Articles

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Interaction of the Antitumor Drug Streptonigrin with Palladium(II) Ions. Evidence of the Formation of a Superoxo-Palladium(II)-Streptonigrin Complex

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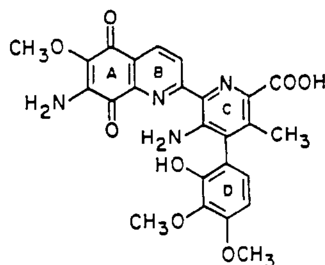
Streptonigrin, a potent antitumor agent of the quinone type, is able to form several types of complexes with Pd(II) ions. Using potentiometric and spectroscopic measurements, we have shown that, at pH 7 in aqueous solution, $[PdCl_4]^{2-}$ reacts with streptonigrin, forming a 1:1 Pd(II)-streptonigrin complex. This complex is able to catalyze the oxidation of NADH by dioxygen. Using EPR measurements, we have shown that NADH reduces the quinone group of the Pd(II)-streptonigrin complex to semiquinone. In the absence of dioxygen, the Pd(II)-streptonigrin (semiquinone) complex is very stable. The subsequent addition of dioxygen yields the formation of a superoxo-Pd(II)-streptonigrin complex, which, as time elapses, releases the initial Pd(II)-streptonigrin complex.

Introduction

Streptonigrin (**1**, SN), a highly substituted and highly functionalized 7-aminoquinoline-5,8-dione, first isolated from *Streptomyces floculus*,[1] has been shown to possess potent cytotoxic



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properties and confirmed broad spectrum antitumor activity. However, it has seen only limited use as an anticancer agent because of its toxicity.[2-4] Nevertheless, it continues to receive attention because of interest in its ability, common to a number of quinone antibiotics, to degrade DNA.[5] It has been shown that streptonigrin reduced in situ by NADH induces single-strand cleavage in covalently closed circular DNA.[6] This reaction requires the presence and activation of dioxygen[6-9] and is inhibited by superoxide dismutase and catalase.[6]

In fact, a variety of antitumor drugs exert their cytotoxic effects, at least in part, by oxidative cleavage of DNA. It has been suggested that these cleavages involve dioxygen and metal ions such as Fe(II) and Cu(II) that are chelated by the drugs. Streptonigrin is also able to chelate Zn(II). Bleomycin and streptonigrin have been presented as typical examples of such drugs.[6,10-12]

However, whereas in the case of bleomycin it has been clearly shown that Fe(II) is necessary to the activity of bleomycin and the structure of the iron-bleomycin complex responsible for the activation of dioxygen is now well documented,[13,14] such precise features of the requirement of a metal ion for streptonigrin activity are still missing. It has been shown that the presence of metal ions such as Cu(II) and Fe(II) enhances the streptonigrin activity and that the addition of EDTA inhibits streptonigrin activity.[6,10]

It is therefore of special interest to elucidate the role of the streptonigrin-metal complexes in the biological action of this antitumor compound. In this paper we report the synthesis and the spectroscopic characterization of a 1:1 Pd(II)-streptonigrin complex that catalyzes the oxidation of NADH by dioxygen. In that complex, the quinone part of streptonigrin is capable of being reduced to semiquinone by biological reducing agents such as NADH. In the absence of O_2, the Pd(II)-semiquinone complex is quite stable. The addition of dioxygen yields a superoxo-Pd(II)-SN complex.

Experimental Section

Purified streptonigrin was a gift from Rhône Poulenc Laboratories and the National Cancer Institute. Contrarily to what has been previously reported,[15] streptonigrin is soluble in water. However, it is first necessary to increase the pH to about 7 to ensure dissolution; the pH of the solution can then be decreased to 3.8. At lower pH values a precipitate appears. Solution concentrations were determined by using $\epsilon_{365} = 14200$ at pH 7.2. The value of ϵ at 365 was determined from 10 solutions prepared from accurately weighted amounts of streptonigrin.

$K_2[PdCl_4]$ and *cis*-$[Pd(NH_3)_2Cl_2]$ were obtained from Johnson Mathey. Unless otherwise stated, HEPES (*N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid) buffer, at pH 7.2, was used. Cytochrome *c* (type VI from horse heart), NADH (grade III), cardiac NADH dehydrogenase, and superoxide dismutase (SOD) were purchased from Sigma Chemical Co. All other reagents were of the highest quality available, and deionized doubly distilled water was used throughout these experiments.

