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Photolysis and photosensitized degradation of the diuretic drug acetazolamide

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Abstract

The phototoxic diuretic drug acetazolamide (**1**) is photolabile under irradiation with UV-B (at 300 nm) light in aerobic. Also photodegradation of **1** with UV-A light (at 337 nm, N_2 laser) and photosensitizing degradation with rose bengal was observed. Two photoproducts were isolated and identified. In both the cases the same photoproducts were obtained. Sensitization reaction involving singlet oxygen lead to decomposition of acetazolamide. A photooxidation with singlet oxygen was determined as the principal photoprocess. Irradiation in presence of histidine and NaN_3 (quenches and scavenges $^1O_2$) induce a pronounced retardation of the photodecomposition rate of **1**, and to the formation of a dimeric photoproduct. Also the generation of singlet oxygen was detected by trap with 2.5-dimethylfuran and furfuryl alcohol. Under argon atmosphere only this dimeric photoproduct was isolated. Acetazolamide has been shown to photosensitize the reduction of nitro blue tetrazolium in PBS, which is more efficient in deoxygenated conditions and quenched in presence of SOD. These results indicate that direct electron transfer occurs from the excited state of **1** to the substrate, and also that superoxide could be involved as an intermediate when oxygen is present. On the basis of our results, photochemical reaction mechanism of acetazolamide is postuled and discussed.

Keywords: Acetazolamide; Photodegradation; Photooxidation; Diuretic drug; Singlet oxygen

1. Introduction

Compounds producing photosensitization in the 290–320 nm range, such as the diuretic drugs, produce reactions within the absorption range of proteins determined by aromatic amino acids, and of nucleic acids on cell cultures or microbiological system [1–3]. Also the intermediate and final products of its photolysis could produce phototoxic effect by direct [4] or photodynamic [5] attack on component of the cellular wall as lipids and affinity for the haemoglobin in non-nuclear cells [6]. Few of the diuretics have been investigated for their photochemistry and phototoxic properties. Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **1**, Fig. 1) is a drug commonly used as diuretic. Cutaneous adverse reactions mediated by light have been described after oral intake of acetazolamide [7]. Phototoxic properties of this drug a similar diuretics were in vitro determined by the means of a photohemolysis test [6,8–10]. The photosensitivity of these drugs in vitro was inhibited by antioxydants and nitrogen atmosphere [1]. These findings indicate a oxygen dependence of the phototoxic action of this drug in vitro [11]. In this context, nothing is as yet known about the relation between the photochemistry and phototoxicity of these drugs. We examined the photolysis of acetazolamide under various conditions, with the main goals of establishing the role of oxygen (especially singlet oxygen) in these photoprocesses and the mechanism of the reaction. The aims of this study were to determine the photochemistry of the diuretic drug acetazolamide (**1**), as also the isolation and characterization of its photoproducts.

2. Experimental details

Acetazolamide (**1**) was extracted from the commercial medicament Diamox® from Lederle laboratory, with a soxhlet extractor with aq. methanol and recrystallized from the same solvent. The purity is 99.5% as determined by ^1H NMR spectroscopy and UV-visible spectrometry. Superoxide dismutase (SOD) were purchased from Sigma (St. Louis, MI, USA), while sodium azide (NaN_3), vitamin C, histidine, furfuryl-alcohol (FFA), 2,5-dimethylfuran (DMF), nitro blue tetrazolium chloride monohydrate (NBT), rose

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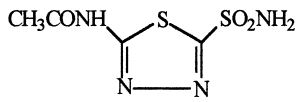


Acetazolamide (**1**)

Fig. 1. Structure of the diuretic drug acetazolamide.

bengal and tetraphenyl porphine (TPP) were purchased from Aldrich (Steinheim, Germany). All analytical or HPLC grade solvents were obtained from Merck (Darmstadt, Germany).

2.1. Photochemical reactions

Acetazolamide (**1**) (CAS 54-31-9) (Diamox®, Lederle) was irradiated at room temperature for 72 h in methanol (0.120 g, 0.335 mmol in 100 ml) with an Rayonet photochemical reactor equipped with 16 phosphorus lamps with a emission maximum of 300 nm (23 mW/cm^2 of irradiance) as measured with a model of UVX Digital Radiometer after 1 h continued illumination, under oxygen atmosphere for 48 h and also under argon. Irradiation was also carried out with a nitrogen laser with spectral output 337 nm (GL-3300 Photon Technology International, New Jersey, USA) and peak power at 5 Hz of 2.4 MW and 1.45 mJ of energy per pulse. In both the cases we obtained the same process of photodegradation.

