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Photoproduction of Superoxide Ion from Near Ultraviolet Light Plus Hydrogen Peroxide; their Synergistic Action on Phage T7

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The ultraviolet radiation of 300–400 nm, also known as near ultraviolet (NUV), can cause a variety of damage to viruses, bacteria and animal cells including DNA strand breaks, formation of pyrimidine dimers and interference in transport and repair systems [1]. The effect of NUV on the inactivation of phages and bacteria is significantly enhanced in the presence of hydrogen peroxide (H_2O_2). The synergistic action of the two agents can be observed at concentrations at which the individual agents may have little or no effect [2]. One reason for the phototoxicity by NUV is implicated with the photooxidation of endogenous tryptophan which, on photolysis, generates H_2O_2 and this agent in turn is photolysed to act synergistically on the biological systems.

Ninety nine percent of a population of phage T7 is inactivated if exposed simultaneously to NUV (4.3×10^4 J m^{-2}) and H_2O_2 (0.3 m*M*). Addition of hydroxyradical (OH·) scavengers, such as sodium benzoate, mannitol or isopropyl alcohol, to the system does not prevent the phage from being inactivated, suggesting that OH· is not the phototoxic agent. However, when the reaction mixture was supplemented with superoxide dismutase (SOD) phage inactivation was significantly reduced. This suggests that superoxide ion (O_2^-) is possibly playing an important role in the synergistic killing of phage T7.

Production of O_2^- from the NUV photolysis of H_2O_2 was detected by the oxidation reaction of NADPH to NADP [3] and the reduction reaction of nitroblue tetrazolium (NBT) to formazan blue [4]. Furthermore, the reduction of NBT and the oxidation of NADPH were prevented in the presence of SOD. Presence of isopropyl alcohol or sodium benzoate in the reaction mixture did not prevent this diminution reaction.

An action spectrum of the reduction of NBT by H_2O_2 showed that the blue colour appeared maximally at 310 nm. Also it was noted that, within the range of pH 7 and 9, as the pH of the reaction mixture was increased the appearance of formazan blue increased proportionately. A sequence of reaction is described [5] to account for the production of O_2^- from the UV photolysis of H_2O_2:

$$H_2O_2 \xrightarrow{h\nu} 2OH\cdot$$

$$OH\cdot + H_2O_2 \longrightarrow H_2O + HO_2^\cdot$$

$$HO_2^\cdot \longrightarrow O_2^- + H^+$$

Is therefore superoxide ion, generated from the NUV photolysis of H_2O_2, responsible for the inactivation of T7 when exposed to these agents? To investigate this T7 was exposed to the agents in the presence of SOD. Phage inactivation was significantly reduced suggesting that O_2^- is the likely phototoxic agent. It is possible that O_2^- is directly reactive with the DNA causing strand breaks or that it is first reactive with a chromophore (perhaps a protein) which then reacts with the DNA. DNA to protein cross links in phage T7 in the presence of NUV plus H_2O_2 have been documented [6].

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In vitro Cytostatic Activity of Palladium(II) and Platinum(II) Halide Complexes with Thiocarbamic Esters

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The thiuram disulfides and dithiocarbamates are widely used in industry, agriculture and medicine [1, 2]. Moreover disulfiram (tetraethylthiuram disulfide) has been used for over 20 years in the treatment of certain patients with chronic alcoholism and the reduced form of the compound, diethyldithiocarbamate anion (DDTC) has been used in the treatment of nickel carbonyl, arsenic and thallium poisoning [3].

Recently DDTC has been found to be an effective agent in attempts to reduce the dose-limiting nephrotoxicity of *cis*-platin [4]. This compound has been shown also to exert a protective effect against a variety of chemically-induced malignant tumors and a unique immunopotentiating activity [5].

Bearing in mind these findings, in recent years we have undertaken to study platinum(II) and palladium-

(II) complexes with thiocarbamate derivatives as ligands, in order to prepare new antitumor platinum metal complexes with a better therapeutic index than *cis*-platin [6, 7].

