Dissociation Kinetics of Peptide Ions†

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The dissociation of peptide ions has been found to have ultrafast components that in many ways are uniquely different from typical unimolecular kinetics. As such, some peptide reactions provide new channels, which do not conform to statistical models of reaction kinetics. When the dissociation rates are in the 100 fs range, they are in a time scale where statistical methods do not yet apply, although molecules that have not yet dissociated will later in time undergo statistical redistribution of their excess energy, which, however, may not lead to noticeable reactivity within the experimental time frames for large peptides and hence are simply dissipative. This work is meant to reconcile the long time statistical results of Lifshitz et al. (2003) with the work of Schlag et al. (1995/6) that suggests an alternate parallel and much faster time scale for dissociation. It is argued that the two sets of results and interpretations augment one another and in fact open up a most interesting new field of peptide kinetics in addition to the unimolecular behavior, which becomes de facto arrested by the shear size of the molecule being unable to find a transition state on any reasonable time scale.

In mass spectrometry, it is an almost universal dictum that the observed breakdown pattern can be explained on the basis of statistical theories such as RRKM or the quasi-equilibrium theory of unimolecular reactions.[1] Exceptions to this dictum were often reversed after more careful theoretical considerations. It was therefore unexpected when Schlag et al.[2,3] proposed that their particular experiments on small peptides provide a very much more rapid behavior. In a beautiful set of experiments, Lifshitz et al.[4] tested some of the smaller peptides used by Schlag et al. in a specially designed mass spectrometer that focused on the measurement of very long rate constants. In these experiments, Lifshitz et al.[4] found that for these small peptides the statistical theory gave excellent agreement with the very long experimental rate constants that they measured. For these same peptides, Schlag, Schanen, and Weinkauf[2,3] measured results that pointed to very fast dissociation rate constants and equally to different decay channels being operative. We here wish to discuss these very important differences. A possible solution to the difference between the two sets of experiments could be that there are two parallel processes being operative and that the two experimental methods employed separately focused on each of these different processes. In fact, the experiments of Lifshitz et al.[4] can be interpreted as not to be able to observe a very fast initial rate as their setup was not optimized for this time regime. In fact, we wish to emphasize here that these two measurements on the same molecules are naturally sensitive to these different mechanisms.

In the background to our discussion is the even earlier paper of Schlag et al.[5] on the unimolecular dissociation of large molecules. In that paper, Schlag et al. noted that mass spectra of peptide ions taken under ordinary conditions show the presence of fragment ions. Sometimes the fragmentation is extensive. However, the sojourn time of the ion in the mass spectrometer is typically shorter than 10^{-5} s. So, the observed dissociation must occur within that time window. On the other hand, the essence of the statistical theory is that the rate of dissociation is the barrier-crossing frequency times the probability that sufficient energy for dissociation is spontaneously localized in the reaction coordinate. This probability is typically computed by the assumption that the internal energy of the molecule is randomized prior to dissociation.[1,4,6,7] If the energy is randomized, it follows that the probability of dissociation decreases exponentially with the size of the parent molecule. This decrease was experimentally well-established already in the early days of chemical activation by Rabinovitch et al.[8] In the case of fragmentation of ions in the mass spectrum, it is this decrease that leads to the observation of the kinetic shift, a concept pioneered and applied by Lifshitz.[7]

The point of Schlag et al.[5] was that if the energy of the molecule is randomized prior to dissociation then even smaller-sized peptides will hardly have enough time to dissociate in the mass spectrometer. For, say, decapeptides, the lifetime will be enormous. At the time of the paper of Schlag et al.,[5] there were no direct measurements of the rates. What is now available are the two sets of results of Schlag et al.[2,3] and of Lifshitz et al.[4] In particular, Lifshitz et al.[4] have measured the lifetimes of Leu-Tyr and of Leu-Leu-Tyr for ions that have been thermalized by many collisions and then activated by excitation at 579 nm. The measured lifetimes are reported as 2.1×10^{-4} and 3.5×10^{-3} s, respectively. In other words, the tripeptide dissociates about 17-fold slower than the dipeptide. Lifshitz et al.[4] have supplemented their experiment with detailed computations based on the assumption that the energy of the molecule is randomized. They conclude that "the peptide length (i.e., its number of degrees of freedom) strongly correlates with the dissociation rate."