The course of the reaction was followed by UV-Vis spectrophotometry using a Milton-Roy 3000 instrument and also by GC and HPLC until the acetazolamide was completely consumed.

After irradiation the solvent was evaporated at reduced pressure (14 Torr) at room temperature and the residue was purified by preparative thin layer chromatography (neutral alumina) using a mixture of methylene chloride/methanol (4:2).

The same conditions were used to the irradiation of a solution of acetazolamide in PBS (0.400 mmol in 100 ml). Next, the solvent was extracted three time with 100 ml CH_2Cl_2 and evaporated and purified as the before irradiation.

Products **2** and **3** were analyzed by ^1H and ^{13}C NMR spectroscopy (Bruker Aspect 3000, 300 MHz) and IR (FT IR-Nicolet DX V 5.07). The GC–MS analyses were performed using a Carlo Erba/Kratos MS25RFA instrument provided with a 25 m capillary column of cross-linked 5% phenylmethylsilicone.

In the determination of quantum yields the photolysis was limited to less than 10% to minimize light absorption and reaction of photoproducts. The photon flux incident on 3 ml of solution in quartz cuvettes of 1 cm optical path was measured by means of a ferric oxalate actinometer and was of the order of 10^{15}–10^{16} quanta s^{-1}.

The HPLC used in all the experiments described herein was a Water Delta Prep 4000 equipped with a 3.9×300 mm, 10 μm Bondapak C18 column using a CH_3CN/H_2O binary solvent system.

2.2. Photodegradation products of **1**

2.2.1. Compound **2**

Yield 75% (methanol-dichloromethane, 2:1); m.p. 269–260°C. IR (KBr pellet) ν: 3460, 3400 (CONH), 2105 (C=N-N), 1655–1648 (NH_2), 1350, 1312, 1165 (SO_2). ^1H NMR ($CDCl_3$) δ: 3.29 (s, 2-H, NH_2), 2.27 (s, 3-H, CH_3), 1.27 (s, 1-H, NH). ^{13}C NMR ($CDCl_3$) δ: 185 (C=O), 177 (C=O), 175 (C-ring), 171 (C-ring), 24 (CH_3). MS *m/z* (%): 255 (M^+, 2%), 228 (65), 211 (39), 185 (30), 129, 129 (34), 102 (50), 73 (28), 60 (45), 43 (100).

2.2.2. Compound **3**

Yield 25% (methanol-dichloromethane, 2:1); m.p.> 300°C. IR (KBr pellet) ν: 3465, 3410 (CONH), 2820, 2140, 2110 (C=N-N), 920. ^1H NMR ($CDCl_3$) δ: 2.30 (s, 3-H, CH_3), 1.20 (s, 1-H, NH). ^{13}C NMR ($CDCl_3$) δ: 171 (C=O),), 165 (C-ring), 163 (C-ring), 20 (CH_3). MS *m/z* (%): 285 (20), 284 (M^+, 40), 241 (M^+-CH_3CO, 22), 227 (8), 194 (5), 180 (44), 164 (8), 85 (55), 43 (100).

2.3. Singlet oxygen detection

In a separate experiment irradiations with a nitrogen laser with spectral output 337 nm (GL-3300 Photon Technology International, New Jersey, USA) and peak power at 5 Hz of 2.4 MW and 1.45 mJ of energy per pulse, were carried out under the same experimental conditions of the photolysis of **1**, in the presence of 2.5-dimethylfuran (2.5-DMF, 5.00 mmol) which is used as a trap for singlet oxygen ($^1O_2$) [12]. This process was followed by gas chromatography and by mass spectrometry.

Another 'trap' method has been successfully used to detect generated $^1O_2$ in a variety of samples [13,14]. This method is based upon following the consumption of a chemical trap (Furfuryl alcohol, FFA) that react with singlet oxygen. The comsumption of FFA was followed by HPLC using a 90:10 H_2O/CH_3CN mobile phase composition. The detection wavelength used for monitoring FFA consumption was λ=222 nm. Rose bengal, a well known $^1O_2$ sensitizer, was used as a standard for comparison with acetazolamide (**1**) for $^1O_2$ formation, under identical conditions of photolysis.

