Photoabsorption of biomolecules and radiation damage – studies in adenine films

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Abstract. The Layer-by-Layer (LbL) technique has recently been developed as a promising method for production of thin films functional molecular heterostructures since the interactions occurring, essentially ionic and hydrogen bonding patterns, are found to be identical to those observed in biological systems. Such films have been shown to be also potentially good mimics of biological membranes. Also, it is possible that a study of biological relevant molecules assembled in LbL films will provide a closer analogue to their role in cellular systems. Thin films of adenine (A) and the polyelectrolyte poly(vinylsulfonic acid sodium salt) (PVS), were prepared by cast and Layer-by-layer (LbL) techniques. In this article, the experimental results on the UV irradiation of adenine cast films are described and the effect of 140 nm irradiation, with an estimated dose of about $8.5 \times 10^{-4}$ W/m$^2$, is evaluated at the molecular level.

Introduction

Synchrotron radiation (SR) has been used for studying photo-induced processes in research areas such as physics, chemistry, astronomy, biology and medicine, among many others. Properties such as high intensity, broad spectral range, high degree of polarization and collimation, make SR a powerful tool for basic and applied research [1]. Since the UV1 beam line at the ASTRID synchrotron light source at the Institute for Storage Ring Facilities (ISA), University of Aarhus, Denmark became available at the end of 2001, we have been studying the Vacuum UltraViolet (VUV) spectroscopy of a wide variety of molecular targets, including aeronomic molecules contributing to global warming and ozone depletion [2]. VUV photoabsorption investigations of several biomolecular targets in the gas phase have also been studied (see e.g. ref. [3, 4]), providing results on the electronic state spectroscopy of these molecular systems. Although a few experiments have been carried out on the effects of radiation on key biological molecular targets, the knowledge of the photoabsorption processes is also extremely necessary to evaluate the role of these molecular systems in physiological environments. In order to assess the risks from radiation damage and to model the effect of radiation on cellular material, a comprehensive understanding of the underlying interactions between the primary radiation (e.g. UV photons) and the biomolecules (e.g., DNA and its constituents) is required. This may, in turn,

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provide information about the molecular pathways that lead from initial deposition of radiative energy to the formation of irreversible biomaterial damage.

Recently the effects of radiation on DNA and other biomaterials have been shown to be a consequence of local damage at the molecular level, which means, site specific reactions in the nucleotide’s bases [5]. The early stages after irradiation (10^{-16} – 10^{-9} s) in the chronology of radiation damage have been well established to be physical and physical-chemistry in nature, which in turn means that the underlying mechanisms can be identified and described at the molecular level. Therefore, understanding mutagenesis as a consequence of radiation damage depends on the detailed knowledge of the spectroscopy and dissociation dynamics of key components in certain initiation reactions and/or of the biomolecular environment constituents [6].

Gas phase experiments often do not represent the medium in which photon interactions occur within the physiological environment. In order to gain some insight into how the spectroscopy and dissociation dynamics of certain molecules (e.g., water) is influenced by its environment, we have started a series of experimental measurements in both liquid and condensed phases [7]. The layer-by-layer (LbL) film technique has recently been developed as a promising method for production of functional molecular heterostructures [8, 9] by alternated adsorption at solid/liquid interface of opposite charged polyelectrolytes, where the interactions occurring are essentially ionic and the hydrogen bonding patterns [10 – 13] are found to be the same as those observed in biological systems. In addition such films are also potentially good mimics of biological membranes [14, 15]. Therefore, it is possible that a study of the presence of biological relevant molecules assembled in LbL films will provide a closer analogue to their role in cellular systems [16, 17]. Recent studies of the UV radiation effect on cast DNA, where films have been prepared by spreading the material solution into a solid substrate and allowing the solvent to evaporate, and LbL films, revealed an increase in the absorbance band intensity centred at 190–200 nm [18]. This band is associated with the electronic transitions of the nucleic bases. In order to infer on the transitions induced by UV radiation and the specific molecular site where damage is taking place, further parallel studies into solid films of nucleic bases were carried out. In this article, the experimental results on the UV irradiation of adenine cast films are described. Taking into account the recent experimental results of Gomes et al. [18], showing that the presence of water molecules plays an important role in DNA damage, efforts have been done to obtain adenine layer-by-layer films in order to control the amount of water molecules present in these films. The conditions required to build adenine LbL molecular films are also addressed here.

