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Ion induced fragmentation of biomolecular systems at low collision energies

V Bernigaud[1], B Manil[2], L Adoui[1], J Y Chesnel[1], J Rangama[1], B A Huber[1], F Alvarado[3], S Bari[3], R Hoekstra[3], J Postma[3], and T Schlathölter[3]

[1] Centre de Recherche sur les Ions, les Matériaux et la Photonique (CIMAP), CEA/CNRS/ENSICAEN/UCBN BP 5133, bd. Henri Becquerel, 14070 Caen Cedex 05, France
[2] Laboratoire de Physique des Lasers, CNRS - UMR7538, Université Paris 13 - Institut Galilée - 99, avenue Jean-Baptiste Clément, 93 430 Villetaneuse, France
[3] KVI Atomic Physics, University of Groningen Zernikelaan 25, 9747AA Groningen, The Netherlands

E-mail: bernigaud@ganil.fr

Abstract. In this paper, we present results of different collision experiments between multiply charged ions at low collision energies (in the keV-region) and biomolecular systems. This kind of interaction allows to remove electrons form the biomolecule without transferring a large amount of vibrational excitation energy. Nevertheless, following the ionization of the target, fragmentation of biomolecular species may occur. It is the main objective of this work to study the physical processes involved in the dissociation of highly electronically excited systems. In order to elucidate the intrinsic properties of certain biomolecules (porphyrins and amino acids) we have performed experiments in the gas phase with isolated systems. The obtained results demonstrate the high stability of porphyrins after electron removal. Furthermore, a dependence of the fragmentation pattern produced by multiply charged ions on the isomeric structure of the alanine molecule has been shown. By considering the presence of other surrounding biomolecules (clusters of nucleobases), a strong influence of the environment of the biomolecule on the fragmentation channels and their modification, has been clearly proven. This result is explained, in the thymine and uracil case, by the formation of hydrogen bonds between O and H atoms, which is known to favor planar cluster geometries.

1. Introduction

Collisions with multiply charged ions are a sensitive tool to study the fragmentation patterns of complex systems like biomolecules. Depending on the projectile charge and its kinetic energy, which drive the interaction, many electrons can be transferred in a single collision as well as a certain amount of excitation energy. This energy is initially transferred mainly as electronic excitation energy, avoiding the direct production of vibrationally excited biomolecular systems. On a longer time scale, however, due to the electron phonon coupling the transferred energy ends up essentially in vibrational excitation of the molecular degrees of freedom, possibly leading to the dissociation of the molecule. An increasing number of gas phase studies devoted to the fragmentation of biomolecules induced by ion collisions have been performed since several years. In the context of biomolecular radiation damage, high energy primary ions loose energy during the interaction with biological tissues and slow secondary electrons and ions are formed. It has

been shown that these particles might induce further damage because they may have energies as high as 100 eV [1]. In order to better understand the biological radiation damage on a molecular level, but also, on a more general scale, to study the fundamental aspects of complex system fragmentation in more detail, it is necessary to quantify excitation, ionization and fragmentation cross sections, to identify the fragmentation dynamics as well as to determine the kinetic energies of the recoil ions. Such studies can be performed in the gas phase allowing for the application of mass spectrometric methods which also may be combined with coincident particle detection techniques [2]. Thus, mass spectrometry involving multiply charged ions is a straightforward technique to analyze fragmentation processes of complex systems (pigments, DNA/RNA building blocks and amino acids for instance).

In a first part of this paper, we will focus on results obtained with isolated neutral biomolecules. We present experiments concerning the multi-ionization and ion induced fragmentation of tetraphenyl iron(III) porphyrin chloride [3]. Fragmentation mechanisms will be discussed in terms of lifetime and charge mobility. In a second example, we will report the interaction of slow multiply charged ions with alanine, which is the only amino acid naturally occurring in two different isomers (called α- and β-alanine). The comparison of the fragmentation spectra from both isomeric forms allows to study the structural sensitivity of biomolecular fragmentation pathways induced by multiply charged ions [4].

