Inner shell excitation, ionization and fragmentation of pyrimidine

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Abstract. The inner shell excitation and ionisation of pyrimidine have been studied at the carbon K edge by near-edge X ray absorption fine structure (NEXAFS) and X ray photoelectron (XPS) spectroscopies. The theoretical predictions of density functional theory (DFT) provide a satisfactory assignment of the complex spectra of this polyatomic molecule. The fragmentation following the C(1s→π*) excitation has been investigated by resonant Auger electron-ion coincidence spectroscopy, which allows a site and state selective study.

1. Introduction

Pyrimidines are an important class of organic molecules because the pyrimidine ring forms the base structure of three nucleic acids (uracil, cytosine and thymine) and their halogenated substituted bases have found applications as radiosensitizers in radiotherapy [1]. A photoabsorption process in a biological system may cause repairable and/or unreparable changes/damages in a biological cell. The macroscopic damage can be tracked down to a microscopic scale, where the initial processes on the elementary constituents are the same as those studied in molecular physics and photochemistry. Therefore it is expected that gas phase experiments of model molecules can provide insight in the physical and chemical properties of biological molecules. This has lead to an extensive investigation of the structure and dynamics of biomolecules and their solvated complexes [2-5].

A recent theoretical study of the electronic structure and chemical properties of DNA/RNA bases [6] has shown that pyrimidine bases inherit certain properties from their parent pyrimidine, while the purine bases do not. Moreover the observation of high relative biological effectiveness of soft X ray in cells [7] has raised the question whether this is due to a specific physical/chemical effect or to the preferential localization of the ionizations in the DNA bases. In the former case this implies a fragmentation process induced by the radiation, while the latter implies that the bond breaks are due to the electron swarms produced following the absorption of the soft X-ray. To answer these questions one has to measure the electron spectra of the inner shell ionized and excited molecules as well as their site selected
fragmentation patterns. For this purpose we have undertaken a detailed investigation of the inner shell photoabsorption, photoionization and photofragmentation of pyrimidine and some of its halogenated substitutes. Here some results of the XPS, NEXAFS and resonant Auger measurements of pyrimidine together with the analysis of the fragmentation following inner shell excitation are reported.

2. Experimental

All the measurements have been performed at the Gas Phase Photoemission beamline of the Elettra synchrotron radiation source, Trieste [8]. The 100% linearly polarised radiation from the undulator (period 12.5 cm, length 4.5 m) is deflected to the variable-angle-spherical grating monochromator, which covers the energy region 13-1000 eV with five interchangeable gratings. The XPS photoemission and resonant Auger spectra were measured using a commercial 6-channel, 150 mm hemispherical electron energy VG analyzer. The axis of the electron analyzer is set at the magic angle in the parallel plane, i.e. in the plane defined by the electric vector of the light and the photon propagation direction, at 54.7° with respect to the electric vector of the light. In this geometry the measurements are insensitive to the photoelectron asymmetry β parameter. The C (1s) spectrum of pyrimidine (Figure 1) was measured at about 100 eV above ionization threshold, using an energy resolution of the analyzer of about 100 meV [9]. This collection energy in the photoelectron spectrum guarantees that PCI effects [10] can be neglected in the data analysis. The energy spectra were calibrated using a mixture of the molecule under study and CO₂, whose XPS peak (C(1s), ν=0) is at 297.69 eV [11]. The C NEXAFS spectrum of pyrimidine (Figure 2.a) was measured using a channeltron multiplier close to the interaction region to detect the ions produced by the decay of the excited molecular states [12]. The ion detector has been placed at about the ‘magic angle’ with respect the photon beam polarization axis, so that the measured cross section is not affected by asymmetry parameters. The NEXAFS spectrum has been obtained scanning the photon energy over the range 283-300 eV. The spectrum was then normalized to the variation of the photon beam intensity due to time and monochromator transmission efficiency. The energy resolution was approximately 75 meV. For the measurements of the site and state selected fragmentation the same hemispherical analyser, set at 90° with respect to the electric vector, and a time-of-flight ion analyzer mounted opposite to it have been used. The characteristic of this set-up as well as the procedure adopted in the measurements have been described recently [13] and will not be repeated here. An important peculiarity of the resonant Auger-ion coincidence spectroscopy is the ability of the technique to select both the initial state and site (by setting the photon energy on a specific NEXAFS peak) and the final ionic state (by selecting the kinetic energy of a particular resonant Auger electron).