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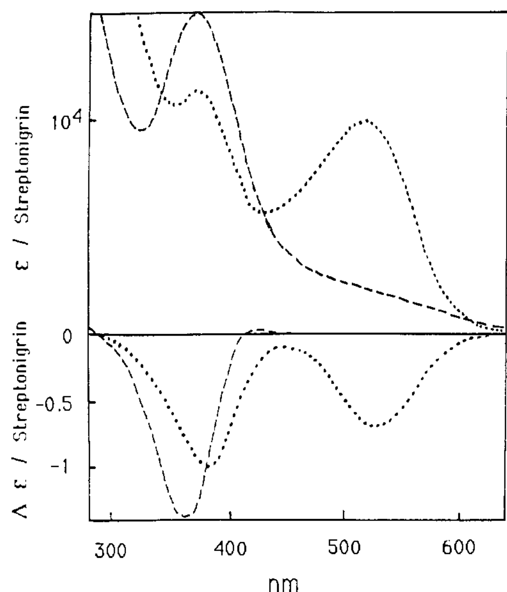

Figure 1. Absorption and CD spectra of the Pd-SN (I) complex (···) and free SN (- - -). Experimental conditions: 400 μM Pd-SN or 100 μM streptonigrin in 0.1 M KCl, 0.01 M HEPES buffer at pH 7.2.

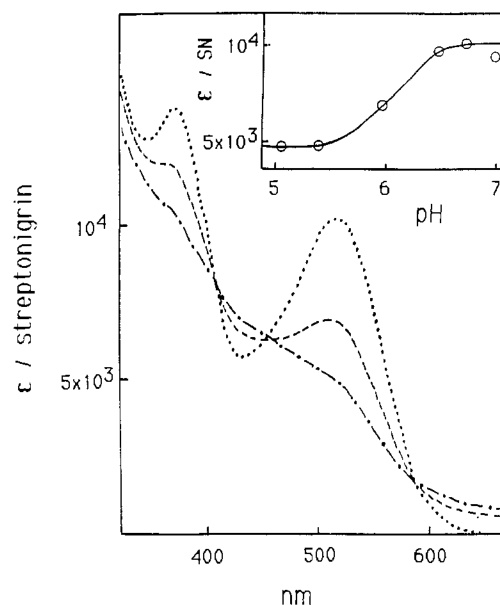

Figure 2. Absorption of the Pd-SN complex as a function of pH. Experimental conditions: 400 μM Pd-SN in 0.1 M KCl, 0.01 M HEPES buffer at pH 6.8 (complex I) (···), 6.0 (- - -), and 5.4 (complex II) (-·-). Inset: ε at 515 nm plotted as a function of pH.

Absorption spectra were recorded on a Cary 219 spectrophotometer, and circular dichroism (CD) spectra, on a Jobin Yvon Model Mark V dichrograph. Results are expressed in terms of ε (molar absorption coefficient) and $\Delta\epsilon = \epsilon_L - \epsilon_R$ (molar CD coefficient). The values of ε and Δε are expressed in terms of [SN], molar concentrations of streptonigrin. Uncorrected fluorescence spectra were recorded at 20 °C on a Jobin Yvon JY3C spectrofluorometer, and EPR spectra, on a Varian CSE 109 spectrophotometer at −180 °C. Potentiometric measurements were obtained with a Metrohm Model E603 pH meter at 25 °C using a Metrohm EA 147 combined glass electrode.

The rate of dioxygen consumption was determined at 25 °C with a YSI 5331 oxygen monitoring system. The reaction was initiated by the addition of NADH to the reaction chamber through the access slot of the oxygen electrode plunger.