In relation with biological radiation damage, the relevance of these studies with isolated molecules in the gas phase is often questioned, because they do not take into account the influence of the natural molecular environment. The so-called environmental effects, i.e. the modification of the fragmentation pattern of an isolated molecule when embedded in a chemical or aqueous environment will be discussed in a second part. We will exclusively focus on the formation of neutral clusters of small biomolecules (nucleobases) and on the fragmentation spectrum after ion irradiation, allowing a comparison between isolated biomolecules and clusters of biomolecules [5]. The cluster approach can be seen as a first step towards molecules surrounded by a more realistic biochemical environment.

2. Experimental set-ups

Two different devices have been used in these fragmentation studies of isolated biomolecules and clusters of biomolecules. Details of these experimental set-ups are given elsewhere [6, 7]. In brief, porphyrin and nucleobases clusters have been produced at CIMAP/GANIL in Caen (France), whereas fragmentation spectra of alanine and isolated nucleobase molecules have been obtained at the KVI in Groningen (The Netherlands). In both cases, keV multiply charged ions were extracted from electron cyclotron resonance ion sources. Concerning the production technique of biomolecular species, isolated targets were produced by evaporation from a powder sample in a stainless steel oven (temperature between 120 °C and 220 °C) and neutral nucleobase clusters in a cluster-aggregation source. Aggregation of the biomolecular vapor occurred at liquid nitrogen temperature in a condensation channel under He atmosphere (pressure in the mbar range). In these experiments, biomolecules or clusters of biomolecules were crossed with a beam of highly charged ions and the charged collision products were extracted from the interaction chamber by a pulsed extraction field. They are analyzed with a time-of-flight (TOF) mass spectrometer (Wiley-McLaren type in Caen, and reflectron type in Groningen) and detected with the aid of a channel plate detector. The signals were registered by a multi-hit capable acquisition system permitting to measure the TOF of one or several positively charged collision products from each collision event in coincidence.

3. Results and discussion

3.1. Fragmentation of isolated molecules

3.1.1. FeTPPCl porphyrin molecules The porphyrin molecule plays an important role as a natural pigment in many biological processes [8]. Many of these natural tetrapyrrolic pigments are similar in their geometrical structure, they differ only in the type of the metal centre and the side groups attached to the porphyrin ring. Thus, when iron, magnesium or cobalt represent the metal centre, the pigments play an important role in heme, chlorophyll and vitamin B12, respectively. In addition, porphyrins find many useful applications as biomarkers or for diagnostic purposes. The tetraphenyl porphyrin molecules, in the present case Tetraphenyl Iron III Porphyrin Chloride (FeTPPCl), are very attractive systems to be studied due to their chromophore structure and high molecular symmetry, and can be considered as model systems for fragmentation experiments as well as for theoretical descriptions. The geometry of the molecule is shown in Figure 1.

In Figure 2, we show a typical mass spectrum of FeTPPCl, when ionized in collisions with

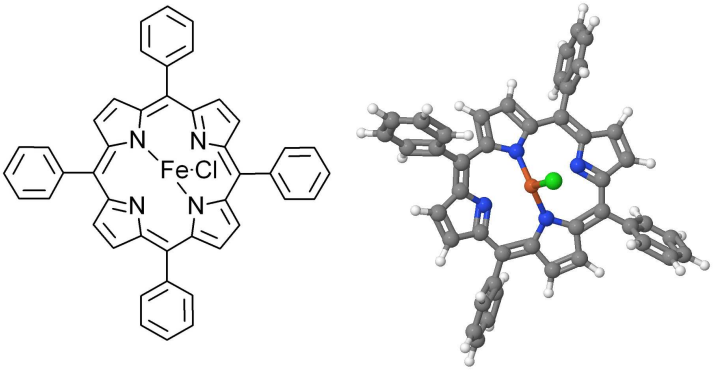

Figure 1. Molecular structure of FeTPPCl ($C_{44}H_{28}ClFeN_4$).

