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Antiarthritic Gold Compounds Effectively Quench Electronically Excited Singlet Oxygen

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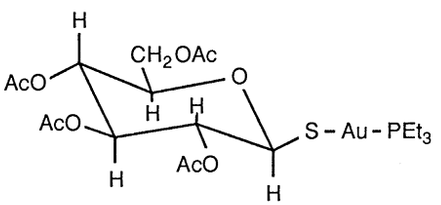
Although certain gold [Au(I)] compounds have been used effectively in the treatment of rheumatoid arthritis for some years, the molecular basis for such therapeutic action has been unclear. One possible mechanism of the action of Au(I) compounds is that they protect unsaturated membrane lipids and proteins against oxidative degradation caused by activated phagocytes that are not properly regulated. In this study it has been shown that superoxide ion ($O_2^{\cdot-}$), a product of activated phagocytes, can be oxidized to electronically excited singlet oxygen ($O_2^1\Delta_g$), an agent that is capable of peroxidation of unsaturated fatty acid derivatives. It has also been shown that antiarthritic Au(I) compounds are effective deactivators of $O_2^1\Delta_g$ with quenching constants on the order of $10^7\ M^{-1}\ sec^{-1}$.

A MAJORITY OF PATIENTS WITH rheumatoid arthritis (RA) who do not respond to nonsteroidal anti-inflammatory drugs benefit from a course of therapy with certain compounds of Au(I), for example, tetra-*O*-acetylglucose-1-thiol gold(I) triethylphosphine complex (auranofin; Ac is acetyl group and Et is ethyl group)



Auranofin

(*1, 2*). Such treatment is more effective in the early rather than the later stages of RA before extensive destruction of joint bone and tissue occurs; it also appears to retard further erosion. The molecular basis of the antiarthritic activity of Au(I) compounds remains unclear, although several possibilities have been discussed that include: (i) inhibition of thiol-dependent proteases such as cathepsins (*3*); (ii) inhibition of cellular function of various phagocytes, for example, chemotaxis of neutrophils (*4, 5*) and superoxide ion ($O_2^{\cdot-}$) (*6*) or oxyradical generation (*7*); (iii) prevention of myeloperoxidase-induced inactivation of α-1-proteinase inhibitor (*8*); (iv) inhibition of release of lysosomal enzymes (*9*); and (v) inhibition of adenosine diphosphate or collagen-induced platelet aggregation (*10*). Most of the current research on RA is guided by the idea that the disease is associated with deficiencies in regulation of the immune response that result in excessive inflammation and irreversible deterioration of joints. Lipid peroxidation and subsequent free radical–promoted degradation of protein (which includes protective proteins such as superoxide dismutase and α-1-antiproteinase, as well as proteins of cartilage and ligament) are thought to be important degenerative processes (*11*). In this report we show that Au(I) compounds might inhibit the peroxidation of membrane lipids.

Superoxide ion, which is produced in abundance by activated phagocytes such as neutrophils and macrophages, has been shown to effect biodegradation (*12*). Initial proposals that this and other toxic effects may result from the formation of hydroxyl radicals from superoxide ion have not received support (*13*), and leave open the question of how the relatively innocuous superoxide ion (*14–16*) could effect lipid peroxidation and other oxidative damage. The view that superoxide ion can be deleterious in biological systems derives support from the existence of superoxide dismutase (SOD) proteins that catalyze the conversion of $O_2^{\cdot-}$ to H_2O_2 and O_2 (ground state) at a rate ($k = \sim 2 \times 10^9\ M^{-1}\ sec^{-1}$), which is even greater than that for the rapid spontaneous second-order process in neutral water ($k = 10^7\ M^{-1}\ sec^{-1}$) (*17, 18*). SOD also displays anti-inflammatory activity. A simple reason for the protective function for SOD could be the existence of another pathway for toxicity (independent of H_2O_2 or $HO\cdot$), specifically, the direct oxidative conversion to electronically excited singlet oxygen ($O_2^1\Delta_g$). We have demonstrated this conversion unambiguously in a simple chemical experiment. Addition of a solution of potassium superoxide in acetonitrile that contains 18-crown-6 ether (*15, 16*) to a solution of excess cerium(IV) ammonium nitrate in acetonitrile rapidly produced singlet oxygen, as shown by the measurement of intense and characteristic emission at 1270 nm with the detection system previously described (*19*) (see Fig. 1) (*20, 21*). In addition, the emission spectrum corresponds to that characteristic of singlet oxygen (*19*). The formation of $O_2^1\Delta_g$ from $O_2^{\cdot-}$ also occurs with other oxidants, for example, with lead tetraacetate, iodobenzene diacetate, and tetranitromethane in acetonitrile. Oxidation of $O_2^{\cdot-}$ to $O_2^1\Delta_g$ within or near the surface of biological membranes, for example, by cytochrome oxidases, is therefore a reasonable possibility. The peroxidation of unsaturated fatty acids by singlet oxygen and the in vivo toxicity of photochemically generated singlet oxygen are both well established (*22*).

In addition to showing that superoxide ion can be converted directly to singlet oxygen by oxidation, we have established a

Table 1. Rate constants for the quenching of $O_2^1\Delta_g$ by various agents according to two methods. Method A: solvent, $CFCl_2CF_2Cl$; $O_2^1\Delta_g$ generated from 1,4-dimethylnaphthalene-1,4-endoperoxide at 30°C. Method B: solvent, benzene; $O_2^1\Delta_g$ generated by self-sensitized photooxidation of rubrene at 30°C.