NADH Dehydrogenase Assay. NADH dehydrogenase activity was determined at 25 °C by modification of a method described previously[16] using cytochrome *c* as the electron acceptor. Streptonigrin and its complex were assayed for their NADH–cytochrome *c* reductase activity by following cytochrome *c* reduction at 550 nm. The difference between the extinction coefficients of reduced and oxidized cytochrome *c* was taken to be equal to 19 600. The reaction mixture contained 0.05 M HEPES buffer at pH 7.2, 81 μM cytochrome *c*, 160 μM NADH, 10 uL^{-1} NADH dehydrogenase, and either 0 or *C* μM free or complexed streptonigrin (*C* was varied from 0 to 400 μM). The reaction was initiated by addition of the enzyme. Enzymatic activity is expressed in units, such that 1 unit is the amount of enzyme that reduces 1 μmol cytochrome *c*/min at pH 7.2 and 25 °C under the reaction conditions specified above. The production of superoxide anion in the experimental samples was corrected from the rate of cytochrome *c* reduction inhibited by SOD (20 μg/mL).

In Vitro Inhibition of P-388 Leukemia Cell Growth. P-388 cells were grown in vitro in RPMI 1640 medium supplemented with fetal calf serum (10%) and 10 μM 2-mercaptoethanol. For the growth studies, tubes are seeded with 4.5 mL of cells (approximately 5 × 10^4 cells/mL); compounds prepared in whole medium are added under a final volume of 0.5 mL (three tubes per concentration). Tubes are incubated at 37 °C for 4 days, and cell numbers are then determined with a Coulter counter. Drug effect is expressed by inhibitory dose (ID_{50}), which is obtained by plotting the logarithms of drug concentration against percent inhibition of cell growth and extrapolating the concentration required to inhibit 50% of cell growth.

Results

Pd(II)–SN Complexes: Spectroscopic Identification. The addition of an aqueous solution of $[PdCl_4]^{2-}$ to streptonigrin in 0.1 M KCl HEPES buffer at pH 7.2 gave rise to the formation of a red complex. This was attested by the appearance of an absorption band at 515 nm and a negative band at 525 nm in the circular dichroism spectrum. In order to determine the stoichiometry of the complex formed, the molar ratio of $[PdCl_4]^{2-}$ to streptonigrin was varied from 0 to 2. Spectral modifications were observed when the molar ratio was varied from 0 to 1, and no further modification was observed when the molar ratio was varied from 1 to 2. In a typical experiment, 400 μM streptonigrin in 0.1 M KCl, 0.01 M HEPES buffer at pH 7.2, was used. In such conditions about 1 min was necessary for equilibration. From these data we can infer that a complex of 1:1 Pd:SN stoichiometry was formed. Figure 1 shows the absorption and CD spectra of the Pd–SN complex thus formed, which will be hereafter labeled I. In the absence of metal ions and in HEPES buffer at pH 7.2, the absorption spectrum of streptonigrin exhibited a band at 364 nm (ϵ_M = 14 200) (Figure 1). According to Sidman, this band can be assigned to a $^1L_b \leftarrow {}^1A$ transition of the quinoid A ring.[17] We have shown that in HEPES buffer the spectrum of streptonigrin depends on concentration.[18] At high concentration, i.e. 750 μM, streptonigrin exhibited a CD signal of the couplet type centered at 380 nm. When the concentration was decreased down to 100 μM, the CD signal of the couplet type changed to a negative band at 370 nm (Figure 1). The CD signal of the couplet type has been assigned to intermolecular associations. At low concentration these associations disappear and the CD spectrum of streptonigrin in the monomeric state exhibits one negative band only. As can be seen in Figure 1, the absorption as well as the CD band at 370 nm is still present in the complex spectra. Moreover, the slight fluorescence of streptonigrin that is obtained through excitation at 370 nm is not quenched by the addition of Pd(II). These observations strongly suggest that the quinoid A ring is not involved in the Pd(II) coordination site.

Similar experiments were performed by using *cis*-$[Pd(NH_3)_2Cl_2]$ instead of $[PdCl_4]^{2-}$. In that case the reaction was slower but ultimately a 1:1 Pd–SN complex with spectral features identical with those described above was obtained. The absorption spectrum of Pd–SN complex I was pH dependent. Figure 2 shows the absorption spectrum of Pd–SN in aqueous solution at different pH values. As can be seen, the intensity of the two bands at 515 and 370 nm decreased as the pH decreased and the color of the solution turned from red to brown. Ultimately at pH 5.4 the absorption spectrum exhibited two shoulders at approximately 490 and 370 nm. In the Figure 2 inset, ε at 515 nm has been plotted as a function of pH. As can be seen, at pH 6 50% of the red