As was pointed out before,[5] as the molecule increases in size, the statistical dissociation rate slows down because of the

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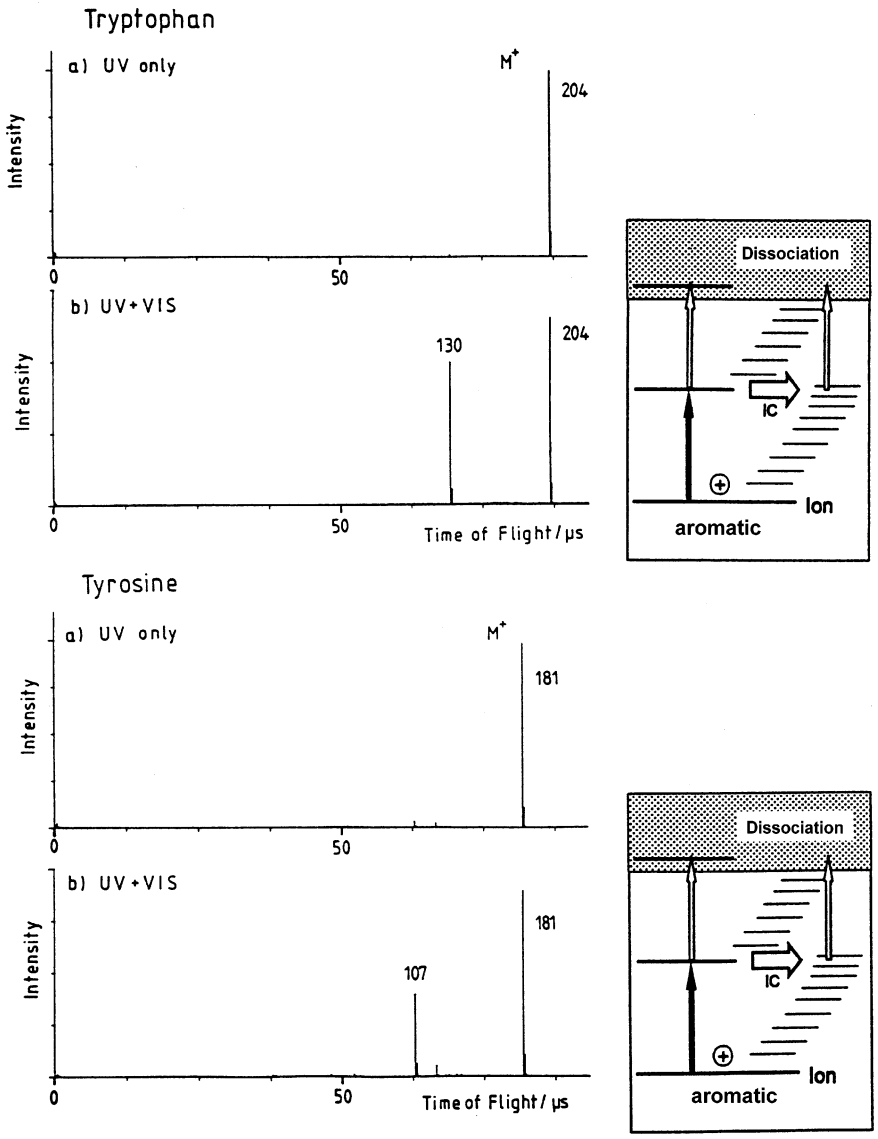

Figure 1. One color excitation of tryptophan (upper part a) and tyrosine (lower part a) leads to intact molecular ions. Two color multiphoton ionization of tryptophan (upper part b) and tyrosine (lower part b) leads to fragmentation since the ion is present to absorb the photon. This demonstrates that the ions themselves absorb light and can dissociate.