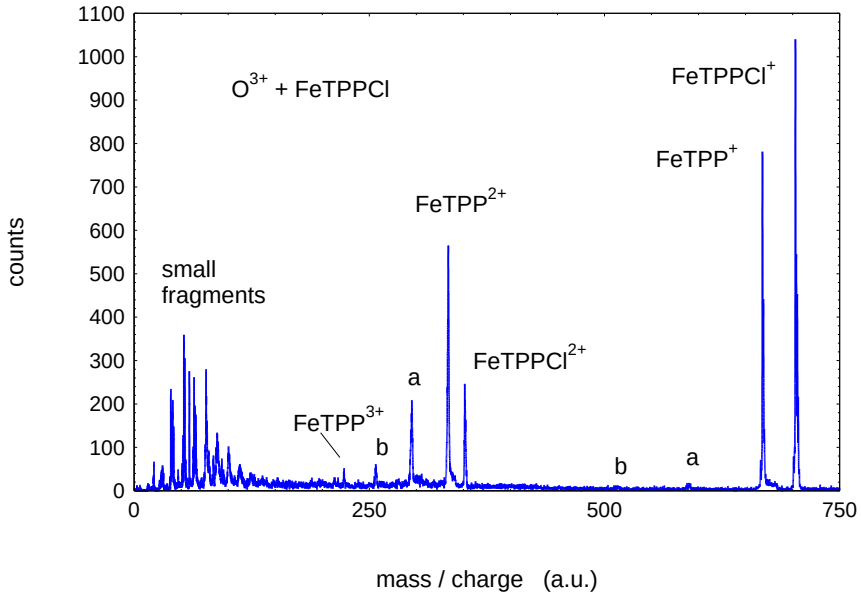

Figure 2. Fragmentation spectrum obtained in 30 keV-collisions of O^{3+} with FeTPPCl molecules. The peaks labeled a and b correspond to the loss of 1 and 2 phenyl groups with the Cl-atom, respectively.

O^{3+} ions at 30 keV. The singly charged intact molecule is found to be the most abundant one in contrast to experiments with electrons [9]. The intact molecule is observed in charge states up to $q = 4$. However, with increasing charge state the loss of the Cl atom becomes more and more important. Furthermore, it is evident that in addition to the loss of the Cl atom also the loss of 1 or 2 phenyl groups (peaks labelled a and b) becomes increasingly important with the charge state of the ionised molecule. Finally, small fragments are observed which are due to a more complete break-up of the multiply ionised molecule.

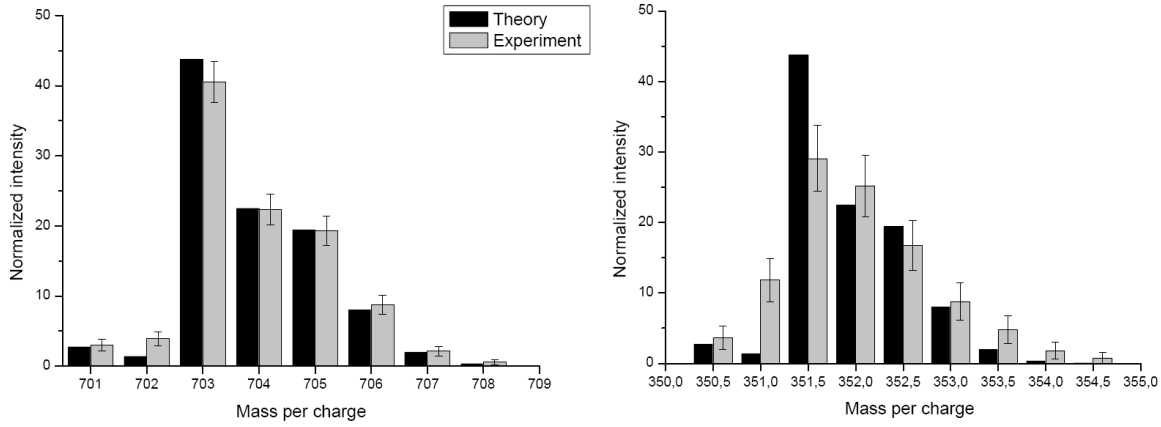

Figure 3. Isotopic distributions for singly (left) and doubly charged (right) $FeTPPCl^+$ ions. The data are extracted from the fragmentation spectrum shown in figure 2.

