Ultrafast, subfemtosecond charge migration in small peptides is discussed on the basis of computational studies and compared with the selective bond dissociation after ionization as observed by Schlag and Weinkauf. The reported relaxation could be probed in real time if the removal of an electron could be achieved on the attosecond time scale. Then the mean field seen by an electron would be changing rapidly enough to initiate the migration. Tyrosine-terminated tetrapeptides have a particularly fast charge migration where in <1 fs the charge arrives at the other end. A femtosecond pulse can be used to observe the somewhat slower relaxation induced by correlation between electrons of different spins. A slower relaxation also is indicated when removing a deeper-lying valence electron. When a chromophoric amino acid is at one end of the peptide, the charge can migrate all along the peptide backbone up to the N end, but site-selective ionization is probably easier to detect for tryptophan than for tyrosine.

Attosecond lasers | charge transfer | protein mass spectrometry

A chemical rearrangement occurs when atoms in a molecule change their specific arrangement. The time scale of chemistry is therefore the time scale for the motion of atoms (1, 2). The forces operating on the atoms determine the path of this motion. In the Born–Oppenheimer approximation, it is the averaging over the much faster motion of the electrons that specifies the potential energy and hence the forces. The physical picture for this approximation is that the electrons instantly adjust to the current position of the nuclei so that the equilibrium arrangement of the bonded atoms is determined as a minimum of the electronic energy. In the Born–Oppenheimer approximation, the system is confined to be in a single stationary electronic state, and if this confinement is strict then there is no electronic time scale that is relevant to chemistry. During a chemical rearrangement, the motion of the atoms can be accompanied by a reorganization of the electronic structure, and recent experimental progress allows for probing this change (3) in real time. However, it is the motion of the nuclei that sets the time scale because the electronic reorganization exactly tracks the shifts in the positions of the nuclei. As the heavier nuclei move, the light electrons immediately adapt.

It is recognized that the energy of the same stationary electronic state can have two distinct minima, where the electronic charge distribution has a quite different character. At the immediate vicinity of either of the two configurations of the nuclei, the motion is bounded, but larger-amplitude motion can connect the two hulls. Major intramolecular charge reorganization follows upon a change in nuclear geometry (4–8). The time scale of the charge shuffling is that of the motion of the nuclei relocating between the two configurations.

Optical excitation can prepare a nonstationary state, and such a state is often known as the optically bright state. The optically bright state is not stationary because propensity rules constrain the configuration that can be accessed by light. The most common restriction is the Franck–Condon principle, which, in the lowest approximation, requires that during the fast electronic excitation the momentum of the nuclei remains unchanged. By using a light pulse that is shorter than the characteristic time for nuclear motion, it is thereby possible to initiate the system in a localized region on a given electronic potential energy landscape. The subsequent motion of the nuclei then can be probed as a function of the time delay between the pump and probe pulses.

Attosecond (1 as = 10−18 s) laser pulses are becoming available (9–14), and one therefore can ask whether it is possible to access and probe nonstationary electronic states. So that more than one light cycle is generated within the duration of the as pulse, the wavelength of the light needs to be rather short, and the frequency will be high. Subfemtosecond (sub-fs) photons have enough energy to ionize atoms and molecules, and shorter pulses also can ionize core electrons (15, 16). In this work, we discuss electronic relaxation that follows prompt ionization by an attosecond laser pulse.

The fragmentation of molecules upon ionization is at the basis of the analytical applications of mass spectrometry. The quasi-equilibrium theory (QET) (17, 18), nowadays recognized as equivalent to the statistical, or Rice, Ramsperger, Kassel, Marcus (RRKM), theory, assumes that any excess energy in the molecule is first equipartitioned amongst all degrees of freedom. Then, a fluctuation localizes sufficient energy in the coordinate that needs to be broken. It follows that the larger the molecule, the less probable the fluctuation that localizes the energy and the slower the rate of dissociation. In particular, biological molecules such as proteins are not likely to dissociate in the time window (<10−5 s) available in an ordinary mass spectrometer (19). Special techniques [e.g., multicharging by electrospray (20)] therefore are needed to dissociate biological molecular ions.