increasing number of degrees of freedom between which the available energy can be partitioned. It has been argued[9] that in large hydrocarbons, molecules with many high-frequency C−H and C−C stretch motions, the number of states of the energy-rich molecule increases with size significantly slower than expected on the basis of simple state counting. It was therefore suggested that very large molecules can still exhibit not small statistical dissociation rates. However, for peptides in particular, there are numerous softer modes. Therefore, on statistical grounds, we expect that larger peptides do dissociate more slowly. The experimental/computational results of Lifshitz et al.[4] indeed provide just the support required for the argument of Schlag et al.[5] By extrapolation of the quoted results, the tetrapeptide Leu-Leu-Leu-Tyr will have a lifetime of about 5.7 $\times$ 10^{-2} s. This means that only one molecule in 10000 will dissociate in the time window of a mass spectrometer. Even for the measured Leu-Leu-Tyr, the yield of fragments in an ordinary mass spectrometer is only 1/1000. Yet, upon single photon or two photon ionization, tetra and larger peptides are observed[10−12] to fragment.

In the 1995/6 experiments of Weinkauf and Schlag,[2,3] fragmentation of tetrapeptides such as Leu-Leu-Leu-Trp or Ala-Ala-Ala-Tyr and of larger peptides was however demonstrated with unexpected ease. Equally important, one could select the bond to be broken by engineering a peptide with a suitable sequence. The particular example of Leu-Gly-Leu-Trp is discussed below. It seems inevitable to conclude that there must be an additional nonstatistical dissociation mechanism for peptide ions.

These considerations also raise the more general question of how and why is a polypeptide ion a special class of nonstatistical behavior unlike more compact molecules. In other words, is it the case that polypeptides constitute a unique class of molecules, unlike most other molecular ensembles? It may be that it is precisely this special property, which makes the behavior of proteins in signal transduction unique and contributes directly to their efficiency over long distances—despite the many dissipative degrees of freedom such molecules possess. To begin such an examination, Baranov and Schlag[13] asked the question as to what molecular property do proteins possess, which many other molecules do not have. They then proposed that there is one unique feature of protein motions associated with their revolvings along their dihedral angles, the so-called Ramachandran angles. These are rotational motions over some 100° or

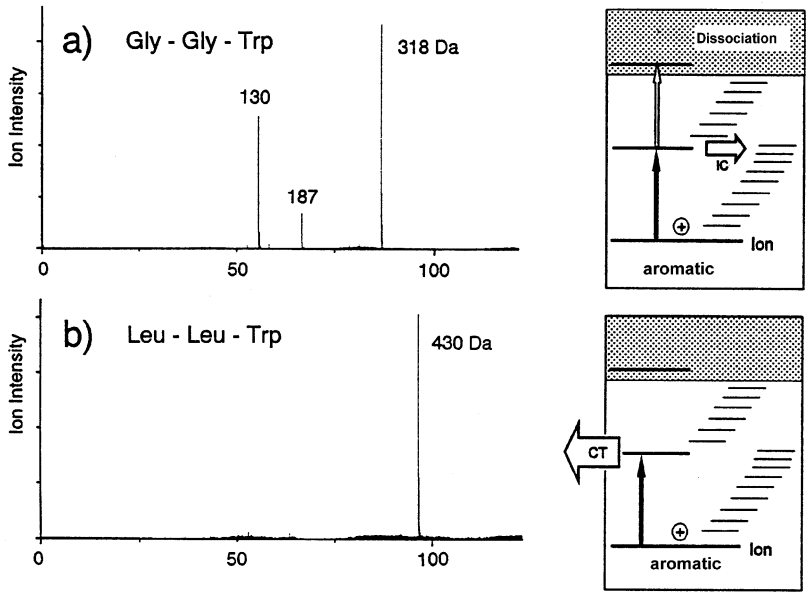

Figure 2. Charge transfer from the aromatic chromophore down the chain: (a) Gly-Gly-Trp shows no charge transfer, and the charge remains on the Trp, which then absorbs light to photodissociate. The fragments at mass 187 and 130 are both typical of the Trp ion; see ref 3. (b) Leu-Leu-Trp shows charge transfer. The ion escapes to the N terminus. This shows charge blockage by the higher energy form Gly as compared to Leu.

