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Photosensitized production of nitric oxide and peroxynitrite from a carbon-bound diazenium diolate and 2-methyl-2-nitrosopropane

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ABSTRACT

The photosensitized generation of nitric oxide from alanosine (3-(hydroxynitrosoamino)-D,L-alanine) by aluminum phthalocyanine tetrasulfonate (AlPcS4) is reported. While nitric oxide (NO) is obtained in nitrogen-saturated solutions, evidence suggest that both NO and peroxynitrite are produced in air-saturated solutions. Enhancement of NO production occurs in the presence of ubiquinone-0. These observations support the idea that NO is produced by the photosensitized oxidation of alanosine. Both NO and peroxynitrite are detected during photoirradiation of AlPcS4 in the presence of 2-methyl-2-nitrosopropane (MNP) and hypoxanthine (HX), but not in the absence of HX, in air-saturated solutions, thus implying that HX is acting as sacrificial electron donor, thus promoting superoxide formation.

1. Introduction

Nitric oxide (NO) is an endogenously produced molecule that has multiple roles in physiological processes, including angiogenesis, wound healing, neurotransmission, smooth muscle relaxation, and inflammation [1]. Nitric oxide's action on physiology is highly dependent on location, source, and concentration [2]. It is produced in vivo by NO synthase (NOS). Low nanomolar NO concentrations are produced by eNOS and nNOS to promote vasodilation and neurotransmission, respectively [3]. The iNOS form is capable of producing micromolar levels of NO, often responding to infection and inflammation [1]. In the presence of superoxide ($O_2^{\bullet}$), NO will react to form peroxynitrite ($ONOO^-$), an even greater oxidant involved in the inflammatory response [4]. Peroxynitrite causes apoptotic or necrotic cell death through nitration of tyrosine residues in proteins, lipid peroxidation, oxidation of critical thiols, DNA strand breaks, NAD depletion and thus energy failure [5]. NO is also a wound healing promoting agent and, due to its antibacterial activity, it is a promising agent for reducing implant-associated infections and promoting tissue regeneration in orthopedic procedures [6,7].

However, nitric oxide has a short half-life (<1 s) in the presence of oxygen and hemoglobin in vivo, arising from its high reactivity with transition metals and heme-containing proteins [8]. Due to the reactive nature of gaseous NO, its short half-life, instability during storage, and potential toxicity, including its influence on the systemic blood pressure, chemical strategies for NO storage and release have been developed in an effort to use NO's pharmacological potential. Several ways of NO release to tissues have been developed. Diazeniumdiolates (1-amino-substituted diazen-1-ium-1,2-diolate, i.e. NONOates) and S-nitrosothiols represent the two most diverse NO donor classes [9,10]. Other classes are organic nitrates and metal nitrosyl compounds such as sodium nitroprusside and potassium nitrosylpentachlororuthenate [9].

The release of NO from several nanocarriers have been developed to avoid systemic NO side effects while transporting the NO source to the selected tissue [11]. The selective delivery of NO to tissues in adequate concentrations is a developing area of research. These include polymeric nanoparticles, micelles, dendrimers, nanogels, gels, gold nanoparticles, silica nanoparticles and liposomes [11]. The possibility of releasing NO before reaching the tissue site is still a major problem in particle-based systems. In addition, the rate of release of NO at the tissue site using those systems is difficult to be controlled and those where controlled release of NO is observed are mostly metal-based nanoparticle systems containing transition metals with the potential toxicity of those remaining to be tested.

One way of selectively release NO at the needed tissue is to use tissue-penetrating light (wavelengths in the near infrared region, NIR) to activate NO release from molecules. A recent technique,

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using a 2 photon laser irradiation where NIR photons are added to produce more energetic photons, and NIR-to-visible up-conversion, which are able to release NO from NO-containing molecules and has been developed and used in NO-containing nanoparticles [12–14]. This technique permits the use of longer, tissue-penetrating wavelengths for the photochemical release of NO at the selected tissue site. The use of liposomes for photodelivering NO from NO-containing chromium complexes has also been reported, where NO is detected outside the liposome [15]. However, a non-tissue-penetrating light wavelength was used [15]. The technical problem to overcome is that those photo-controllable NO donors, where all of them contain transition metals, may exhibit systemic toxicity due to release of transition metal ions. In addition, those systems do not generate peroxynitrite, a species which should enhance the toxic activity of NO.

Cupferron, a carbon-bound diazenium diolate, is able to produce nitric oxide photochemically [16] and upon enzymatic oxidation [17]. A natural product with carbon-bound diazenium diolate structure, without the potential carcinogenicity of cupferron [18], is alanosine, Fig. 1. Furthermore, the possibility of generating carcinogenic nitrosamines, as could occur after photolysis of nitrogen-bound diazeniumdiolate ions [19], has not been reported for carbon-bound diazeniumdiolates. Since other NO-containing compounds release NO by photosensitization [20], we were prompted to test the photosensitized generation of NO from this type of compound. In this work we report the photosensitized release of NO from alanosine using NIR radiation. Evidence supports the generation of peroxynitrite from air-saturated dye-alanosine solutions and from air-saturated 2-methyl-2-nitrosopropane (MNP) solutions containing a sacrificial electron donor. Although the photosensitized generation of NO from MNP has been reported previously [20], evidence suggesting the photosensitized production of peroxynitrite by MNP in the presence of the sacrificial electron donor, hypoxanthine (HX), is described here. In the present work we have used MNP to contrast the behavior of alanosine. The photosensitized production of NO could be used in photodynamic therapies of malignancies, where NO or peroxynitrite are used as toxic agents, and where nanoparticle carriers containing both the NO source and the photosensitizer, are transported to the desired tissue.