The experiments of Schlag, Weinkauf, and coworkers (21, 22) on the dissociation of oligopeptide radical ions after excitation/ionization of a chromophore provide two criteria for the presence of a fast time scale. The first is that dissociation is prompt (23), which is not expected for the (tetra and larger) peptides that they used, particularly because even the small dipeptide or tripeptide ions that are collisionally activated in an ion trap are observed to have quite long decay times (24). The second observation is that the ions dissociated in a bond-selective manner. It is expected that in large molecular ions, the bond that breaks is the one near to which the charge is localized. By choosing the sequence of amino acids that constitute the peptide, Schlag and Weinkauf observed fragmentation of different bonds. They interpreted their experiments as showing that the initial ionization is localized at the chromophore. This ionization creates a hole that can then propagate along a sequence of amino acids that, depending on their ionization potential (IP), can energetically assist or hinder the migration. Schlag and Weinkauf studied hole transport, the subject of the present work. Aspects of long-range electron transfer are reviewed in ref. 25.

Our early model computations (26) were consistent with the idea of a “charge-directed reactivity.” In a more elaborate model (27, 28), we also allowed for the energetic effect known from...
solid-state physics as “Coulomb blocking.” The Coulomb repulsion is the energy released when one of two electrons that are both localized on a given site moves apart. Coulomb blocking is blocking the propagation of electrons, and in terms of electronic structure theory it is a correlation effect because it depends on the position of two electrons. The importance of correlation effects in hole migration in molecular ions has since been further demonstrated in more refined computations of the electronic structure (29–31). Molecular dynamics simulations by Schlag et al. (32) have sought to model the charge propagation as induced by steric requirements of aligning the bonds along the peptide chain to facilitate charge transfer (32, 33). The resulting time scale is of the order of many tens of femtoseconds (fs) as expected for a rotation of a bond.

**Results and Discussion**

In this work, we discuss the possible finite duration of a purely electronic reorganization that results from ionization. We conclude that there is a hundreds of as to a few fs electronic relaxation process, with duration depending primarily on the local symmetry of the initial hole density and how deep in the valence band it is. We illustrate the results for the same tetrapeptides that were experimentally examined by Schlag and Weinkauf, but the mechanism that we discuss is general.

We examine two key assumptions made by Schlag and Weinkauf: Is the ionization local, and is there charge reorganization after the ionization, and, if so, on what time scale? In line with our original model, the present fully detailed density functional type computations demonstrate that a purely charge migration process, a process too fast to be accompanied by motion of the nuclei, is possible. Our results are close in spirit to the notion of electronic relaxation that has been discussed for ionization of core electrons in the x-ray region as used in (photo) electron spectroscopy chemical analysis (ESCA). For the purpose of ESCA (34) steady-state spectroscopy, what matters are the changes in electron binding energies and the appearance of satellite lines (35, 36). Compton spectroscopy (37) also can probe the orbitals, and the concept of Dyson orbitals (38–40) is used in this context. Here we propose to follow in time the evolution of the hole density from inception until both dephasing and the onset of nuclear motion-induced relaxation terminate the purely electronic coherent motion.

The electronic reorganization that we discuss is overlooked in the simplest view of electronic structure theory, sometimes known as the aufbau or building-up principle (41). In building-up one first sets up orbitals and then proceeds to fill them up with electrons, taking the Pauli exclusion principle into account thereby allowing only up to two electrons per orbital. In this picture, ionization is a removal of an electron from an orbital that is occupied in the neutral parent molecule. The orbitals themselves are unchanged by this reduction in the number of electrons. The IP is then just the orbital energy, a result that is stronger than this demonstration, and is known as Koopmans theorem (42). The lowest IP is when the ionized electron is removed from the highest occupied molecular orbital (HOMO) of the parent. The positive ion is typically not formed in its equilibrium geometry. On the tens-of-femtoseconds time scale, the ion will relax while lowering its electronic energy by adopting a different arrangement for the nuclei. The excess energy is to be found in the vibration of the bonds, which is the energy that can cause the dissociation of the ion in mass spectrometry. In charge transfer literature (e.g., ref. 43), it is the $\lambda^*$ reorganization energy of the isolated molecule. In terms of ordinary spectroscopy, the nuclear rearrangement upon ionization makes necessary the distinction between a vertical IP when the nuclei are frozen and the adiabatic IP when the ion is in its equilibrium nuclear configuration. We are concerned here with a faster time scale when it is the electrons that reoptimize their orbits because of the removal of an electron, whereas the nuclei perhaps do not yet have the time to move.