so, which have the unique property of almost no rotational barrier—in fact, even less of a barrier than the methyl groups have in ethane. In a series of molecular dynamics (MD) calculations, Sheu and Yang et al.[14] showed that after local excitation the mean first passage time for this rotational motion was only some 100−120 fs, making it one of the fastest protein motions on record. A time scale of 110 fs has recently also been experimentally observed for pure dihedral motion of trialanine by Hamm et al.[15] In fact, this time is faster than the vibrational coupling to the other higher lying phonon modes of the system (IVR) and may well outstrip any IVR times. In this special sense, such rapid motions circumvent in a very special way the entropic barrier to dissociation of large molecules. It is motion on such a time scale that precedes any statistical ansatz, which requires the precedence of IVR—typically some 2 ps or so. Hence, any chemistry attached to this dihedral motion would not be expected to follow statistical behavior.

To test the charge mobility on such an ultrarapid time scale, Lehr et al.[16] in a femtosecond pump/probe experiment photoionized 2-phenylethyl-*N*,*N*-dimethylamine, a model system for charge transfer in a peptide chain. For the downhill charge transfer in the cation, they demonstrated an 80 fs charge mobility as a result of motional rearrangement. In a set of experiments on tyrosine and tryptophan (Figure 1), it was shown that multiphoton excitation of either of these bare chromophoric molecules can lead to dissociation of the ion. This is not in itself surprising, but what was surprising is that leucine attached to the tryptophan would immediately transfer the charge to the N terminus of the leucine but attaching glycene would not (Figure 2). Rather, in the case of Gly, the charge remained on the tryptophan and produced dissociation there. This experiment was repeated with a series of amino acids with similar results, indicating facile charge transfer from the excited aromatic amino acid at the C terminus to the N terminus under the further condition that the ionization potential (IP) of the subsequent amino acids was energetically downhill from that of the chromophore at the C terminus. Because tyrosine on the other hand has a higher IP than tryptophan, it was observed that both glycene and leucine facilitate rapid charge transport to the N terminus.

One of the further puzzles was the range of IPs one would encounter down the chain. Sitting on any given amino acid, there is a left/right asymmetry in that it faces an NH site in one direction and a CO site in the other direction. Calculations[13,17] show that this leads to an energy difference of some 0.4 eV, a large barrier for any facile charge flow. Hence, any transport between typical amino acid sites would have to tunnel through a large barrier—the basis of the superexchange model. In a series of calculations, Baranov and Schlag[13] discovered that this energy is not constant over the entire dihedral angle, but as the CO groups of neighboring amino acid sites approach to within some 2.8 Å, this energy disappears an the energetic states of two neighboring sites become isoenergetic; in fact, it goes into a degenerate state—much like sp^3 hybrids in typical molecules. Hence, it appears that the facile motion along the Ramachandran angles is here seen as a necessary precursor for any rapid charge transport down the chain. This mechanism appears to provide a foundation for the observed rapid and highly efficient charge transport down the peptide chain.

In so far that it is experimentally observed that such a charge transport outstrips thermal reaction rates, it must be that it requires a peptide sequence such that local IPs permit this process. To be technically correct, we must of course use charge transfer states (CT) after the original ionization to explain the process, since the electrons down the chain do not go into the vacuum state but are merely transferred to the nearest neighbor. See Remacle et al.[18−20] for more technical details.