The irradiation of **1** was also carried out in presence of histidine, 1,4-diazabicyclo [2.2.2] octane (DABCO) and sodium azide (NaN_3) as singlet oxygen quencher, and superoxide dismutase (SOD) as oxygen superoxide scavenger.

Acetazolamide (**1**) was also irradiated in the presence of rose bengal or also of tetraphenyl porphine (TPP) with an Osram HQL 250 W medium pressure Hg lamp using a potassium chromate solution (100 mg/L) as a filter allowing λ>400 nm and maintaining all other conditions. In this way, the reaction of singlet oxygen with **1** can be studied.

2.4. Electron transfer mechanism detection by reduction of NBT

Under the same condition of the photololysis of acetazolamide ([**1**]=5.1×10^{-5} M), the photoreduction of nitro blue tetrazolium ([NBT]=5.1×10^{-5} M) was followed in presence of NBT in presence and absence of oxygen, as a function of the irradiation time by determining the increase in absorbance at 560 nm due to the formation of diformazan product (15, 16).

3. Results

The phototoxic diuretic drug acetazolamide (**1**) is photolabile under aerobic and anaerobic conditions in methanolic and also in buffered aqueous medium (ph 7.4). The photolysis of **1** was followed by monitoring the disappearance of the 266 nm band at 5 min intervals. The results is shown for a methanolic solution (1×10^{-3} M) of **1** in Fig. 2.

The quantum yields was Φ=0.24 in aerobic conditions, with the formation of the photoproduct **2** (yield 75%) and **3** (yield 25%) (Scheme 1).

Degradation of **1** was also observed when the irradiation of acetazolamide was carried out in the presence of rose bengal or also of tetraphenyl porphine (TPP) using a potassiumchromate solution (100 mg/l) as a filter allowing λ>400 nm and maintaining all other conditions. Only the compound **2** (yield 23%) was detected as photoproduct of the reaction in this experiment.

Acetazolamide photosensitize the oxidation of either 2.5-dimethylfuran (efficient acceptor for $^1O_2$ independently of the pH of the system) [17] or histidine [18,19]. Trapping of singlet oxygen with DMF leads to the formation of hexene-2.5-dione (28%), *cis*- and trans-3-oxo-1-butenyl acetate (11% and 40%, respectively), and 2-methoxy-5-hydroperoxide-2,5-dimethylfuran (20%) [12], as detected by GC-MS. This result demonstrates that compound **1** possesses type II photodynamic activity, and also is itself susceptible to photosensitized oxidation. This observation is consistent with the fact that the photodegradation of **1** under aerobic conditions is affect by the addition of singlet oxygen and oxygen superoxide quenchers, such as Vitamin C, sodium azide, and of SOD. Under these conditions the yield of photodegradation of acetazolamide was reduced to 20% of **2** and 10% of **3** (under addition of Vitamin C), 40% of **2** and 20% of **3** (under addition of sodium azide) and 50% of **2** and 20% of **3** (under addition of SOD).

Acetazolamide has been shown to photosensitize the reduction of nitro blue tetrazolium (NBT) in PBS solution (pH 7.4, 30°C). This reaction was more efficient in deoxygenated conditions and in presence of SOD. This result (Fig. 3) is consistent with the report [16] that in the presence of oxygen will suppress the photochemical reduction of NBT to formazan by simple mass action.

4. Discussion

In the present investigation, it was observed as a normal diminution of the absorbance at 270 nm and enhancement at 210 nm (Fig. 2) demonstrated the photodegradation of **1** with significant photolability under irradiation at 300 nm and also 337 nm in aerobic conditions.