The self-consistent field approximation retains the orbital picture but makes each electron move in an optimized field. In this approximation, the field in which the electron moves does depend on how many electrons there are. Therefore, the orbitals of the neutral and the cation are not exactly the same. It follows that the ionic species defined by removing one electron from the neutral is not stationary. The stationary orbitals are those defined by the variational self-consistent field procedure applied to the ion. Say the electron is instantly ionized from the HOMO of the neutral. Then the hole charge density will evolve in time because the HOMO of the neutral is not a stationary orbital of the cation. Rather, the HOMO of the neutral can be expressed as a linear combination of (stationary) orbitals of the cation. When ionization is by an as pulse, the nuclei do not have time to move, and the stationary electronic orbitals are the orbitals of the cation computed at the geometry of the neutral.

The electronic relaxation arises because the self-consistent field for the motion of the electrons in the neutral and the cation is not the same. To initiate such a change in the field, it is not necessary to ionize the neutral. It is sufficient to electronically excite it. For higher excited states, the promoted electron moves in an orbital that is further from the core, giving rise to a Rydberg state. The self-consistent field for the core electrons in a high-Rydberg state is effectively that of the ion, particularly so if the orbit of the Rydberg electron is nonpenetrating (44). For lower excited states of the neutral, the change in the mean field will be smaller and the relaxation will be reduced.

For the electronic closed shell that is the parent neutral, a single Slater determinant wave function has two electrons of opposite spin per space orbital (45). The cation is a radical, but we can constrain its orbitals to be doubly occupied except for the singly occupied molecular orbital (MO) containing the lone electron. This arrangement is the “restricted” self-consistent field scheme. However, it is also possible to allow a relaxation whereby electrons of different spins have different orbitals (45), known as an unrestricted self-consistent field scheme. The unrestricted scheme enables electrons of opposite spin to keep away from one another and thereby incorporates the average role of the Coulomb repulsion. Our results below are that the first type of relaxation, where an orbital of a hole is deforming because the mean field for the motion of the electrons is changed, is often faster and can occur on a sub-fs time scale. The relaxation where the orbitals of the cation split apart is slower, often requiring up to few fs.

In this work, we focus on the results for those tetrapeptides studied experimentally by Schlag, Weinkauf, and coworkers (21, 22). We studied such tetrapeptides as Trp-Ala-Ala-Ala where the aromatic amino acid is at a terminal position. The equilibrium geometry, determined by the computation, is that of the extended conformer of tetrapeptide. The results for the different peptides are not quite similar (see Fig. 1). In the tetrapeptides where Trp is the chromophore the HOMO of the neutral is localized on the Trp, and it is $\approx 0.5$ eV ($1 \text{ eV} = 1.602 \times 10^{-19}$ J) higher in energy than the HOMO-1. These two orbitals are very much similar to those of the aromatic amino acid itself and have a local $\pi$ character with respect to the plane of the ring. When tyrosine (Tyr) is the chromophore (see Tyr-Ala-Ala-Ala in Fig. 1 Lower), the HOMO of the neutral is only $\approx 0.1$ eV above the HOMO-1, and each one is localized at a different end.