Experimental set-up

2.1 Condensed phase measurements

The set-up used for film samples characterisation in the VUV photoabsorption region consisted of a vacuum chamber containing a holder which supports up to three CaF\(_2\) sample discs and one reference disk mounted on a MDC SBLM-266-4 push-pull linear motion. Synchrotron radiation passes through the sample and a photomultiplier was used to measure the transmitted light intensity. The incident wavelength was selected using a toroidal dispersion grating with 2000 lines/mm providing a resolution of \(\sim 0.075\) nm at FWHM. In both type of samples, the UV beam light passes through the samples and the transmitted intensity, \(I_t\), was measured at 1.0 nm intervals. For wavelengths below 200 nm, a flow of He gas is flushed through the small gap between the photomultiplier and the exit window of the gas cell to prevent any absorption by air contributing to the spectrum. The minimum and maximum wavelengths between which scans are performed, 115 – 320 nm (10.8 – 3.9 eV), are determined by the windows of the gas cell (LiF entrance and CaF\(_2\) exit) and the grating, respectively. The synchrotron beam ring current is monitored throughout the collection of each spectrum in order that spectra can be corrected for any changes in incident photon flux during the period of spectral accumulation. Each sample measurement was accompanied by a background scan recorded with the reference disk. The apparatus is calibrated using O\(_2\) and SO\(_2\). The Schuman-Rünge absorption band of O\(_2\) (6.9 eV – 9.5 eV) [19] is used to calibrate the absolute cross section because its broad nature minimises the effect of any changes in energy resolution. SO\(_2\) is used to calibrate the energy scale as it has absorption bands
with clearly defined sets of sharp absorption peaks in the ranges from 3.8 eV to 5.1 eV [20] and from 5.15 eV to 7.25 eV [21].

2.2 Films preparation

Films were obtained from adenine (A) and the polyelectrolyte poly(vinylsulfonic acid sodium salt) (PVS), both from Sigma-Aldrich. Aqueous solutions were prepared dissolving these materials in ultrapure water with 18 MΩcm resistivity supplied by a Millipore system (Milli-Q, Millipore GmbH). The polyelectrolyte monomer concentration was estimated to be 10^{-2} M and for adenine a concentration of 7×10^{-3} M.

Cast films were prepared by spreading the aqueous solutions of adenine into CaF_2 substrates. The films were led resting for a 24 hours period in order to allow water to evaporate. The substrates have been hydrophilized in a H_2SO_4/H_2O_2 (7:3) solution for 10 minutes previously to film deposition.

Layer-by-layer (LbL) films were also prepared from these materials and its production comprised the following steps: i) immersion of the substrate in a cationic solution (adenine) during 5 seconds; ii) substrate + cationic macromolecule layer washing in an aqueous solution with the same pH of the cationic solution; iii) immersion of the substrate + cationic macromolecule layer into the anionic solution (PVS) during 5 seconds; iv) substrate + cationic macromolecule/anionic macromolecule bilayer washing with an aqueous solution with the same pH of the anionic solution. By repeating steps i) to iv), a large number of bilayers can be deposited. In figure 1 the procedure sequence for production of layer-by-layer films is schematically shown.

Cast and LbL films were prepared and characterised with VUV synchrotron radiation. Cast films were irradiated with UV 140 nm wavelength with an estimated dose of about 8.5 × 10^{-4} W/m^2.

![Figure 1. Schematic sequence of layer-by-layer (LBL) technique for membranes’ production.](image-url)
Results

As it was already mentioned, recent studies of ultraviolet irradiation of DNA cast and LBL films showed an increase of the absorbance band centred at 190–200 nm [18]. This band has been associated with the adenine absorption peaks at 207 nm (5.90 eV) and at 179 nm (6.80 eV) and to the thymine absorption peaks at 208 nm (5.86 eV) and 173.5 nm (7.04 eV) [22], corresponding to ($\pi \rightarrow \pi^*$) transitions. In order to clarify the role of adenine as far as DNA damage is concerned, studies on the UV irradiation of adenine films were performed. Figure 2 shows the VUV absorption spectra of a cast adenine sample after and before irradiation at 140 nm with an estimated dose of about $8.5 \times 10^{-4}$ W/m$^2$.

![Figure 2. VUV absorbance spectra of a cast adenine film after and before irradiation at 140 nm with an estimated UV dose of about $8.5 \times 10^{-4}$ W/m$^2$.](image)

These spectra show that the irradiation has a strong influence in the molecule’s electronic states since some of the intensity peaks are changed. In order to quantify the effects of radiation, spectral bands were fitted to Gaussian curves and the positions of the bands were compared with the literature available data [23 – 26]. Table 1 shows the calculated and relative peak areas before and after irradiation. The relative peak areas are obtained dividing the peak area by the total spectrum area without baseline. From table 1, it is noticeable the same proportion of decrease in the peak at 236 nm to the increase of the peak at 218 nm. This can be closely related to intramolecular rearrangement leading to change in the molecule’s conformation, which can be attributed to the rupture of $C_4=C_5$ bond. We also noticed a significant decrease in the structure at around 236 nm but can only resume ourselves to some speculation on the change observed based on the previous assumptions. Therefore, our next investigations are to perform FTIR spectroscopy measurements in order to evaluate which sort of bond (and/or bonds) might be affected and which are probably being formed.
Table 1. Adenine absorption features, assignments, peak areas and relative peak areas after and before irradiation.