The results indicate that O_2 is directly involved in the photolysis of acetazolamide. The main photodecomposition product formed in aerobic solution of **1** was the compound **2**. The study of the influence of oxygen radicals on the

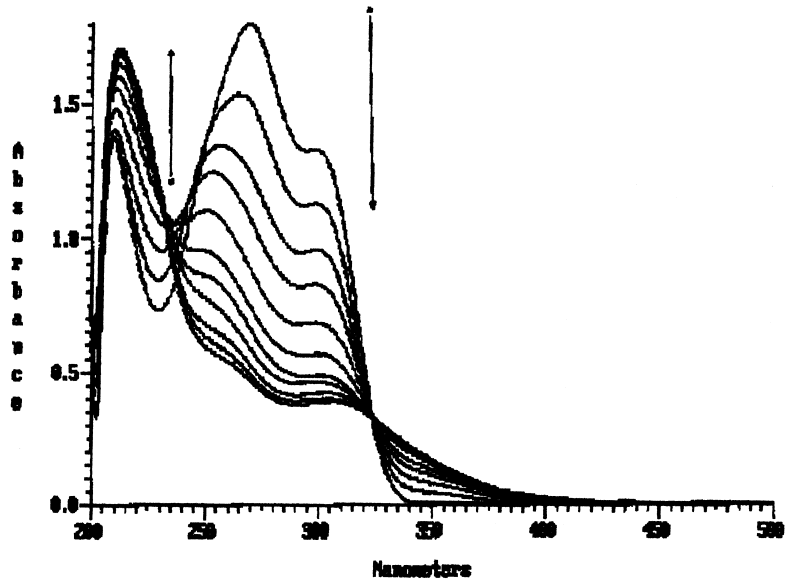
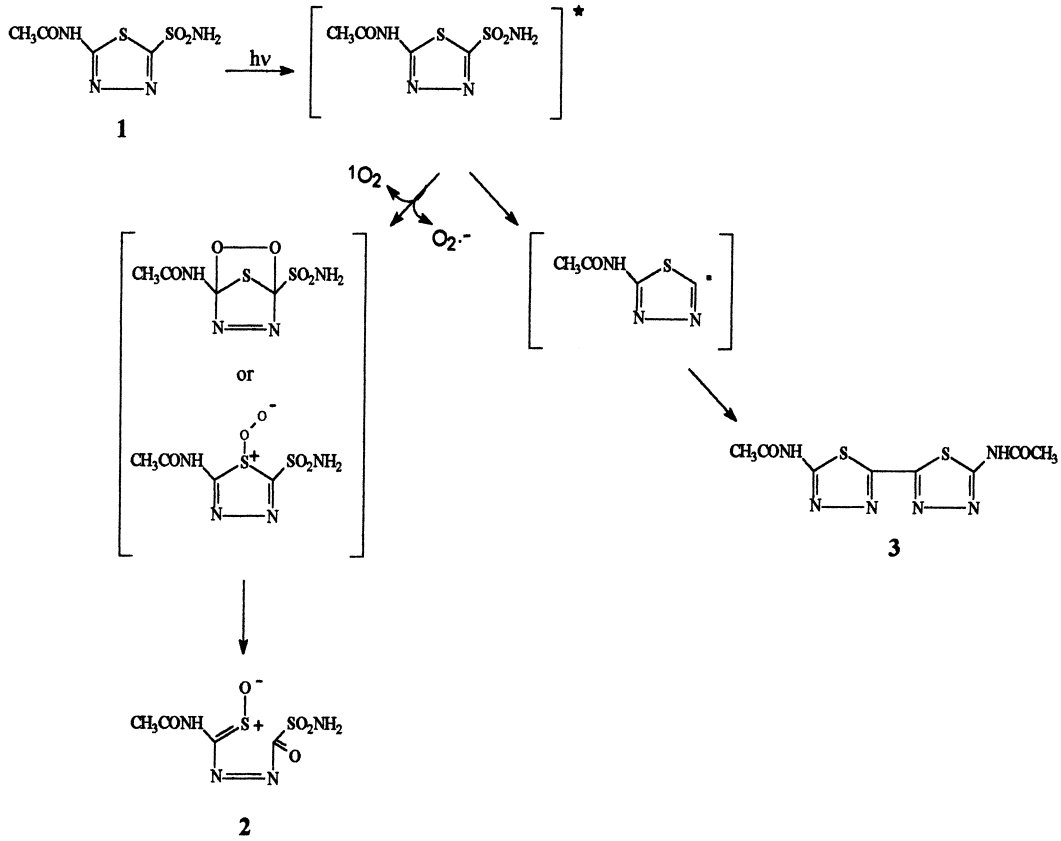


Fig. 2. UV monitoring of the photolysis of acetazolamide.