The present note reports a new series of platinum-(II) and palladium(II) complexes containing as ligands dithiocarbamic esters of the type $L = R_1R_2N{\cdot}CSSR$ ($R_1 = R_2 = R = Me$, L = TMDT; $R_1 = R_2 = Me$, R = Et, L = DMDTE; $R_1 = R_2 = R = Et$, L = TEDT; $R_1 = R_2 = Et$, R = Me, L = DEDTM). Whereas by reacting PtX_2 and PdX_2 (X = Cl, Br) with TMDT and DMDTE, complexes of 1:1 molar ratio, MLX_2, and 1:2, ML_2X_2 have been isolated, with DEDTM and TEDT only 1:1 adducts have been obtained. On the basis of the IR data the ligands act as monodentate through the thiocarbonyl group in the 1:2 complexes; for the 1:1 a tetracoordination by one bidentate (S,S) dithiocarbamic ester molecule and two halides is suggested.

The cytostatic activity was evaluated on KB cells according to protocols suggested by the National Cancer Institute (Bethesda) [8].

Results of an *in vitro* assay are expressed as concentration of the compound in culture medium (μg/ml) required to inhibit growth by 50% (ID_{50}).

Some of the compounds showed significant cytostatic activity in the preliminary test. Particularly $Pd(DMDTE)X_2$ (X = Cl, Br), $Pt(DMDTE)Br_2$ and $Pd(DMDTE)_2Br_2$ have ID_{50} values in the 0.5–1 μg/ml interval. The ligands alone did not show any activity.

From the preliminary results the palladium complexes seem to possess a higher cytostatic activity than platinum analogues.

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Peroxides of Vanadium and Related Metals in Biological and Medicinal Chemistry

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Ions of vanadium and other transition metals of d^o or low d^n electron configuration are present in substantial amount in selected human tissues and blood. Vanadium is one of the elements recently recognised as essential for mammals [1]. In a very low concentration [2] it is thought to be widely distributed in tissues, but its biological function remains unknown. Complexes of vanadium and related metals with peroxo group, stabilized in specific ligand fields [3], exist in solid state and aqueous solutions. Such compounds of biologically important metals can help us understand the metal interactions with dioxygen moiety in living matter [4]. We have prepared and characterized a number of peroxo complexes of vanadium, niobium, tantalum and lanthanides, some containing various heteroligands (*e.g.*, oxalates, amino-carboxylates). Antitumor activity (ILS 25–32%), using L1210 murine leukemia test systems, has been found for some of these vanadium complexes [5]. They represent a new type of antitumor metal agents, quite different from a previously reported vanadocene dichloride [6]. A change in toxicity has been observed among analogous peroxo and nonperoxo niobate complexes. The relationship between the chemical properties of these compounds and their biological effects is studied by observing properties of complex peroxo species in aqueous solutions. Proton and ^{13}C NMR spectra of heteroligands offer an indirect evidence for peroxo group presence in the metal ligand sphere. Individual resonance patterns and specific chemical shifts are observed in saturated deuterium oxide solutions for particular complex polyhedra present in the solid state. Peroxo → metal charge transfer band is pH dependent and distinct for different ligand spheres. Redox potentials measured upon oxidation by various oxidants depend upon the type of the complex, showing significant differences (≥300 mV). Vanadium complexes are particularly interesting in this respect because of a conceivable intramolecular redox process [7]. Under proper tuning by heteroligands the reactivity of coordinated peroxo group is expected to be modified, and eventually undergo one electron oxidation. Antitumor activity and toxicity of vanadium complexes can accordingly be associated with free radical processes [8], in addition to previously observed perturbation of enzymes involving phosphate metabolism, and perhaps sodium pump. For such speculations more reliable analyses of vanadium in tissues are of primary importance [9].