treated in the simulation under periodic boundary conditions, reveal one example of a sequence of residues in which the helix is destabilized by water insertion. This result independent supports the work of Sundaralingam and Sekharudu,[1] who first observed this phenomenon in diverse protein crystal structures and postulated water insertion to be an important mechanism in protein unfolding.

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