The density for the HOMO of neutral Trp-Leu-Leu-Leu-Leu is shown in Fig. 1 Upper. In the corresponding cation, the HOMO is delocalized with weight at both the Trp and the N ends and no significant population on the peptide bridge (see Fig. 2). As

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shown, the densities are the same in the restricted and unrestricted schemes for the cation. What differs between the two sets of computations are the orbital energies. The results shown in Figs. 1 and 2 are for the density of the orbital, and the two colors indicate the plus and minus phases of the wave function. Taking account of the phase shows that the even and odd linear combinations of the two orbitals of the cation as shown in Fig. 2 are two orbitals localized at the chromophore and at the N ends of the peptide, respectively. For the Trp peptides, the HOMO is localized at the chromophore end, as shown for Trp-Leu-Leu-Leu in Fig. 1 Upper. Ionization of the highest electron creates a cation with a localized hole. To time-propagate the hole density, the HOMO orbital of the neutral is projected on the $|\varphi_i\rangle$'s, the (stationary) MOs of the cation as computed for the equilibrium geometry of the neutral. This expansion defines the nonstationary initial hole orbital

$$|\psi(0)\rangle = \sum_{\lambda=1}^{N} \langle \varphi_{\lambda} | \varphi_{\text{HOMO}} \rangle |\varphi_{\lambda}\rangle.$$  \[1\]

There are $N$ atomic basis orbitals where the number $N$ specifies the quality of the basis set, e.g., $N = 810$ for Trp-Leu-Leu-Leu. There are $N$ stationary states of the effective one-electron Hamiltonian, the MOs, that are linear combinations of these $N$ atomic basis orbitals (LCAO). The HOMO of Trp-Leu-Leu-Leu is orbital 147 so that there are many more basis orbitals than electrons, and therefore many MOs are unoccupied. The MOs of the ion also are expressed as linear combination of the $N$ atomic orbitals. We impose symmetric orthogonalization of the atomic orbitals, and so the overlap amplitude $\langle \varphi_{\alpha}|\varphi_{\text{HOMO}}\rangle$ between the HOMO and $|\varphi_{\lambda}\rangle$, the ($\lambda$th stationary) MOs of the cation is conveniently expressed in terms of the expansion coefficients of the HOMO of the neutral and the MOs of the cation as

$$\langle \varphi_{\alpha}|\varphi_{\text{HOMO}}\rangle = \sum_{i=1}^{N} \langle \varphi_{\alpha}|\varphi_{i}\rangle \langle \varphi_{i}|\varphi_{\text{HOMO}}\rangle = \sum_{i=1}^{N} \epsilon_{\lambda\alpha} a_{i\alpha} a_{i\lambda}.$$  \[2\]

Fig. 3 shows typical results for the weights, $|\langle \varphi_{\alpha}|\varphi_{\text{HOMO}}\rangle|^2$, as a function of the orbital energy $E_{\lambda}$ of the $\lambda$th MO of the cation. Fig. 3a shows the weights for the HOMO of the Trp-Leu-Leu-Leu neutral. It is seen that by far the highest overlap is with the HOMO and HOMO-1 of the cation. This finding is qualitatively to be expected from the results shown in Figs. 1 and 2. Note also that there is not much weight of the HOMO of the neutral on the unoccupied orbitals of the cation. As we discuss below, this result means that the ionized neutral does not evolve significantly into excited states of the cation. The results for the HOMO of Trp-Ala-Ala-Ala (data not shown) are similar because the HOMO in both cases is essentially an orbital of Trp. Fig. 3b shows the weights of a deeper-lying orbital of the neutral on orbitals of the cation. We chose orbital 100 of Trp-Ala-Ala-Ala for which the HOMO is orbital 111. The difference between the weights of the two orbitals is reflected in the time evolution as shown in Fig. 5 and explains why ionization from lower-lying valence orbitals typically results in a slower relaxation.

When the cation is computed in the unrestricted scheme, its orbitals also carry a spin label, say, $\alpha$ or $\beta$. Fig. 4 shows the weights of the HOMO, orbital 147, of the neutral of Trp-Leu-Leu-Leu, separately on the $\alpha$ and $\beta$ of the cation. The removed electron is assigned spin $\beta$ so the overlap is highest with the lowest unoccupied MO and HOMO orbitals of this spin. The effect of the correlation is seen in that the higher-most orbitals of spin $\alpha$ of the cation are localized on the leucine (Leu) bridge and thereby kept away from the higher orbitals of spin $\beta$. The result of the correlation is that the HOMO of the neutral overlaps with lower MOs of spin $\alpha$ of the cation, as shown in Fig. 4b.

