Infra-red photodissociation of protonated amino-acids and peptides in the gas phase

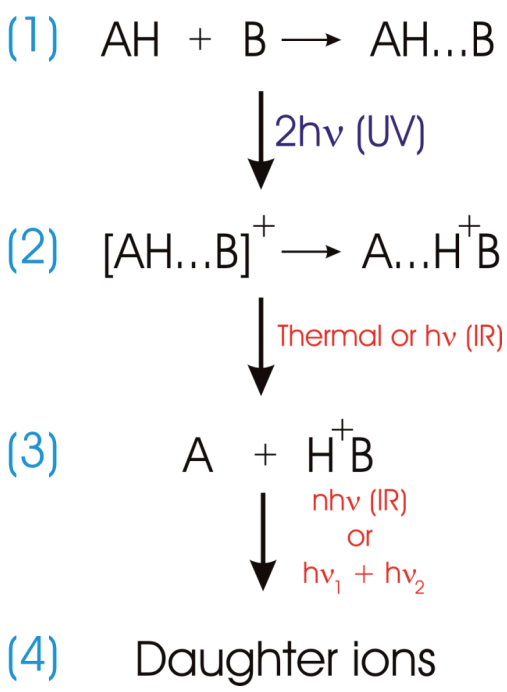
N. A. Macleod, T. de Boer, N. Minns, L. C. Snoek and J. P. Simons
Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK

E. Marzluff
Department of Chemistry, Grinnell College, Grinnell, Iowa, USA

Main contact email address N.MacLeod@rl.ac.uk

Introduction

The majority of molecules of biological relevance contain functional groups which can be protonated at physiological pH. Detailed spectroscopic examination of such species under isolated, gas phase, conditions has been hindered by the practical difficulties of producing sufficient number densities of ions in the gas phase. To overcome this problem, we have recently developed a photo-chemically based method (see scheme 1) capable of producing spectroscopically useful numbers of protonated ions in the gas phase [1].



Scheme 1. Photo-chemical production scheme and infra-red (multi-) photon dissociation.

Briefly, complexes between a suitable proton donor (e.g. phenol or indole) and proton acceptor (e.g. the amino acids lysine or γ-amino butyric acid, GABA) are produced in a molecular beam. 2-photon ionization (of the donor chromophore) produces an ionic complex in which proton transfer is an energetically favourable process. The resulting complex, consisting of a protonated ion hydrogen bonded to a radical species, can be probed using infra-red dissociation. Alternatively, spontaneous dissociation of the internally excited complex produces the bare protonated ion which can be detected through a one- or two-colour multi-photon process.

Results

Infra-red spectra (detected via evaporation of the phenoxy fragment) of the cationic complexes between phenol and a number of amino acids are shown in figure 1. The appearance, in all cases, of a band at 3560 cm^{-1} due to the OH stretch of the acid group indicates the absence of any strong interactions between this group and the functional groups of the flexible side-chain. This is in contrast to the neutral amino-acids where structures with interactions between the acid group and the other parts of the molecule are among the most energetically favourable [2].

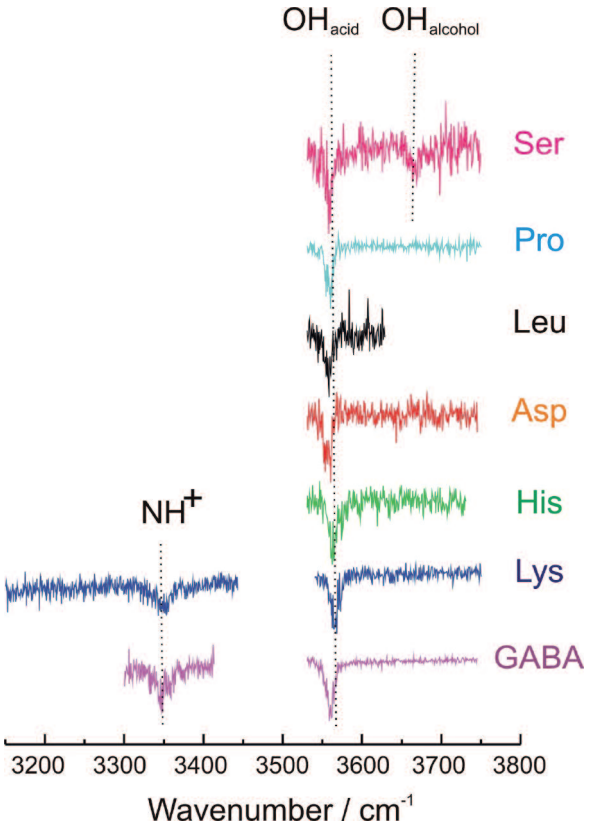


Figure 1. Infra-red photo-dissociation spectra of the cationic complex between phenol and a number of amino-acids.

Figure 2 shows the infra-red photo-dissociation spectrum of the cationic complex between phenol and lysine; also shown are computed spectra (B3LYP/6-31+G*) for the energetically favourable conformers of protonated lysine and their complexes with a phenoxy radical. Computed structures and relative energies (MP2/6-311++G** in $kJmol^{-1}$) are shown in figure 3.