In Figure 3, we compare the experimentally measured fine structure of the intact molecular ion peak with the distribution obtained by taking into account the natural isotopic distribution of the elements contained in the molecule. Individual peaks have been deconvoluted and integrated. Whereas for the singly charged species $FeTPPCl^+$ (left) the general agreement between experiment and theory is rather good, in the case of the doubly charged molecule the measured intensity of the most prominent peak at $m/q = 351,5$ is strongly reduced in favour of the peak at $m/q = 351$. This indicates that one hydrogen atom is very likely to be emitted after single ionization. When the projectile charge is increased, up to 6 H-units (or 3 H_2 molecules) may be lost in a single electron capture process. The loss of a pair number of H-atoms is more likely, than of an odd number. This finding is in good agreement with observations made by electron impact studies [9].

Due to the large number of degrees of freedom, energy can be stored for a long time in the molecule before finally leading to its dissociation. When regarding the observed peak forms more precisely, for some of the fragments we find a tail towards longer drift times, as to be seen in Figure 4 for the doubly charged ion $FeTPP^{2+}$. These contributions are due to a decay which occurs within the extraction region, i.e. on a time scale of the order of several microseconds. The first shoulder in Figure 4 can be attributed to the following decay process:

$$FeTPPCl^{2+} \rightarrow FeTPP^{2+} + Cl \tag{1}$$

The observed shoulder ends at that time which one calculates for the case, that the process 1 occurs at the end of the first electric field of the extraction region of the Wiley-McLaren TOF-system. The time spent in the second field is much shorter and yields a much lower signal with a time window which will reach out to the peak of the intact doubly charged molecular ion. A second delayed decay process is observed, which is characterised by a much larger shoulder towards longer drift times. It is attributed to the delayed electron emission by singly charged

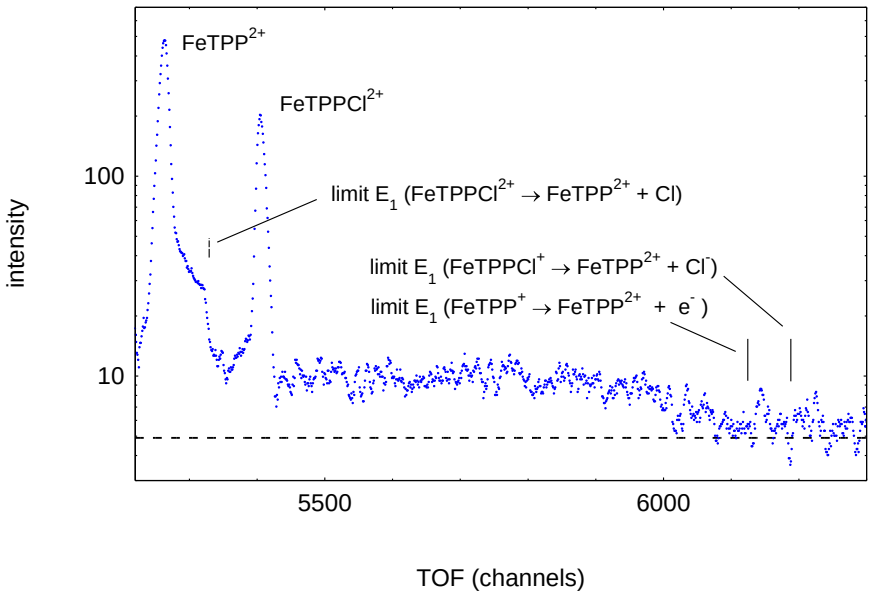

Figure 4. Mass spectrum range showing the tail structure of the dicationic $FeTPP^{2+}$ molecule. The horizontal dashed line indicates the background level.