During an ionization, the nuclei are not moving so that the orbitals of the cation are stationary, and the time-propagated hole orbital is determined by appending the time-dependent phase to each orbital of the cation in Eq. 1 of the hole orbital

$$|\psi(t)\rangle = \sum_{\lambda=1}^{N} \langle \varphi_{\lambda} | \varphi_{\text{HOMO}} \rangle \exp(-iE_{\lambda}/\hbar) |\varphi_{\lambda}\rangle.$$  \[3\]

The sum in Eq. 3 is over all $N$ orbitals of the cation whether these are occupied or not, meaning that it is not only the ground state of the cation that contributes to the time evolution. The weights $|\langle \varphi_{\alpha}|\varphi_{\text{HOMO}}\rangle|^2$ as a function of energy $E_{\lambda}$ plotted in Figs. 3 and 4

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**Fig. 1.** Orbital densities for the HOMO (Right) and HOMO-1 (Left) for the two tetrapeptides TrpLeu$_3$ (Upper) and TyrAla$_3$ (Lower) computed at the B3LYP/6–31G(d,p) level of density functional theory. ([At the consistent field level the densities are robust, and the plot will look the same when computed with the more compact B3LYP/6–31G(d,p) basis.] For TrpLeu$_3$, the HOMO-1 is $\approx 0.5$ eV below the HOMO, whereas in the TyrAla$_3$ peptide, the two HOMOs are nearly degenerate (0.1 eV apart).

**Fig. 2.** MO densities for the TrpLeu$_3$ cation computed at the equilibrium geometry of the neutral in the restricted (Upper) and unrestricted (Lower) schemes and using the B3LYP/6–31G(d,p) basis. The lowest unoccupied MO (LUMO) in the unrestricted scheme corresponds to the HOMO of the restricted scheme. Note that the HOMO (Upper Right) and HOMO-1 (Upper Left) of the restricted and HOMO (Lower Left) and LUMO (Lower Right) of the unrestricted needs to be subtracted to describe a hole that is similar in density to the HOMO of the neutral (see also Fig. 3).
illustrate the contribution from orbitals that are unoccupied in the ground state of the cation.

Fig. 5 shows the hole dynamics in Trp peptides as computed by using the restricted scheme orbitals of the cation. For ionization from the HOMO, that is localized on the Trp chromophore and has a clear local $\sigma$ character whereas the HOMO of TrpLeu$_3$ is a $\pi$ local symmetry. Note also that both are almost identical to the corresponding MOs of Trp and therefore are very similar in the TrpLeu$_3$ and TrpAla$_3$ peptides. The deeper MO 100 has the highest weights on MOs of the cation of local $\sigma$ character localized at the Trp end. When the corresponding hole is time propagated, the time scale for its motion to the N end is in the range of 10 fs. The HOMO of TrpLeu$_3$ is of local $\pi$ symmetry and has high weights on two MOs of the cation (restricted) delocalized at both end and $\sim$3 eV apart in energy. This result explains the sub-fs time scale for charge migration (see Fig. 5) as shown for the higher-most orbitals. A sub-fs pulse is quite likely to ionize from several valence orbitals, and so the resulting relaxation will contain both faster and slower components. For Tyr, the HOMO also has a very high width in energy, but the near-degeneracy of the HOMO, which is localized at the N end (see Fig. 1), and of the HOMO-1, which is localized at the C end, means that it may not prove easy to experimentally prepare a localized initial.
although the natural hole orbitals provide the fewest orbitals needed for convergence when representing the hole density, the weights of the natural orbitals also change with time. Toward a more detailed comparison, we carried out computations for glycine (Gly) and thereby verified that the time scale for the relaxation of lower valence orbitals are comparable with the relaxation time of the natural orbitals in Gly as reported by Cederbaum and coworkers (31).

The slower relaxation as electrons of different spin recede from one another is shown in Fig. 7, which is published as supporting information on the PNAS web site. This “Coulomb-blocking” effect examined in our model computations (27, 28) of charge migration has since been reported by Cederbaum and coworkers (31, 46). We also have included the role of Coulomb blocking in computing electron propagation along molecular wires in real time (47).

