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Photo-absorption Studies of Chromophores at ELISA Storage Ring

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Abstract: Absorption of gas-phase biomolecules has been studied at the electrostatic ion storage ring ELISA. It is demonstrated that the storage ring technique with long storage times and suitable particle detection is ideal for studies of the photo-physics of chromophores. Here we discuss the absorption characteristics of the chromophores of the Green Fluorescent Protein (GFP) and compare our gas-phase absorption measurements with absorption profiles of the protein and chromophores in liquids. It is argued that the absorption characteristics of GFP are mainly ascribed to intrinsic chemical properties of the chromophore.

Key words: chromophore; protein; photo-absorption; storage-ring

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1 Introduction

In this communication we demonstrate how the electrostatic ion storage ring in Aarhus, ELISA[1] may be used to perform photo absorption measurements of bio-molecules. More specifically, we studied chromophores of the well known Green Fluorescent Protein (GFP), which is a relatively small protein whose natural function is to convert blue light to green light[2—5]. This single chain protein, consisting of 238 amino acids, has triggered a revolution in molecular biology since it may be fused into other proteins and by its special absorption and emission characteristics be used for continuous monitoring of gene expression and developments in living cells[2—5]. By substituting amino acids in the protein one can make new fluorescent proteins (mutants) with different absorption and emission characteristics. Naturally, the photo physics of these proteins has attracted a great deal of attention.

The absorption properties of these proteins are remarkable. The proteins exhibit strong absorption and the absorption spectra are broad. Moreover, the spectra of emission are significantly redshifted upon absorption, which forms the basis for many applications. To fully understand the photophysics of these proteins, several pieces of information must be collected. The electronic and conformational structures of the chromophore molecule in the ground and excited states are responsible for the initial absorption (wavelength and intensity). The response of the protein is given by an interplay between the photo excited chromophore and the environment in the protein. Typically, the interaction with the protein determines whether isomerization can take place or not. The surroundings in the protein can also deliver or take away protons from the chromophore and hence change the charge state and electronic structure of the chromophore.

Many studies of the photophysics of such biosys-

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tems are performed in solutions. It is, however, clear from observed shifts of the absorption maxima that the environment provided by the protein is not identical to that of aqueous solutions. To shed light on the intrinsic properties of chromophores, we have initiated a series of studies of gas phase chromophores. In the present communication we focus on the results obtained with the wild-type GFP chromophore. The chromophore of the native GFP is formed from three amino acids at residues 65—67, which are Ser-Tyr-Gly. We used the model chromophore shown in Fig. 1, which was also used in an experiment by Webber et al[6].

For several reasons, the ELISA storage ring, which is shown in Fig. 2, is ideal for studies of such heavy bio-molecular ions: First, ELISA is based on electrostatic storage and hence basically has no mass limitation. Second, it provides storage of seconds, which is needed to monitor the consequences of photo absorption which may proceed over a long time-scale. Third, photo products (photo-dissociation fragments) have kinetic energies of ~keV and may conveniently be detected by standard particle detectors. Finally, ions that were born hot in the ion source, or made hot by collisions during the acceleration and injection, will have time to cool down by infrared emission before a given measurement starts.

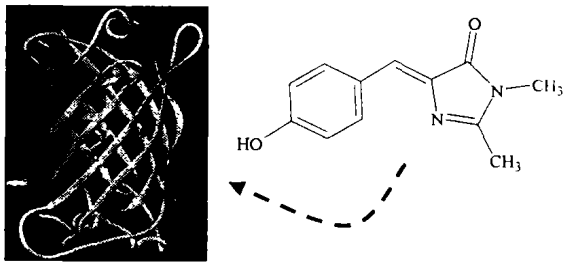

Fig. 1 The protein (GFP) and the structure of the neutral gas-phase GFP-model chromophore used in the present work. Anions are created when the proton is removed from the hydroxyl group and cations are created when a proton is attached to the imidazolidinone ring containing the two nitrogen atoms.

