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Recyclization Rate of a Photocleaved Peptide from Multiscale Simulation

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Protein folding dynamics is of fundamental importance in biology, as it regulates protein biological activity.[1] Shedding light on the dynamical conformational changes that convert a polypeptide chain into a three-dimensional protein structure has been the objective of a vast number of investigations.[1−6] Experimental studies of protein (un)folding require a triggering event that initiates the conformational dynamics. Traditional stopped-flow mixing techniques have a time resolution of a few milliseconds and thus are not able to elucidate important fast folding processes.[2] The latter can be conveniently investigated using recently developed ultrafast phototriggers.[2,3,6] An idea pioneered by Hochstrasser[2,3] is to synthesize cyclic polypeptides containing a photocleavable disulfide (S−S) bridge. This technique had been limited to protein dynamics on the nanosecond time scale by the high recombination rate of the S· radicals. In contrast, Kolano et al.[6,7] recently measured remarkably long (millisecond) lifetimes for a β-turn cyclic tetrapeptide (for the structure, see Figure 1).

Detailed insight into the mechanisms governing the S−S ↔ 2S· photocleavage, (un)folding dynamics, and recombination of the S· radicals on the atomistic and electronic structure level can only be gained from computer simulation. To date, only classical molecular dynamics (CMD) simulations using empirical interaction potentials have been performed.[6] Crucially, however, CMD is unable to describe either the initial S−S photocleavage or the recombination, as it does not treat electrons explicitly. In fact, it precludes the breaking and formation of covalent bonds in general and thus does not take into account any intra- or intermolecular chemical processes that may affect the lifetime of the disulfide bridge.

Here we present multiscale simulations of the tetrapeptide in which (nonadiabatic) ab initio MD ((na-)AIMD)[8] was linked to CMD in a three-stage cycle (also see Figure 1): (i) the initial photocleavage was simulated by nonadiabatic QM/MM dynamics (na-QM/MM);[9] (ii) the slow conformational dynamics was described by CMD; (iii) below a threshold distance between the two S· radicals, recombination was enabled by switching to ground-state QM/MM MD. In particular, we investigated the differences between solution and vacuum, as the condensed-phase impact on the (un)folding process remains poorly understood. Viscosity effects on photocleavage and protonation of the resulting cysteinyl radicals could play a crucial role.

The photodissociation of the S−S bridge in the $\pi\sigma^*$ excited state was simulated with CPMD[10] using na-QM/MM[9] employing a cubic 13.23 Å QM box containing the S−S bridge and the C_β hydrogens, the BLYP functional, and plane waves truncated at 70 Ry with norm-conserving pseudopotentials.[11] For each system, 10 nonadiabatic simulations of at least 1 ps were performed microcanonically in the Born−Oppenheimer mode with a time step of 2 au using initial conditions sampled from a canonical ground-state run at 300 K. Simulations in acetonitrile, CH_3CN were carried out in a cubic 45.90 Å periodic supercell using the same force-field parameters as in ref 6. For the CMD simulations, we used the GROMACS program[12] with the OPLS force field[13] for the peptide and a time step of 1 fs. Switching between QM/MM and CMD was done in such a way as to guarantee continuity of the coordinates and velocities (but not the forces) by passing on this information from one code to the other.[14]

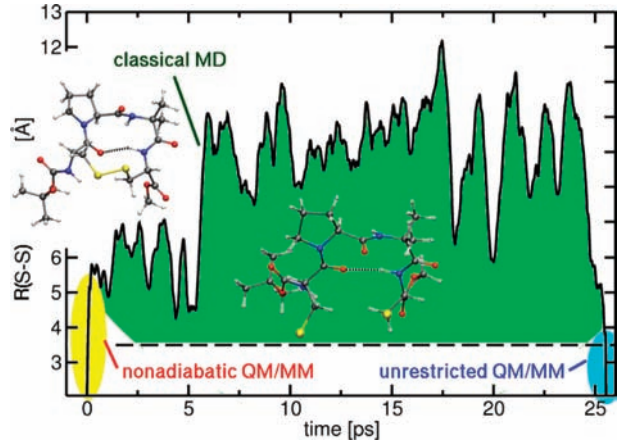

Figure 1. S−S distance as a function of time after photoexcitation, from a typical three-stage simulation of the cyclic disulfide-bridged tetrapeptide *cyclo*(Boc-Cys-Pro-Aib-Cys-OMe). The insets show the (left) closed initial and (middle) cleaved peptide structures.

Prior to the dynamics, we validated the underlying potential energy surfaces for the isolated peptide by comparison with time-dependent density functional theory (TDDFT) and second-order approximate coupled-cluster (CC2) ab initio calculations. The restricted open-shell Kohn−Sham (ROKS) vertical excitation energy of 4.10 eV compared well to the TDDFT value of 4.21 eV, while CC2 yielded 4.86 eV. The pump pulse used in the experiments had an energy of 4.66 eV.[6,7] Furthermore, ROKS and TDDFT excited-state energy profiles along the S−S minimum-energy path showed good agreement.