The HOMO and HOMO-1 of Tyr-Ala-Ala-Ala are localized at the N and the C ends, respectively (Fig. 1), and they are 0.1 eV apart. Their weights on the cation are shown in Fig. 3. With a broad ultrafast pulse, it may only prove possible to excite a linear combination of these orbitals.

Schlag and Weinkauf reported that ionization of Trp-Leu-Leu-Leu leads to dissociation at both ends as is consistent with our computed facile charge migration from Trp to the N end. For Tyr-Ala-Ala-Ala, ionization results in dissociation very preferentially from the N end. Our computations show that the HOMO is localized at the N end but that it is close in energy (Fig. 1) with the HOMO-1 that is localized at the Tyr end. The computed results are that dissociation may not be a direct probe of charge migration in the Tyr-Ala-Ala-Ala tetrapeptide.

The state \( |\psi(t)\rangle \) (Eq. 3) is not stationary, but it does have a mean energy

\[
\langle E \rangle = \langle \psi(t)|H|\psi(t)\rangle = \sum_{\lambda=1}^{N} |\langle \phi_{\lambda}|\psi_{\text{HOMO}}\rangle|^2 E_{\lambda},
\]

where \( H \) is the Hamiltonian for the orbitals of the cation. The dispersion in the energy of this state is

\[
\langle \psi(t)| (H - \langle E \rangle)^2 |\psi(t)\rangle = \sum_{\lambda=1}^{N} |\langle \phi_{\lambda}|\psi_{\text{HOMO}}\rangle|^2 (E_{\lambda} - \langle E \rangle)^2.
\]

On the basis of the time-energy uncertainty principle, this spread in energy is a convenient single number measure (48, 49) of how fast the state will evolve in time: the more spread the initial hole orbital is over the orbitals of the cation, the faster it will leave its initial location. The spreading is determined by two factors in Eq. 5. A higher width meaning faster relaxation is favored when cation orbitals whose energy differs from the mean contribute to the sum. Also, a higher width is favored when cation orbitals of different energies contribute with about equal weight. If a single orbital dominates the sum in Eq. 5, then its energy is necessarily about equal to the mean, and the width is small. For molecular systems, the width also admits the interpretation as the inherent uncertainty in the potential energy for the motion of the atoms.

In this work, we discussed electron dynamics after prompt ionization. Molecules and clusters with enough energy to ionize need not necessarily do so promptly (50). The longest time scale between excitation and ionization is for thermonic emission where the available energy is first equipartitioned between nuclear and electronic degrees of freedom. Faster, subpicosecond processes occur when the energy is partitioned only among electronic states. This result can be realized for states excited by resonant collisional electronic energy transfer (51) or from electronic excitation with short (few to tens of fs) laser pulses (52). Such electronic energy redistribution can be initiated with as pulses. Then the spectrum of the ionizing electrons is the probe for the electron dynamics.

The electronic energy serves as a potential energy for the motion of the nuclei. Prompt ionization from topmost valence orbitals results in nonstationary electronic states whose energy can have a width of >1 eV. A nonstationary state can be represented as a linear combination of stationary states, as shown in Figs. 3 and 4. This finding means that the evolution of atomic motions can take place simultaneously on different potential energy surfaces with possibly different dynamics on different electronic states (53, 54). Thus, although the lowest surface of the cation is reported to have potential energy barriers for hole migration of the order of 0.4 eV (32, 33), this result need not prevent the ultrafast motion reported here because the inherent uncertainty of the potential energy is higher than or comparable with the barrier heights.

Materials and Methods

The computations were carried out by density functional theory (55) calling upon GAUSSIAN 03 package (56) using DFT/B3LYP with two basis sets 6–31G(d,p) and the more extended 6–311+G(d,p). The larger basis set was used because cations sometimes have a more diffuse electronic density. The radical cation has an open shell, so we also used the unrestricted scheme where electrons of different spin are not confined to move in the same orbital (45).

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