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Spectroscopic characterization of human cystatin C and its mutants

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Human cystatin C (HCC) is a small *β*-protein consisting of 120 amino acidsthat is found in all nucleated cells. Physiologically, its function is to regulate the catalytic activity of cysteine proteas. This protein is also associated with two types of amyloid disorders. First one is hereditarycystatine C amyloid angiopathy (HCCAA), which is related to the L68Q mutation and it is causing brianhemorrages [1]. The second disorder is connected with deposition of amyloid *β*-fibrils where the wild-type cystatin C is present as co-precipitant [2,3].

The aim of our studies was the spectroscopic characterisation of the native and mutated forms of human cystatin C in solution.Particullary mutants V57N, V57P, V57G, V57D and L68Q were tested. The secondary structure content in the broad range of temperatures, in solution was evaluated on the basis of Fourier transformed infrared spectroscopy (FTIR) and circular dichroism spectroscopy (CD). Additionally the overall fluerescence and dynamic light scattering (DLS) was also recorded.

Fourier self–deconvolution procedure was used to assign all the components of the Amid-I band to particular secondary structures [4]. Obtained data was compared with CD-based percentage content of secondaty structures, for which spectra were recoreded in temperature range from 5 to 70 ºC.The obtained results have shown that there are differences in content of secondary structures between wild type of HCC and its mutants. For example the L68Q mutant contain the most percentages of *β*-sheets of all tested proteins wheras V57P mutant has the richest *α*-helix content.

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Hydrogen migration in formation of NH($A^3\Pi$) radicals in photodissociations of isoxazole and pyridine molecules

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Absorption of radiation by hydrocarbon molecules can initiate their isomerization that usually provokes extensive deformation of molecular structure and reorganization of chemical bonds. In this rearrangement, hydrogen migration plays an important role, because a hydrogen atom or proton migrates from one site to another within a molecule [1] what may lead to opening of new reaction channels, frequently including dissociation [2-5]. The hydrogen movement occurs typically on a femtosecond time scale [3] and is faster than the molecular bond breaking in dissociation. It can therefore control chemical-bond-breaking and new bond-forming processes in the biological radiation damage [5], combustion [6], or catalytic studies [7].

In this context, the five and six-membered heterocyclic hydrocarbons are ideal candidates to characterize the hydrogen migration mechanisms in their dissociation. Studies of these fundamental molecular processes are important, especially from the viewpoint of the DNA helix damage by the ionizing radiation. In particular, the five-membered ring of isoxazole molecule (Figure 1) may be discerned in the deoxyribose sugar of DNA. On the other hand, pyridine, the six-membered heterocyclic molecule (Figure 2), may be recognized in the nucleic bases, adenine and guanine.

In the present study, the H atom migration was observed in the photodissociation processes of the isoxazole and pyridine molecules in the gas-phase, applying the photon-induced fluorescence spectroscopy (PIFS) [8]. The measured fluorescence emission spectra revealed the excited NH($A^3\Pi$) radicals detected by observation of their $A^3\Pi \rightarrow X^3\Sigma^-$ bands (Figure 1) together with emission from the CH, CN and H excited dissociation fragments [9, 10]. Neither isoxazole nor pyridine molecules contain structural units built on NH group. Thus, observation of the NH($A^3\Pi \rightarrow X^3\Sigma^-$) fluorescence gives a clear evidence of the hydrogen migration prior to the photodissociation.

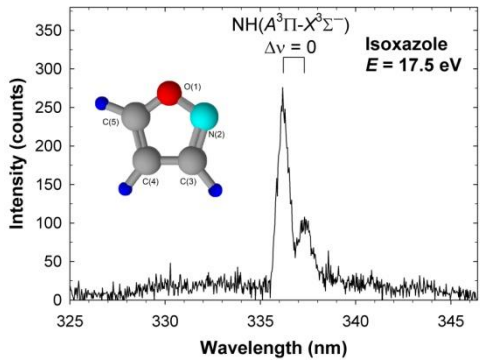
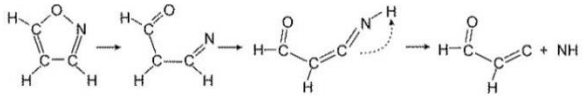


Figure 1. Emission spectrum of the isoxazole molecules showing the NH($A^3\Pi \rightarrow X^3\Sigma^-$) bands.

In the studies of the photodissociation of isoxazole molecule we have performed the density functional and *ab initio* quantum chemical calculations [5] to propose the mechanism of hydrogen relocation and formation of the NH fragment:



The photon energy dependences of the measured fragmentation yields show that the highly excited states, the super-excited states, of the target molecules produced in the photon absorption are intermediate states in fragmentation. The fluorescence yields of the excited NH($A^3\Pi$) and H(n=5) fragments measured in pyridine in the 16-70 eV region (Figure 2) show pronounced excitation bands that rise at about 10 eV above the first ionization potential of pyridine (9.26 eV). The dissociation of the super-excited states proceeds by opening of the molecular ring and breakup of the molecular chain with formation of several excited fragmentation products.

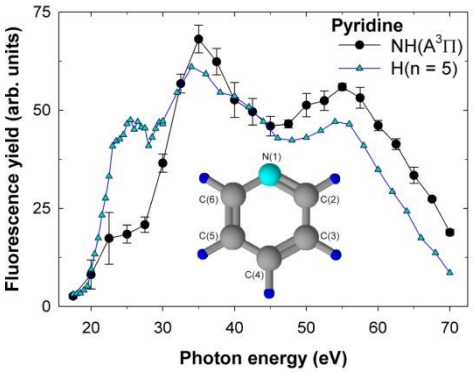


Figure 2. The NH($A^3\Pi$) and H(n=5) fragmentation yields obtained in the 16-70 eV photon energy range.

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