Pyrimidine is in the form of liquid at room temperature, with enough vapour pressure to produce the gas density needed for gas phase experiments. The sample was purchased from Sigma-Aldrich, with purity always higher than 99% and used without further purification. The compound was kept in a glass vial outside the vacuum chamber and introduced in the interaction region via a leak valve.

3. Results

The C(1s) XPS spectrum of pyrimidine is shown in figure 1. The spectrum is composed of three main peaks, corresponding to the ionization of the three non equivalent carbon atomic sites in the molecule, the C2, C4/C6 and C5. Each one of these XPS peaks is most likely the convolution of an undefined number of overlapping and unresolved vibrational progressions. The resulting asymmetric lineshape of each peak has been approximated in the fitting procedure by an asymmetric Gaussian function with the position of the centroid, the area and the low/high energy side widths used as free parameters. This procedure provides a good description of the spectra, with only small discrepancies on the tails of the peaks. The centroid of each peak has been assumed as the binding energy of the state and is reported in Table 1 together with the estimated areas from the fitting. In the same table the results of a DFT calculation [9] and previous
calculations of F. Wang et al.[6] are reported. The different binding energies of the non equivalent C atoms are mainly due to the presence of the two electron-attractive N atoms, that have the overall effect of shifting the binding energies towards higher values with respect to the benzene case [14]. Indeed the C2 carbon atom located between the two N atoms is affected by the larger binding energy shift, while the C5 being the farthest one has the smallest shift. These observations can be rationalized in terms of the inductive and resonant models of the charge screening in aromatic molecules.

Table 1. C(1s) experimental binding energies and relative intensities of pyrimidine. The theoretical values of [9,6] have been arbitrarily aligned to the C2 site of pyrimidine for comparison with the experimental results. The uncertainty in the theoretical calculation is estimated to be around 0.1 eV.

| C(1s) state | Binding energy (eV) | Relative Intensity | Theory (eV) | Ref [9] | Ref [6] |
|------------|---------------------|--------------------|------------|---------|---------|
| CO₂(C1s, v=0) | 297.69 ± 0.02 | -- | 291.20 (C5) | 290.98 (C5) |
| pyrimidine | 291.09 ± 0.12 | 0.52 ± 0.04 | 292.24 (C4) | 292.11 (C4) |
| | 292.08 ± 0.02 | 1.00 ± 0.05 | | |
| | 292.48 ± 0.03 | 0.53 ± 0.07 | 292.48 (C2)* | 292.48 (C2)* |

* zero of the theoretical binding energy scale

In the inductive model an electron attractive substituent removes charge density from the neighboring sites, while the weaker resonant effect involves the π conjugated bonds along the ring. It is interesting to note for example that an analysis based on the difference electron density maps, obtained by subtracting the valence electronic density of a “standard” calculation from the valence electronic density of the
corresponding “core hole” calculation, shows that a contribution to the screening of the C2 hole comes from the farthest C5 atom [9]. This contribution is explained by a resonant effect involving the C5 atom in para position with respect to the C2 hole.

The portion of the C(1s) NEXAFS spectrum of pyrimidine between 284-288 eV is shown in Figure 2.a. The spectrum is dominated by the strong $\pi^*$ resonance representing the transition of the 1s electron to the lowest unoccupied, antibonding, molecular orbital. The peaks labeled 1', 1'' and 1''' have been assigned by a DFT calculation [12] to the transition from the three inequivalent carbon sites, C5,C4/C6 and C2 respectively. At higher photon energy the NEXAFS spectrum shows peaks due to a mixed contributions of $\sigma^*$ and $\pi^*$ orbitals corresponding to excitation of Rydberg or mixed valence-Rydberg orbitals [12]. The present experimental and theoretical spectra are in good agreement with previous results [15], even though the higher resolution of the present measurement allows the observation of some extra features on the $\pi^*$ that can be assigned to vibrational progressions, as already observed in benzene [16]. The decay of the inner shell excited pyrimidine via resonant Auger electron emission has been studied at the energies which correspond to the excitation to the $\pi^*$ orbital of the three non equivalent sites. The results are collected in figures 2.b,c and d. The final states in the resonant Auger decay are singly charged ion states, thus the resonant Auger spectra have been compared with the PES spectrum (figure 2.e) of pyrimidine recently reported and assigned [17]. In figures 2.b-d the three resonant Auger spectra display different intensity among themselves and with respect to the PES spectrum.