Scheme 1.

decomposition of acetazolamide (irradiations in the presence of singlet oxygen quenchers) indicate that $^1O_2$ may lead to degradation of **1** by oxygenation (Scheme 1). Although the oxygenated product **2** may also be formed by reactions with oxygen molecular O_2 or $O_2^{\cdot-}$ this way was descarted from the results obtained of the addition of singlet oxygen quenchers and SOD as oxygen superoxide scavenger on the irradiation.

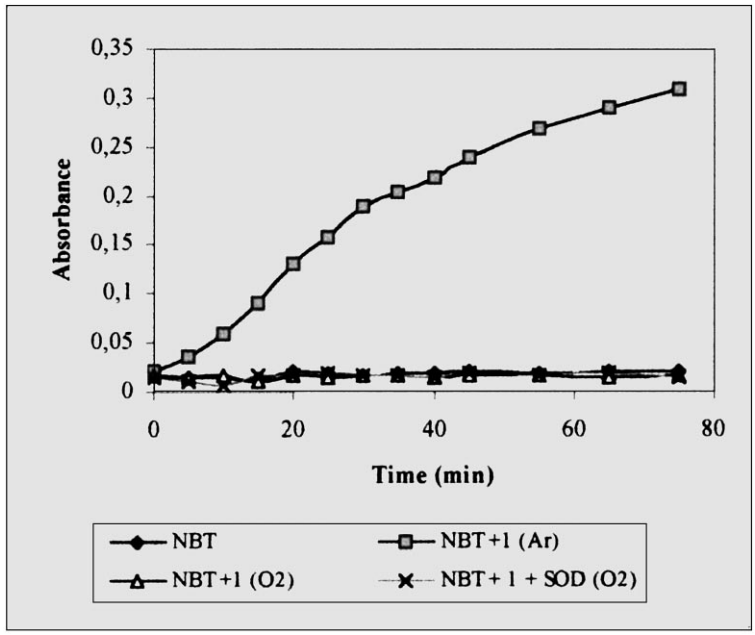
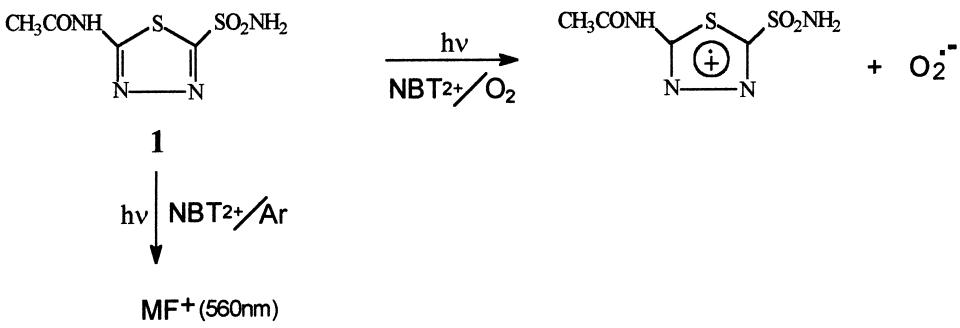


Fig. 3. Photoreduction of NBT (5.1×10^{-5} M) sensitized by acetazolamide (**1**) (5.1×10^{-5} M) in PBS solution.



Scheme 2.

The fact that the photodegradation process was also efficient under oxygen atmosphere and it is essential to produce photoproduct **2** is relevant to understand the mechanism of oxygen-dependent photobiological effects of **1**. With the determination of $^1O_2$ and $O_2^{\cdot-}$ in the photolysis of **1** and its participation in the photodegradation of acetazolamide we can look forward to a better understanding of the role of singlet oxygen generated in the photolysis of these type of drugs in the biological systems [20]. Also, we should be able to use both $^1O_2$ generators and $^1O_2$ quenchers to alter the rates and directions of biochemical reactions.

Acetazolamide has been shown to photosensitize the reduction of NBT in PBS, which is more efficient in deoxygenated conditions and in the presence of SOD. These results indicate that the direct electron transfer occurs from the excited state of **1** to the substrate, specially oxygen. Naturally, superoxide could be involved as an intermediate when oxygen is present (Fig. 3). On the basis of these results, a photochemical reaction mechanism of acetazolamide and NBT is postuled in Scheme 2.

Another photochemical process appear to compete is the formation of the free radical intermediate from the cleavage of the group sulfonamide of the ring thiadiazole. This intermediate produce a dimerization to yield the compound **3** (Scheme 1), it does not require oxygen.

Acknowledgements

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