Communication

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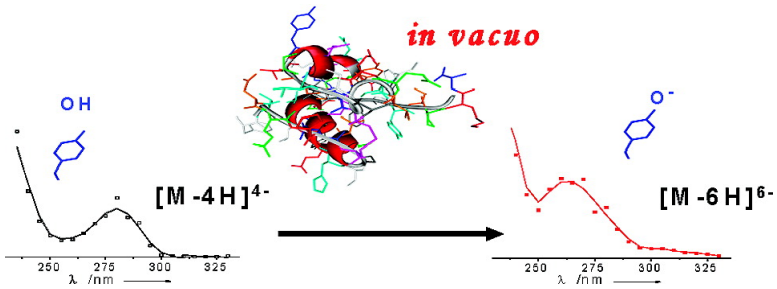
J. Am. Chem. Soc., **2007**, 129 (27), 8428-8429• DOI: 10.1021/ja072818r • Publication Date (Web): 19 June 2007



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Published on Web 06/19/2007

Ultraviolet Spectroscopy of Peptide and Protein Polyanions in Vacuo: Signature of the Ionization State of Tyrosine

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Molecular charge distribution and charge-transfer play crucial roles in the selectivity and function of biologically active molecules. To improve our understanding of these mechanisms at a fundamental level, peptides, proteins, or their aggregates may be studied in vacuo, free of the influence of counterions and solvent molecules. Proton transfer and zwitterionic structures have been probed in the gas phase by ion mobility, electric deflection, and mass spectrometry-based experiments.[1] Recently, laser infrared spectroscopy has provided fingerprints of salt-bridge interactions for amino acids and small peptides.[2] Laser-induced fluorescence appears also as a promising technique to monitor structural changes or fluctuations in unsolvated derivatized peptide ions.[3] Although the first electronic spectra of amino acids and small peptides were reported long ago,[4] the gap to using electronic absorption spectroscopy as a tool for probing the structure and electronic configuration of gas-phase polypeptides or proteins has not yet been bridged. We have recently shown that UV excitation of polypeptides and DNA polyanions induces an electronic excitation of the ions followed by electron detachment,[5] which may involve a resonant tunneling through the repulsive Coulomb barrier.[6] The electron detachment yield can then be used to monitor the electronic excitation spectrum of the precursor ions,[5,7] even for ions with a large number of degrees-of-freedom which would not dissociate after absorption of a single photon. In this Communication, we present the UV spectroscopy of trapped polyanions of different variants of angiotensin peptides and of different charge states of insulin. The gas-phase spectra show bathochromic shifts and provide a direct diagnostic of the ionization state of the tyrosine chromophores containing peptides or proteins.

The experimental setup consists in an ion-trap mass spectrometer that was coupled to a UV−vis tunable OPO laser.[8] Angiotensin II variants ([Val^5]-Angio (DRVYVHPF) and [Asn^1,Val^5]-Angio (NRVYVHPF)) and bovine insulin were purchased from Sigma-Aldrich. Angiotensin peptides were dissolved at a concentration of 100 μM and insulin at a concentration of 150 μM in H_2O/CH_3-CN (50/50, v/v) and directly electrosprayed. The ions were injected into the trap, mass selected, then laser-irradiated during 500 ms (10 laser shots). Action spectra were systematically recorded as a function of the laser wavelength in the range 220−330 nm. The detachment yield is given by ln((parent + oxidized product)/parent)/λ/P_l, where λ is the laser wavelength and P_l is the laser power. UV−vis spectra in solution were recorded using an AvaSpec-2048 fiber optic spectrometer, and an AvaLight-DH-S deuterium−halogen light source.

First measurements were performed on the doubly deprotonated species of two variants of angiotensin II. According to the reported pK_a values of the amino acids (see Supporting Information), [Val^5]-Angio is deprotonated at the carboxyl groups localized at the C-terminal and on the side chain of the Asp^1 residue. The replacement of the acidic Asp^1 residue by the nonacidic Asn^1 in [Asn^1,Val^5]-Angio causes the charge site to relocate. The second deprotonation occurs now at the Tyr^4 leading to a tyrosylate group.

For both angiotensin variants, the main observed desexcitation channel after laser irradiation is electron detachment, giving rise to the appearance of oxidized product ions. Experiments performed as a function of the laser power show that this detachment is a one photon process (see Supporting Information). The electron detachment yields measured for the two variants as a function of the laser wavelength in the 220−330 nm range, are shown in Figure 1a. For [Val^5]-Angio, the absorption occurs below 300 nm, with a band between 260 and 300 nm due to π excitations of the tyrosine chromophore. For [Asn^1,Val^5]-Angio, a modification of the absorption spectrum occurs with a first band between 290 and 330 nm attributed to the ionized tyrosine residue. This bathochromic shift is also observed in solution[9] as revealed in Figure 1b by the absorption spectra of the two angiotensin variants recorded as a function of pH. At pH = 5.3, the tyrosyl group is neutral. At pH = 13, at which value tyrosyl group is deprotonated (for both variants), the spectra for the two variants are red-shifted (see Figure 1b). The red-shift reflects the destabilization of the molecular orbitals due to the negative charge on the hydroxyl oxygen leading to a smaller HOMO−LUMO gap in tyrosylate as compared to tyrosyl.[10] Indeed, the main difference between the two experimental UV spectra displayed in Figure 1a is reproduced in calculated time-dependent density-functional theory (TDDFT) absorption spectra for isolated tyrosine and tyrosinate species (see Supporting Information). In particular the onset at $\lambda \approx 300$ nm for [Val^5]-Angio and the two bands centered at $\lambda \approx 262$ and $\lambda \approx 312$ nm for [Asn^1,-Val^5]-Angio are well reproduced. Furthermore, the shifts are influenced by the environment of the chromophores, as outlined by the differences observed between gas phase and solution spectra. This was expected and also was observed in solution for different solvents.[11]