Quencher	$k_q\ (M^{-1}\ sec^{-1})$	
	MethodA	Method B
Auranofin	0.75×10^7	0.2×10^7
$(C_2H_5)_3PAuSCH_3$	4.5×10^7	3.7×10^7
$(C_6H_5NHCS_2)_2Ni$	7.2×10^9	4.4×10^9
β-Carotene	1.1×10^{10}	1.5×10^{10}

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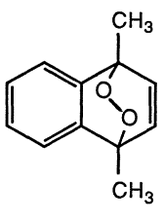
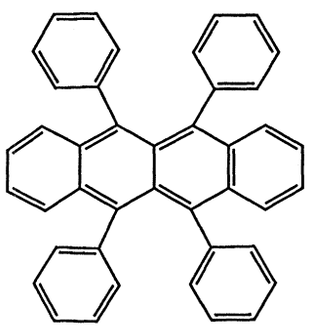
link between Au(I) compounds and singlet oxygen. It was suspected that Au(I) compounds might be effective quenchers of singlet oxygen because of the high atomic number of gold ($Z = 79$) and the possibility of heavy atom–promoted intersystem crossing by spin-orbit coupling, the efficiency of which increases as Z^4. Further, "chemical" quenching of singlet oxygen by Au(I) is also possible by charge transfer or by oxidative addition to form an unstable Au(III) peroxy adduct that decomposes to Au(I) and triplet oxygen. Studies on the quenching of singlet oxygen by auranofin (*23*) and the simpler analog $(C_2H_5)_3PAuSCH_3$ (*24*) have confirmed that these Au(I) compounds are good quenchers. Rate constants for the quenching of singlet oxygen were determined by two different methods and in two different solvents, the results of which are summarized in Table 1. β-Carotene was included in this study because it is the most efficient quencher known for singlet oxygen; its rate of quenching is essentially diffusion-controlled (*25, 26*).

In method A, singlet oxygen was generated by spontaneous decomposition of 1,4-dimethylnaphthalene-1,4-endoperoxide in $CFCl_2CF_2Cl$ at 30°C in the presence of rubrene (as the $O_2^1\Delta_g$ acceptor) and the



1,4-Dimethylnaphthalene - 1,4-endoperoxide



Rubrene

various quenchers. The conversion of rubrene to its endoperoxide was followed spectrophotometrically at 520 nm (*27*). The quenching constants were calculated as previously described (*28*). In method B singlet oxygen was generated in benzene from triplet oxygen by rubrene photosensitization,

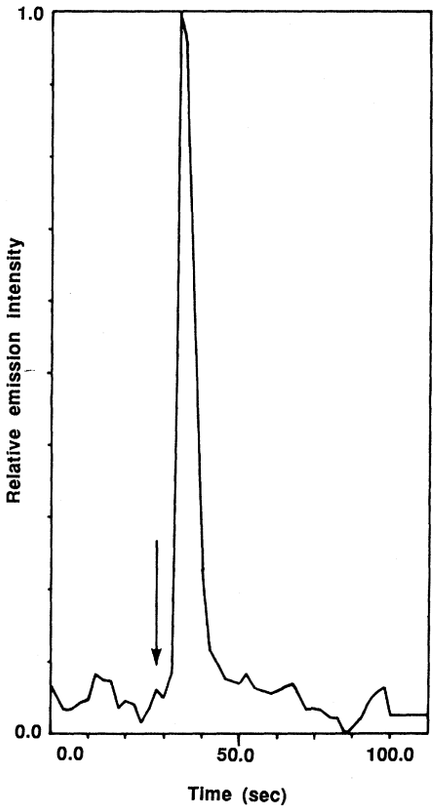


Fig. 1. Emission intensity at 1270 nm as a function of time from the reaction at 23°C of potassium superoxide (solubilized by 18-crown-6 ether in acetonitrile) with a solution of cerium (IV) ammonium nitrate in acetonitrile. The arrow indicates the approximate time that the solutions were mixed in the optical reaction cell that was located at the entrance slit of a high-sensitivity near–infrared emission spectrometer (*19*).

with rubrene also serving as the $O_2^1\Delta_g$ acceptor. The rate of endoperoxide formation was measured in the presence of quencher (*28, 29*). The results obtained by the two methods were comparable and clearly demonstrated that the two Au(I) compounds studied are effective quenchers of $O_2^1\Delta_g$, and have rates just a factor of 10^2 to 10^3 below those for β-carotene and the most efficient known metal-containing quencher $(C_6H_5NHCS_2)_2Ni$ (*28*), both of which have rates that are near the diffusion-controlled limit (*30*).

It seems possible that a considerable portion of the gold absorbed by the body during a course of therapy [~2 g, most of which is in the Au(I) form] could be associated with membrane surfaces or interiors. If this is the case, a definitive protective effect of gold against $O_2^1\Delta_g$-mediated lipid peroxidation can be expected. This may be a factor in the therapeutic efficacy of Au(I) compounds in RA. To our knowledge, no mention of this possibility occurs even in the most recent primary or review literature (*31–33*). Although attempts to detect singlet oxygen production in activated polymorphonuclear leukocytes in vitro have been negative (*34*), additional work in this area is needed. The use of β-carotene in the treatment of RA is complicated by its rapid metabolism to vitamin A.

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