molecules which have already lost the Cl-atom before 2, or to the direct emission of a Cl^- anion (reaction 3) :

$$FeTPPCl^+ \rightarrow FeTPP^+ + Cl \qquad \text{and} \qquad FeTPP^+ \rightarrow FeTPP^{2+} + e^- \tag{2}$$

or

$$FeTPPCl^+ \rightarrow FeTPP^{2+} + Cl^- \tag{3}$$

These processes are surprising as a singly charged system emits a negative particle, thus increasing its own charge. Due to the low statistics, the slightly different limits indicated in Figure 4 cannot be used to distinguish between both processes. However, from an energetic point of view the reaction 2, corresponding to the delayed electron emission by an ion, should be favored.

3.1.2. α and β-alanine molecules In a second experiment, the interaction of keV He^{2+} and O^{5+} ions with isolated α and β isomers of the alanine amino acid (see Figure 5) has been studied [4]. A comparison of the fragmentation spectra for both isomers is shown in Figure 6, produced in collisions with He^{2+} at 40 keV. Firstly, we note that the intact charged molecule is not visible for α-alanine and is very weak also for β-alanine. Thus, nondissociative ionization is rather unlikely for this molecule, in constrast to the case of the FeTPPCl porphyrin molecule (see Figure 2), as discussed before. The most striking difference between both spectra is found for the dominant fragment cations. For α-alanine, the dominant products are H^+ ($m/q = 1$) and more particularly $NH_2CH_3CH^+$ (labeled B in Figure 6), which is formed by a single rupture of the C-C_α bond (see Figure 6). For β-alanine, the H^+ cations is the dominant fragment, but instead of $NH_2CH_3CH^+$, we observe the product $NH_2CH_2^+$ at $m/q = 30$ (referred to as A in Figure 6). This fragment is formed by the cleavage of the C_α-C_β bond. Cation A is almost absent for α-alanine, whereas fragment B is almost absent for β-alanine. This observation is also confirmed by experiments with O^{5+} ions and indicates a strong isomer dependence of characteristic fragmentation channels

leading to strongly altered branching ratios. Another aspect of this study concerns the kinetic energy of atomic fragments (H^+, N^+, O^+ and C^+) formed after the collision with multiply charged ions. These more in depth information on the fragmentation dynamics was obtained from the two-particle correlation plots. Fragment ion kinetic energies were found to exceed 6 eV (He^{2+} impact) and 15 eV (O^{5+} impact). In the context of radiation damages, these high values imply that the interaction between ions and proteins might produce sufficiently energetic secondary ions to provoke further damage to neighboring DNA.

Nevertheless, as already mentioned above, these conclusions should be taken with care, because the influence of the environment of the biomolecule is not taken into account in such studies.

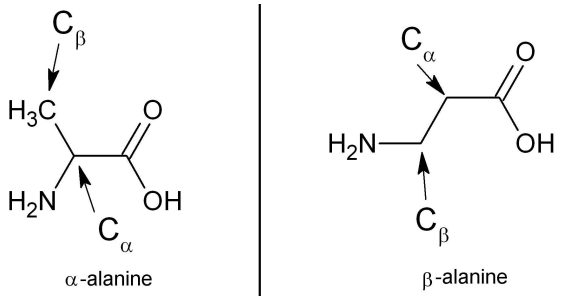

Figure 5. Molecular structure of α- and β-alanine (position isomery).