In the same way, we recorded the electronic spectrum of the gas-phase multi-deprotonated insulin for different charge states. Spectra are strongly charge-state dependent. Results for the [M-4H]$^{4-}$ and [M-6H]$^{6-}$ ions are compared in Figure 2a. Insulin contains four acidic [Glu] residues (plus one carboxyl function at the C-terminal) and four tyrosines (sequence given in Supporting Information). The absorption observed between 300 and 330 nm and the band centered at 260 nm reveal the ionization of at least one tyrosine residue in the [M-6H]$^{6-}$ species. Figure 2b displays the evolution of the electron detachment yield as a function of the charge state of insulin at $\lambda = 305$ nm. It is nearly zero for charge states 4- and 5-. For these two species, deprotonation occurs only on the carboxy group at C-terminal and Glu residues; in particular for 4- no absorption is observed above 300 nm (see Figure 2a).

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10.1021/ja072818r CCC: $37.00

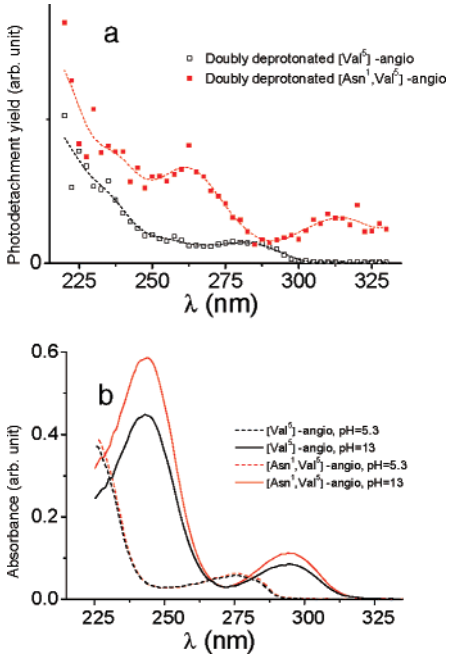

Figure 1. (a) Photodetachment yield as a function of wavelength for the doubly deprotonated $[M\text{-}2H]^{2-}$ ions of the two gas-phase variants of angiotensin II. Dashed curves are smoothed curves of the experimental data. (b) Absorption spectra of the two variants of angiotensin II in solution at pH = 5.3 (100 μM in H_2O/CH_3CN (50/50, v/v)) and at pH = 13 (100 μM in H_2O + 0.1 M KOH).

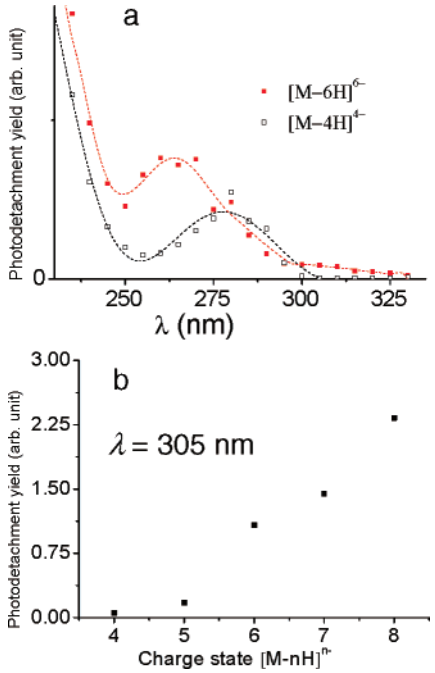

Figure 2. (a) Photodetachment yield as a function of wavelength for the gas phase $[M\text{-}4H]^{4-}$ and $[M\text{-}6H]^{6-}$ ions of insulin. Dashed curves are smoothed curves of the experimental data. (b) Photodetachment yield measured at $\lambda = 305$ nm, as a function of the charge state n of the $[M\text{-}nH]^{n-}$ insulin ions.

For 6-, 7-, 8- charge states, a detachment is observed. The yield linearly increases as the charge increases, which we interpret as a subsequent ionization of the tyrosine residues. The electron detachment yield can then be used as a measure of the tyrosylate content in proteins, as performed with absorption in solution.[12]

In conclusion, we used electron detachment to report the first electronic excitation spectra of polypeptides and of a protein in vacuo and demonstrated the feasibility of UV spectroscopy on large isolated biomolecular ions. Experiments provide a clear signature of the ionization of the tyrosyl group. Moreover, electron detachment from a tyrosylate leads to the formation of a tyrosylate radical. Both the ionization of tyrosyl into tyrosylate and tyrosylate radical are key events that regulate the catalytic properties of many proteins. For instance, tyrosyl residues drive an important role in redox reaction associated with ferritin, photosystem II, or cytochrome oxidase.[13] The current results demonstrate the possibility of probing these mechanisms in vacuo and of monitoring biologically relevant processes in isolated proteins or proteins associated to noncovalent complexes.

Supporting Information Available: Intrinsic p*K*a values of ionizable groups found in proteins, amino acid sequence of bovine insulin, electron detachment yield measured as a function of the laser power at $\lambda = 260$ nm for the charge states 4- and 6- of insulin and the two variants of angiotensin II, laser power curve as a function of the wavelength, calculated TDDFT spectra of tyrosine and tyrosinate. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA072818R