| λ (nm) | ΔE (eV) | Assignment [23] | Assignment [26] | Peak Area before irradiation | Peak Area after irradiation | Area deviation | Relative Peak Area before irradiation | Relative Peak Area after irradiation |
|--------|---------|----------------|-----------------|-----------------------------|-----------------------------|---------------|---------------------------------|----------------------------------|
| 266    | 4.66    | 3 1A'           | S₁(n→π*)        | 0.30 ± 0.08                | 0.38 ± 0.04                | 0.08±0.12     | 0.17                            | 0.19                             |
| 236    | 5.25    | ---             | S₂(π→π*)        | 0.54 ± 0.07                | 0.16 ± 0.06                | 0.38±0.13     | 0.31                            | 0.08                             |
| 218    | 5.69    | 4 1A'           | S₄(n→Ryd)       | 0.23 ± 0.08                | 0.57 ± 0.08                | 0.34±0.16     | 0.13                            | 0.29                             |
| 202    | 6.17    | 5 1A'           | S₅(n→Ryd)       | 0.38 ± 0.05                | 0.52 ± 0.06                | 0.14±0.11     | 0.21                            | 0.26                             |
| 190    | 6.53    | 6 1A'           | S₆(n→π*)        | 0.32 ± 0.07                | 0.35 ± 0.04                | 0±0.11        | 0.18                            | 0.18                             |

The preparation of LBL of nucleic bases films is not a straightforward procedure, mainly because they are small neutral molecules, much smaller and less charged than normally used polyelectrolytes, which makes adsorption from solution difficult [9]. Concerning the interactions accounting for molecules adsorption, ionic and secondary interactions such as hydrogen bonding and hydrophobic interactions have been reported [10 – 13] and a classification of the various types of LBL films in terms of mechanisms responsible for adsorption, has been proposed by Oliveira et al. [24]. Recently, Marletta and co-workers [27] have shown that small molecules can be used in the production of LBL films. Changing the nucleic bases pH solution, molecules can acquire electrical charge allowing its adsorption at solid/liquid interface to take place. Layer-by-layer films of adenine alternated with PVS were successfully prepared from aqueous solutions at different pHs between 2 and 6. At pH = 3 adenine acquires positive electrical charge and LBL films can be obtained in agreement with the results of Figure 3 where we show that absorbance at 257 nm versus the number of bilayers increases linearly, therefore indicating that LBL films were successfully produced. At pH = 2, adenine continues electrically charged but its solubility in aqueous solutions is seen to increase which points that no LBL films can be produced.

Figure 3. Absorbance at a fixed wavelength as a function of the number of bilayers for LbL films of adenine/PVS at pH=3.
Conclusions

Synchrotron radiation should be used as an important source for future investigations of biological relevant molecules. In this article it was shown that the UV radiation induces changes in the adenine molecule covalent bonds and that VUV spectroscopy can be used to detect the induced changes and to some extent localize the damage. Moreover, it can also be used to monitor the adenine adsorbed amount during the formation of LbL films. In the present article, it was demonstrated for the first time the conditions that leads to the formation of adenine LbL films as a function of the pH solution.

Future directions

The main goal at short/medium term is the understanding of the processes which occur when well controlled membranes are exposed to radiation. For that, biological membranes and interfaces can be simulated by sequential layers obtained by the layer-by-layer technique using common polyelectrolytes, functional polymers and biological molecules as a result of alternated adsorption from aqueous solutions of molecular species having opposite electrical charges. In order to infer information about the membrane build up mechanism, it will be necessary to characterise the adsorption kinetics of the biological layers and the formation of self organized sequential layers. Since the adsorption process comes from solution, and greatly dependent on solution parameters, adsorption kinetics should be fully characterized in terms of solution concentration, pH, ionic strength and temperature, which also influence the film final structure and characteristics. Adsorption models of macromolecules should also be addressed to interpret the experimental results. Optimised membranes can be used as a mimic to study the effect of radiation damage in soft condensed biological materials when submitted to several radiation sources mainly X-rays, neutrons, electron beams, neutral particles beams and UV radiation both in a controlled environment and, to simulate the cell environment, in a solid/liquid interface. The description of radiation damage at the molecular level in these artificial (mimic) membranes can be performed by the traditional techniques of materials characterisation, particularly adapted for in situ measurements. In addition, the LBL technique allows the incorporation of radiation sensitive polymer layers in the biomimetic membrane during its building-up process, which in turn will allow to quantify the radiation damage with the real radiation dose. Therefore, tissue equivalent materials can be built using the LbL technique. However, as far as we are aware, no studies concerning this approach have been done until now, which in turn means that several experimental and theoretical investigations are usefully needed.

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