2. Materials and methods

2.1. Materials

Alanosine 3-(hydroxynitrosoamino)-D,L-alanine, Fig. 1, was obtained from the NCI DTP Repository (Rockville, MD). The dye aluminum phthalocyanine tetrasulfonate (AlPcS4) was purchased from Frontier Scientific. The compounds ubiquinone-0 (UBQ-0), ferricytochrome c, MNP, HX, L-tyrosine, carboxy-PTIO, superoxide dismutase (SOD, from bovine erythrocytes) and 3-nitrotyrosine were purchased from Sigma-Aldrich Co. All solutions were prepared in phosphate buffer and used the same day. Deionized and Chelex-treated water was used in the preparation of all stock and sample solutions. Chelex treatment of water and buffer was monitored using the ascorbate test, as described by Buettner [21]. Care was always taken to minimize exposure of solutions to light.

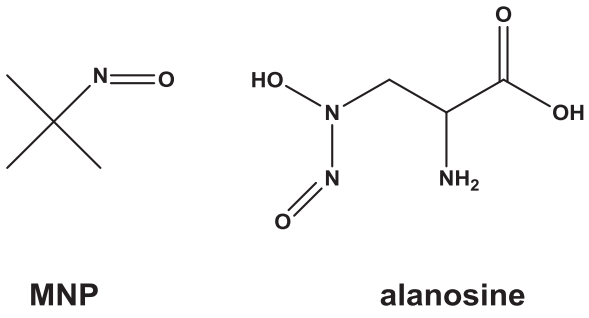

Fig. 1. Alanosine and MNP structures.

2.2. Methods

2.2.1. Sample irradiation for EPR analysis

The NO probe, carboxy-PTIO, was used to detect NO formation from the production of the carboxy-PTI EPR spectrum, as reported previously [22–24]. Air- or N_2-saturated samples containing AlPcS4 (with absorbance of 1 at 675 nm), alanosine (or MNP, in the presence and absence of HX), in the presence or absence of ubiquinone-0 and carboxy-PTIO in 50 mM phosphate buffer (pH 7.4) were irradiated at 675 nm in a 1 cm light path Pyrex cuvettes with continuous stirring for different periods of time. At the end of each period, samples were then transferred into N_2- or air-saturated EPR flat quartz cells ($60 \times 10 \times 0.25$ mm) and placed in the EPR instrument cavity for analysis. A 1000 W xenon arc lamp coupled to a Spectral Energy GM 252 high-intensity grating monochromator with a bandwidth of ± 20 nm was used as the irradiation source. EPR spectra were recorded on a Bruker ER-200D spectrometer at 100 kHz magnetic field modulation. EPR line intensities were determined from the peak-to-peak derivative amplitudes times the square of the peak-to-peak widths.

2.2.2. Sample irradiation in the NO electrode chamber

Nitric oxide production rates were monitored using a NO-specific electrochemical probe (ISO-NOP) inserted in a thermostated NO chamber (World Precision Instruments, Sarasota, FL) at 37 °C. The chamber was either saturated with air or purged with high purity nitrogen followed by injection of 1.00 mL of an air- or nitrogen-saturated solution containing from 0 to 1 mM alanosine or 0–3 mM MNP, 10 μM AlPcS4 and 0 or 500 μM UBQ-0 in 50 mM phosphate buffer (pH 7.4). This was followed by immediate exclusion of all gas bubbles out of the sample, through the chamber capillary. The sample was continuously stirred using a spinning bar. Data acquisition was started before irradiation. The sample was then irradiated at 670 nm using a B&W Tek diode laser with a constant power of 255 mW. Basal voltage was calibrated to zero every day. Voltage output corresponding to a 20 μM NO solution was checked every day, and the electrode membrane was replaced in case there was no agreement with previous outputs within 10%. The electrode was calibrated daily with known concentrations of $NaNO_2$ by reacting this salt with KI in sulfuric acid medium. NO production data were collected in a computer, and the initial rates of NO consumption (RNO) were measured. RNO values reported are averages of 3 determinations for each type of sample.

2.2.3. Peroxynitrite formation

Peroxynitrite formation was detected indirectly by its reaction with L-tyrosine to produce 3-nitrotyrosine, as described previously [25]. For this purpose, micromolar amounts of L-tyrosine were included in the air-saturated samples to be irradiated and its nitro-substituted product detected at 274 nm using HPLC. HPLC analyses were performed using a HP Zorbax SB-C18 (4.6×250 mm) column and eluted with a solvent mixture of 95% ammonium acetate (pH 4.7) and 5% methanol. An Agilent 1100 analytical HPLC system with absorption detection at 276 nm and a flow rate of 0.8 mL/min was used. The retention times of L-tyrosine and 3-nitrotyrosine peaks were determined using commercial standards. All determinations were repeated at least three times, and the average of these determinations is reported.