The left panel of Figure 2 shows the S−S distance as a function of time following photoexcitation for selected na-QM/MM trajectories in vacuum and CH_3CN. In vacuum, the S−S bond is fully dissociated within 120 fs, increasing in length from 2.05 to 5.56 Å. In CH_3CN, only 10% of S−S bonds dissociate, while for another 80% the S−S distance grows in the first ∼50 fs, after which the peptide bounces back from the solvent, resulting in a damped oscillatory motion that restores the S−S bond. The lifetime of the S· radicals for these "unsuccessful" photocleavage events is thus on the order of 100 fs, which is many orders of magnitude shorter than the experimental values ranging from nanoseconds to milliseconds.[2,3,7] However, one should bear in mind that such subpicosecond processes cannot be resolved by the experimental techniques employed. What is the recombination time of the successfully cleaved molecules? Following the na-QM/MM simula-

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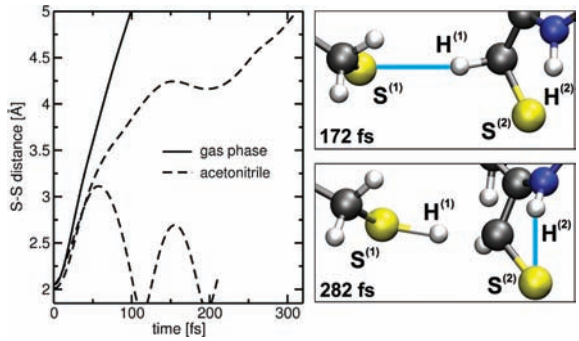

Figure 2. (left) Time evolution of the S−S distance for a selected trajectory in vacuum and for two selected trajectories in CH_3CN. (right) Snapshots of an na-QM/MM trajectory in CH_3CN after (top) 172 and (bottom) 282 fs, illustrating intramolecular hydrogen transfer.

tions of the S−S photocleavage, the slow conformational dynamics was simulated with CMD. To allow for recyclization, we switched to spin-unrestricted ground-state QM/MM MD when the S−S distance fell below the threshold value $R_0 = 3.5$ Å.[15] In the gas phase, the ensemble-averaged time required to reach R_0 for the first time after photoexcitation was 47.2 ps; 80% of molecules recyclized within another 141 fs on average (see Figure 1 for a typical example), while the remainder did not close.[16] Thus, our recombination rates in vacuo are much shorter than the ones measured experimentally for this peptide in solution,[7] but they are consistent with the results of Volk et al.[3] Can our simulations explain the surprisingly long lifetimes reported by Kolano et al.?[7] We performed additional simulations in which we prepared the system in the statistically dominant triplet state when switching back from CMD to QM/MM. Analogous to the singlet simulations, the S−S bridge rapidly adopted the triplet minimum distance of ∼2.6 Å. Relaxation to the singlet ground state via intersystem crossing is expected to be slow, which may contribute to the long recyclization rates observed experimentally. However, the key to reconciling theory and experiment could in fact lie with the remaining simulation in CH_3CN not discussed so far, which exhibits intramolecular hydrogen transfer saturating one of the S· radicals, thereby preventing recombination and leading to a much prolonged lifetime of the other S·. The top-right panel of Figure 2 shows a snapshot at 172 fs after photoexcitation, at which point the S−S bond has broken and the $S^{(2)}$ atom is attracted by the $H^{(2)}$ atom while $H^{(1)}$ first forms a hydrogen bond with $S^{(1)}$ before being transferred onto $S^{(1)}$ some 110 fs later (Figure 2, bottom-right).

In conclusion, we have gained unprecedented insight into the phototriggered unfolding of a polypeptide using a multiscale modeling approach that connects nonadiabatic ab initio MD to classical MD in a three-stage manner. In particular, our simulations address and offer a solution for the controversy regarding the recombination rate of the S· radicals resulting from photocleavage of the S−S bridge. By comparison of simulations in vacuo and in solution, we have found that photocleavage is strongly hindered in the condensed phase, with a high percentage of unsuccessful photocleavage attempts. For a small portion of solvated molecules, however, an intramolecular H-transfer that saturates one of the S· radicals and thus prevents recombination has been observed. This chemical quenching mechanism may indeed be responsible for the surprisingly long S· lifetimes measured experimentally for this system. We expect the H-transfer rate to be highly system-specific, which would explain the apparent discrepancies between experimental recombination rates for different polypeptides.

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(15) An additional series of simulations run with a 4.0 Å threshold yielded no major changes.
(16) The ground-state QM/MM runs were stopped when a S−S distance less than either 2.2 Å (closed peptide) or 5.0 Å (open peptide) was reached.

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