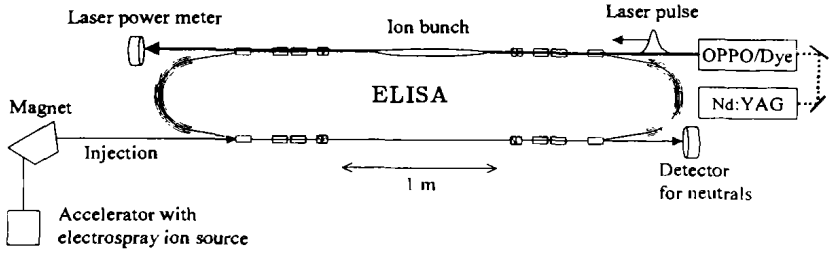

Fig. 2 The electrostatic ion storage ring ELISA equipped with an electrospray ion source, a pulsed laser and a detector for neutral products.

2 Experiment

An electrospray ion source was used for the production of biomolecules. The source, which is shown schematically in Fig. 3, was constructed and built recently in Aarhus[7]. In brief, it may be operated in a DC mode with typically 10^5 to 10^6 ions/s or in a pulsed mode with 10^3 to 10^4 ions in a bunch (repetition rate 10 Hz). Anions were formed by electrospraying a chromophore sample dissolved in an ammoniated water/methanol (1 : 1) solution ($pH = 9$). For cations we used a solution of water/methanol (1 : 1) with HCl ($pH = 3$). The mass spectra of the ion source were remarkably clean with an unambiguous peak at the correct mass of 215 amu for anions (deprotonated ions) and a peak at 217 amu for cations (protonated ions).

In the ion source, a cylindrical ion trap accumulated ions for 0.1 s before they were accelerated as an ion bunch to a kinetic energy of 22 keV. The ions were selected according to their mass to charge ratio by a magnet and injected into the storage ring. About 5 ms after injection, the ions were irradiated by a laser pulse of 3 ns duration in the straight section opposite the injection side. An OP-

PO (optical power parametric oscillator; Lambda Physik), pumped with the third harmonic of an Nd : YAG laser (Coherent) was used to create ~ mJ laser pulses in the region 430 — 630 nm. Frequency-doubled light from a dye laser (Lambda Physik), pumped by the second harmonic of the Nd : YAG laser, was used for wavelengths shorter than 430 nm. Tuning the laser wavelength to an absorption band resulted in the production of neutral particles after electron emission or bond dissociation. Neutrals formed in the straight section opposite the laser-interaction region were counted by a particle detector (see Fig. 2) and constituted the absorption signal.

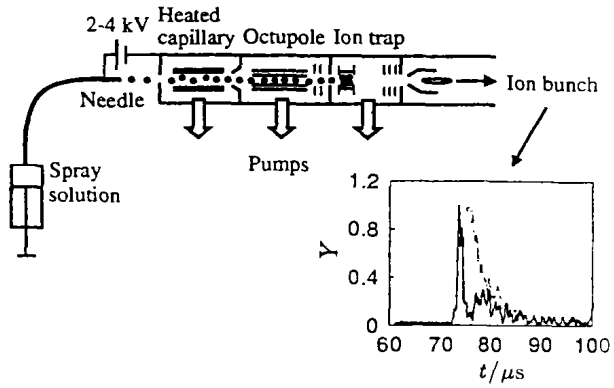

Fig. 3 The principle of the electrospray ion source. The inserted figure shows measured intensities as a function of time. Ion bunches of 2—5 μs were obtained with He as a buffer gas in the ion trap.

3 Results and discussion

The measured absorption bands are shown in Fig. 4, where we compare the absorption of the chromophore in three different media: The protein (GFP)(top)[2], in vacuo(middle)[8,9], and in aqueous solutions of different pH (bottom)[8,9]. The protein absorption spectrum shows the two usual absorption bands that are ascribed to a neutral chromophore in the protein (maximum at 395 nm) and a deprotonated chromophore (maximum at 477 nm).