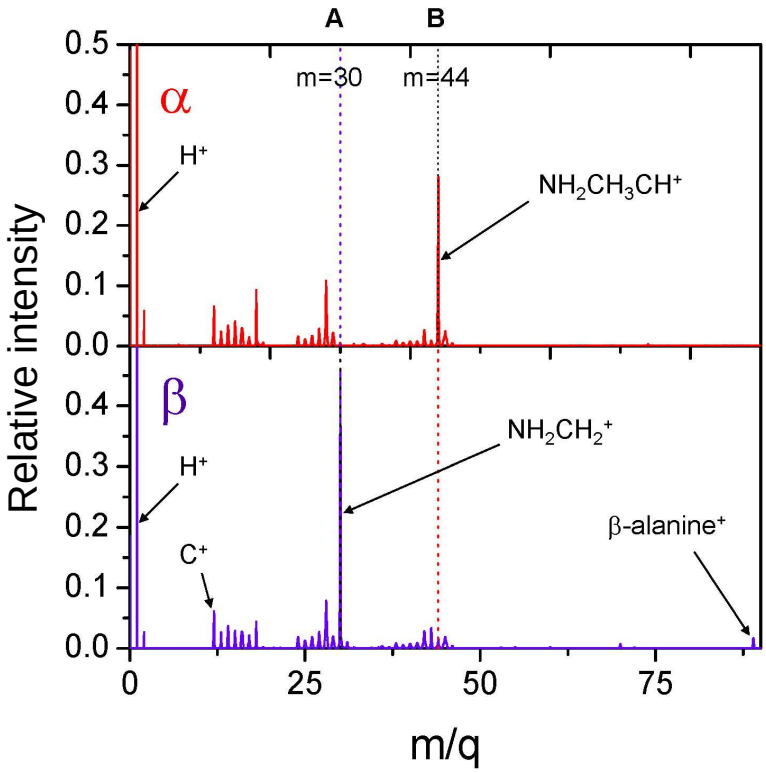

Figure 6. Mass spectra of product ions from 40 keV He^{2+} collisions with α- (top) and β-alanine (bottom). A and B indicate the fragments $NH_2CH_3CH^+$ and $NH_2CH_2^+$, formed by cleavage of the C-C$_\alpha$ and C$_\alpha$-C$_\beta$ bond in α- and β-alanine, respectively.

3.2. Fragmentation of nucleobases clusters

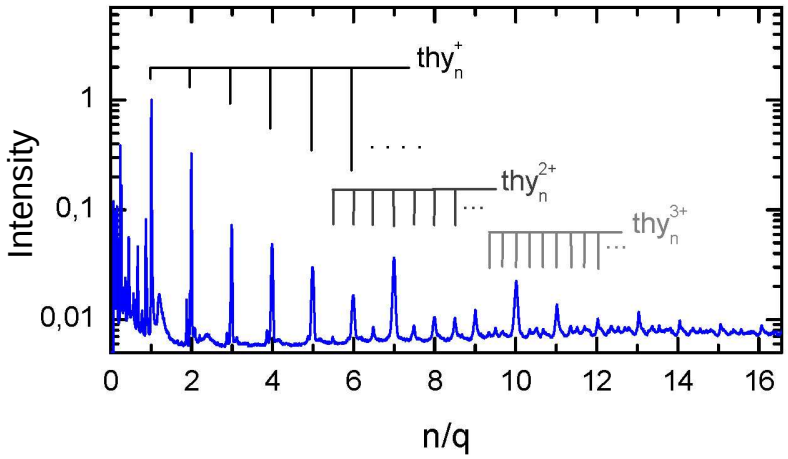

Figure 7. Mass spectrum of thymine clusters, ionised in collisions with O^{5+} projectiles at 50 keV. Vertical lines indicate the position of multiply charged clusters in charge states up to 3.

In order to analyse the influence of a chemical environment, we compared, in a first step, the interaction of keV ions with isolated nucleobases and clusters of nucleobases. In Figure 7, we show a typical charged cluster distribution of thymine molecules formed after ionization by O^{5+} at 50 keV. The spectrum exhibits singly, doubly and even triply charged clusters. A detailed analysis allows to identify structures in the spectrum (magic numbers) and to determine appearance sizes as a function of the charge state.