The reason for such highly efficient and rapid transport is to be found in the very fast sub-IVR motions of the dihedral angles, which makes peptides and proteins highly special in the family of molecular systems, perhaps without parallel. Such a rapid transfer of reactivity over great distances is different from any statistical theories, including transition state rate theories (TST), and any such applications of TST must be used with caution in proteins. This process here represents a special distal kinetics, which is different from conventional proximal kinetics. Nevertheless, once the charge and the connected energy has arrived at its final site, it has all the time at its disposal to now initiate

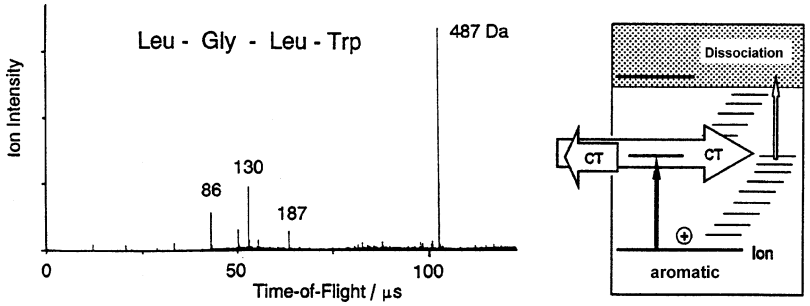

Figure 3. Two color UV−vis mass spectrum of Leu-Gly-Leu-Trp: No charge transfer is found to the N terminus. Glycine blocks charge transfer. See text. The resulting fragmentation pattern is counterstatistical since the charge cannot go to the lowest energy state, which is the N terminus.

a statistical process. Hence, the statistical results observed by Lifshitz et al.[4] are the logical long time end result of this rapid process.

It must be noted that the rapid process has another very interesting prediction that the charge, and hence the reactivity, would get stuck along the sequence if the energetics were appropriately chosen, and thus not find the final state of lowest energy predicted by any statistical or TST model. For this test, the tetrapeptide Leu-Gly-Leu-Trp was studied. Here, clearly the N terminus Leu is the lowest site, but if all of the attached amino acids were Gly, no charge transport was observed (Figure 2). This experiment provides the very important result that the charge travels to the Gly and then gets stuck—and chemical reaction occurs here, not at the thermodynamically more stable N terminus. This result as shown in Figure 3 has many important implications in that it is a clear violation of statistical behavior. It also clearly shows that charge transport proceeds through the chain and not by some other inter- or intramolecular process. This " cork" experiment is crucial to the understanding of this new distal chemistry.

Large angle rotation as here discussed is a unique property of proteins and thus places protein ions in a class by themselves for the long-range transduction of charge. It is a worthwhile challenge to relate this to the efficient transduction of energy—a property, which is observed in many biological experiments involving proteins and may account for some of the unique preference for protein-based systems. The model also makes the opposite prediction, namely, that if such a large angle rotation is impeded, efficient charge transport would cease. Detailed MD calculations[21] of the mean first passage times, but in a water environment, revealed that water makes a hydrophobic shell around the peptide of some 6 Å in diameter. This water barrel is seen in the computations to seriously interfere with these large angle rotations—interestingly not in their mean first passage times—but rather in a drop in efficiency by almost 2 orders of magnitude. Water, although flexible on a longer time scale, is here rigid on the subpicosecond time scale of the dihedral motions. This drop in efficiency means that charge mobility in water now is entirely different in that it decays rapidly as the charge proceeds down the chain. An exponential decrease in charge mobility for proteins in water is well-known[22] but is here not explained by tunneling but rather by motional hindrance of the very large amplitude dihedral motions of the peptide chain.

To conclude, we here wish to argue that the experiments of Lifshitz et al.[4] and the experiments of Schlag et al.[2,3] represent different mechanisms for the dissociation of peptide ions, both of which can apply in their respective—slow and fast—time domains. In fact, for peptides of reasonable biological size, the unimolecular mechanism, although always operative, is only dissipative in character and hence will not produce any chemical reaction in large systems. It would be of considerable interest to characterize the branching ratio between the two pathways, but the considerable separation of time scales presents a challenge. The alternative slow decay channels of a hot polyatomic molecule are part of the hurdle. Theoretically, one expects that the prompt path will increase in importance as the molecule gets bigger and/or the energy is higher. More experimental work on this central issue for unimolecular chemical kinetics is clearly indicated.

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