The absorption spectrum of the gas phase chromophore anion reveals that the absorption band has a maximum at 479 nm. Thus, there is almost no difference between the absorption bands obtained with the protein and the chromophore in vacuo. It is seen that the absorption maximum for the anion form of the chromophore is shifted to 426 nm when recorded in an alkaline aqueous solution. This large shift may be explained by hydrogen bond interactions, which localize the active electrons of the chromophore in the anion form.

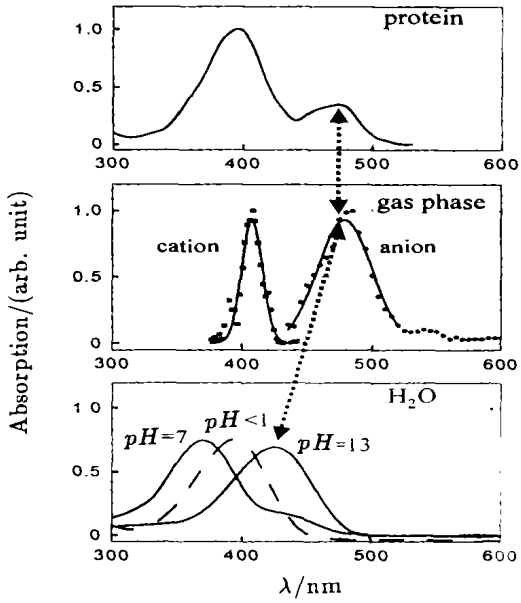

Fig. 4 Measured absorption bands in three different media, see text.

The similarity between the absorption band of the gaseous anion and the second absorption band of the protein is striking. In the protein, the chromophore is covalently attached to an α-helix that runs up the axis of a hollow cylinder formed by eleven β-strands[2,10]. Our results indicate that the actual environment of the chromophore inside the protein cavity is much closer to vacuum than to bulk solution. In other words, the electron delocalization in the Aequorea victoria GFP chromophore matches the one of the gaseous anion. This does not preclude, however, that the protein environment does affect the displayed photophysics. Indeed, the charge state of the chromophore prior to excitation is directly determined by its local environment of proton donating and proton accepting groups, which explains why the photophysics of a

single chromophore can differ significantly from protein to protein and between mutants[2—5].

The chromophore cation in the gas phase exhibits an absorption maximum at 406 nm. It is not possible to make direct comparisons with protein data as this chromophore charge state is not present in GFP. However, we may compare with the measurement performed in solutions. Here the absorption in the acidic solution ($pH<1$) has a maximum at 396 nm, i. e. a blueshift of only 10 nm with respect to the gas-phase case. It may be argued that the shift is significantly smaller for cations than for anions because of less electron delocalization for GFP-chromophore cations[8].

To summarize, a sensitive absorption technique for studies of gas-phase bio-molecules has been used by coupling an electrospray ion source with the ELISA storage ring. With this technique we studied the absorption characteristics of the GFP chromophore anion and cation. We present evidence that the exact location of the absorption band of the anionic form of the GFP chromophore is almost purely ascribed to the intrinsic chemical properties of the chromophore rather than to a result of interactions with amino acid side chains in its vicinity. Absorption measurements with model chromophores of mutated GFP proteins are currently being performed in our laboratory to investigate to what extent our findings apply to other systems.

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在 ELISA 储存环上研究成色基团的光吸收

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摘　要：在电子储存环 ELISA 上研究了气相生物分子的光吸收. 通过研究发现，储存时间长并具有合适的粒子探测系统的储存环是进行成色基团光物理研究的理想工具. 讨论了绿色荧光蛋白质(GFP)的光吸收特性，并将气相光吸收测量谱与液体中蛋白质和成色基团的吸收形貌进行了比较，认为 GFP 的吸收特性可以归因于成色基团的固有化学性质.

关 键 词：色基；蛋白质；光吸收特性；储存环