The intensity of a resonant Auger transition depends on the overlap of the wave functions of the intermediate and final states, i.e. the C(1s')+π* and the ionized valence orbitals. Interestingly, the resonant Auger decay seems to favor the population of final ionic states with similar localization of the charge

![Figure 2](image.png)

**Figure 2.** a) expanded view of the C(1s) NEXAFS spectrum [12] in the region of the $\pi^*$ transition; transitions labeled 1', 1'' and 1''' have been assigned to the excitation of the C5, C4/C6 and C2 carbon sites into the $\pi^*$ orbital; b-d) the resonant Auger electron spectra measured at photon energies corresponding to the excitation of the 1', 1'' and 1''' NEXAFS features are compared to the photoelectron spectrum measured at 21.22 eV photon energy [17].
distribution as in the intermediate state. For example, according to the Mulliken population analysis [17], the HOMO state of pyrimidine receives a major contribution from the lone pair orbitals of the nitrogen atoms. Very little superposition with the C(1s)\(^{-1}\)\(^\pi\)*, core hole state is expected, and indeed observed (see figures 2.b-d at about 9.7 eV binding energy). On the contrary, the HOMO-1 orbital, with a stronger contribution from the C5 and C2 atoms, shows a preferential population from the resonant Auger decay of the 1' and 1'' NEXAFS states, localized on the C5 and C2 site, respectively, according to our calculations [12].

Figure 3. The mass spectra of pyrimidine measured in coincidence with resonant Auger electrons at selected kinetic energies, labeled 1 to 9 in figure 2.b.

At a few selected energies of the resonant Auger electron (labelled 1-9 in figure 2.b), which correspond to several different final states of the singly charged ion, the fragmentation patterns of pyrimidine have been measured. In figure 3 the fragmentation patterns measured at the 1'' position of the NEXAFS spectrum is shown. Three groups of charged fragments of decreasing masses appear in the spectra of figure 3 as the kinetic energy of the resonant Auger electrons is decreased, i.e ion states of increasing binding energy are selected. The parent molecule corresponds to the peak at m/e=80, while the fragments due to the loss of
one or two HCN groups appear at m/e=53 and 26, respectively. Within the statistics and sensitivity of the present experiments, no fragmentation of the molecule is observed for binding energies <11.5eV, while at binding energies >12 eV the full fragmentation occurs. This is consistent with the picture proposed by Schwell et al. [18], who set at a binding energy >11 eV the appearance potential for the loss of a neutral C2H2 fragment, which represents the lowest and weak fragmentation channel. In the same work, those authors set at 12.27 and 14.2 eV the thresholds for the appearance of the C3H3N+ and HCCH+ fragments, corresponding to the peaks at m/e=53 and 26, respectively. The observations reported in figure 3, can be easily understood in terms of the increasing residual energy available for the fragmentation of the singly charged molecule as ion states of increasing binding energy are selected. No substantial differences are observed in the fragmentation patterns by moving the photon energy to the 1′′ and 1′ NEXAFS states, i.e. making a selective excitation of different molecular sites. This allows us to conclude that the fragmentation of pyrimidine following inner shell excitation depends only on the final state of the singly charged ion reached in the relaxation process.

4. Conclusions
The electron spectra produced by C(1s) ionization and excitation with synchrotron radiation have been studied. The spectra have been assigned by comparison with DFT calculations [9]. The measured values of the chemical shifts of the three inequivalent C atoms in the XPS has been explained in terms of both inductive and resonant effects. The resonant Auger spectrum shows a selective population of the final states of the singly charged ion and the site and state selected fragmentation patterns appear to depend only on the final state of the singly charged ion. This set of data represents the benchmark for an extensive study with the same techniques of the halogenated pyrimidines, which aims to provide information useful to disentangle the mechanisms of the radiation damage of soft X-ray in cells.

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