The experimental spectrum shows two bands at 3560 cm^{-1} and 3350 cm^{-1}, assigned to the OH stretch of the carboxylic acid and an NH stretch of the protonated amine group respectively. A rise in the baseline at the lower frequency end of the spectrum indicates the onset of an intense and broadened band due to an NH stretch which is involved in a strong hydrogen bond. The presence of a solitary NH stretch in the 3300-3400 cm^{-1} region implies a structure in which the NH_3^+ group is involved in two strong hydrogen bonds.

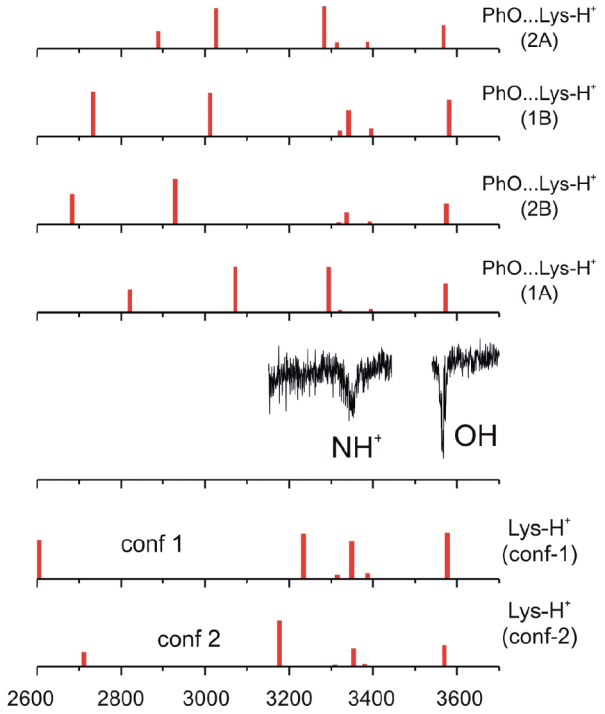


Figure 2. Experimental and computed infra-red spectra of the cationic complex between phenol and lysine. Computed frequencies (B3LYP/6-31+G*) have been scaled by 0.976 (OH) and 0.956 (NH) to account for anharmonicity.

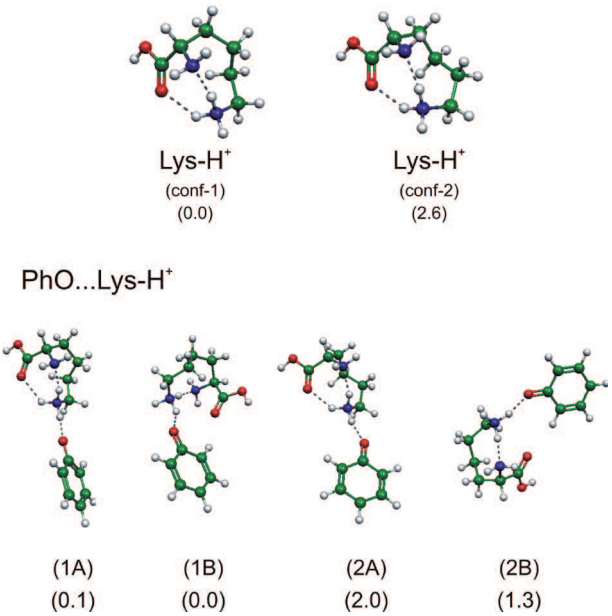


Figure 3. Computed structures of protonated lysine and its complex with the phenoxy radical. Relative energies, in kJmol^{-1}, are from MP2/6-311++G single-point calculations on the B3LYP/6-31+G* optimised geometries.**

While the phenoxy "tag" can account for one of these interactions, there must also be a second, intra-molecular, interaction. This is confirmed by the quantum chemical calculations on protonated lysine where all the energetically favourable structures (figure 3) have a folded arrangement in which the protonated amine group hydrogen bonds to both the α-NH_2 group and the carbonyl oxygen of the acid group. Two such structures are found which differ only in the configuration of the flexible side-chain; alternative structures are destabilised by at least 10 kJmol^{-1}. No structures with an extended type structure are found. This contrasts with neutral lysine where extended and folded structures have very similar relative energies.

Complexation with the phenoxy radical has only a minimal effect on the geometry of protonated lysine. Comparison of the experimental and computed spectra allows an assignment to the "B" type structures in which the phenoxy hydrogen bonds to the NH which was hydrogen bonded to the carbonyl oxygen.

Conclusion

Protonated amino-acids have been produced in the gas phase using a photochemical production method and analysed using infra-red photodissociation spectroscopy. High-level quantum chemical computation allows the identification of a folded structure for protonated lysine in the gas phase which contrasts markedly with the behaviour of the neutral species.

References

1. N. A. Macleod and J. P. Simons. *Phys. Chem. Chem. Phys.* 2004, **6**, 2821.
2. L. C. Snoek, R. T. Kroemer, M. R. Hockridge and J. P. Simons. *Phys. Chem. Chem. Phys.* 2001, **3**, 1819.