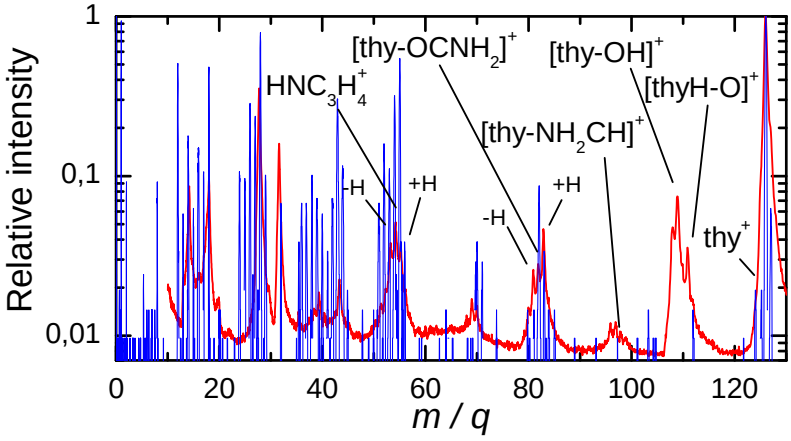

Figure 8. Mass spectra measured in O^{5+} thymine collisions. Blue spectrum, measured with higher resolution, corresponds to the isolated thymine molecule. The red curve (lower resolution) is obtained with thymine clusters as target. In the cluster case, new fragmentation channels are opened characterized by the loss of an O-atom, an OH-group or a NH_2CH molecule.

In Figure 8, we compare the fragmentation spectra for isolated molecules, obtained at the KVI-facility, with those for the corresponding cluster system. From this comparison, it becomes clear that new channels open up in the cluster case which correspond to the formation of nucleobases which have lost an OH group. This new channel can be explained by intermolecular

hydrogen bonds between different cluster constituents which form most likely planar systems (see Figure 9). These bonds in turn weaken the intramolecular bonds to O and H atoms. Thus, when embedded in an ensemble of other biomolecules, the fragmentation pattern changes and new pathways open up, a behaviour which one might expect also in a natural environment.

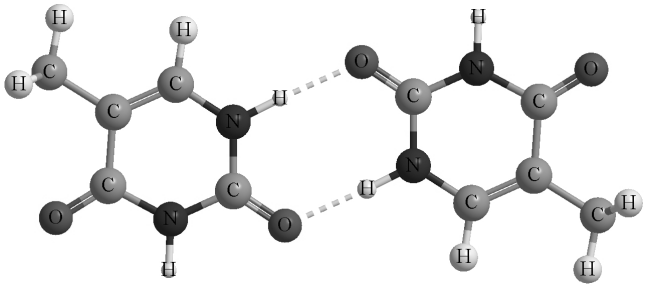

Figure 9. Geometry of the most stable thy_2 dimer. Hydrogen bonds are indicated by dotted lines.

4. Conclusion

Low energy ions colliding with molecules of biological interest may create severe damage, also at rather low collision energies as demonstrated in the gas phase experiment with FeTPPCl molecules. Multi-ionisation has been observed up to $q = 4$, however, only for $q = 1$ the intact molecular ion is the most intense species. For higher charge states most likely evaporation of the Cl-atom or phenyl rings is observed. These evaporation processes may occur on a subnanosecond timescale or in the μs range, depending on the internal energy. Surprisingly, also the processes of delayed electron emission by a positive ion (or to a lower extend the emission of Cl− ions) are observed, increasing the charge state of the residual ion.

Furthermore, we have observed a pronounced isomer dependence of this multiply charged ion induced fragmentation of isolated alanine molecules. Depending on the molecule geometry, very different fragmentation pattern are measured. In addition, the large kinetic energies of the produced fragments demonstrate their potential for further damaging neighboring molecules.

Finally, a comparative study of the fragmentation of isolated molecules and those embedded in surrounding clusters cleary shows the openening of new fragmentation channels which are due to the cleavage of intermolecular bondings, which are formed in planar clusters of nucleobases.

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