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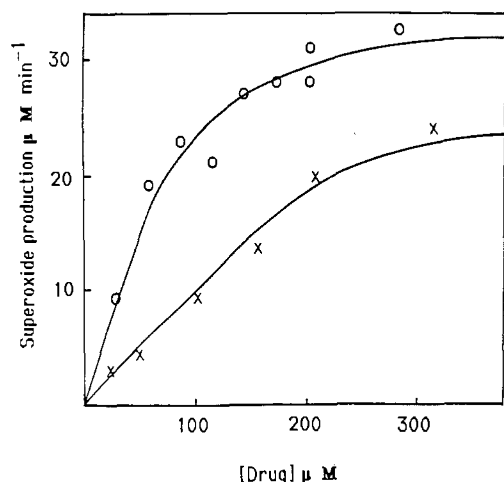
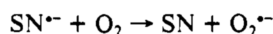


Figure 3. Effect of drug concentration on superoxide formation by NADH dehydrogenase. Superoxide formation was detected spectrophotometrically by the rate of superoxide dismutase-inhibitable cytochrome *c* reduction. The reaction mixture contained 0.05 M HEPES buffer at pH 7.2, 81 μM cytochrome *c*, 160 μM NADH, 10 uL^{-1} NADH dehydrogenase, 0 or 20 μg/mL of superoxide dismutase, and the indicated amount of drug (×, streptonigrin; O, adriamycin).

complex (I) has turned to the brown one, which will be hereafter labeled II. Three isosbestic points were present at 408, 455, and 585 nm.

Complex II formation can be obtained directly by addition of $[PdCl_4]^{2-}$ to an aqueous solution of streptonigrin at pH 5.5. In that case, we observed that the addition of 1 equiv of $[PdCl_4]^{2-}$ released 1 mol of protons/mol of streptonigrin.

Catalytic Properties of Pd-SN Complex I. (A) Reduction of Streptonigrin by NADH. Like other antitumor compounds of the quinone type (for instance anthracycline), streptonigrin was reduced to semiquinone by NADH in the presence of NADH dehydrogenase. The reoxidation of the semiquinone by dioxygen yielded superoxide radical formation. In the absence of enzyme, streptonigrin cannot be reduced by NADH. As can be seen in Figure 3, streptonigrin increased superoxide formation by NADH dehydrogenase in a dose-dependent fashion that appeared to follow saturation kinetics. The formation of superoxide radical was not detected in the presence of superoxide dismutase. This means that the free superoxide radicals formed according to the equation



$$SN^{\bullet-} + O_2 \rightarrow SN + O_2^{\bullet-}$$

were immediately dismutated by the enzyme. For comparison the data obtained with the anthracycline adriamycin have also been reported.

In previous studies, we have shown that the complexation of anthracyclines such as adriamycin, daunorubicin, and carminomycin by metal ions such as Fe(III) and Pd(II) inhibits their reduction by NADH in the presence of NADH dehydrogenase.[19-22] We checked whether the same phenomenon occurred in the case of Pd-SN complex I. The opposite result was obtained: Pd-SN was still reduced by NADH, and moreover, this occurred even in the absence of NADH dehydrogenase and the presence of the enzyme had no effect on the kinetics of the reaction. This conclusion was attested by the following experiments.

(B) Reaction of Pd-SN Complex I with NADH. The addition of NADH to Pd-SN (I) in HEPES buffer gave rise to a shift of the absorption band from 515 to 475 nm (Figure 4). In a typical experiment 100 μM NADH was added to 60 μM Pd-SN in 0.1 M KCl HEPES buffer at pH 7.2. Under those conditions, the shift of the absorption band occurred within 30 s. When the experiment was performed in the *absence of* O_2, the new species (I′) characterized by an absorption band at 475 nm (the solution was brown) and an EPR signal at $g = 2.005$ (Figure 5) was quite stable and no modifications of the spectra were observed within several hours. This result strongly suggests that the quinone has been reduced to a semiquinone. When O_2 *was added to I′*, the EPR signal first changed very rapidly: the negative part of the new signal exhibited two peaks separated by about 15 G. We hereafter labeled as I″ the new species obtained by addition of

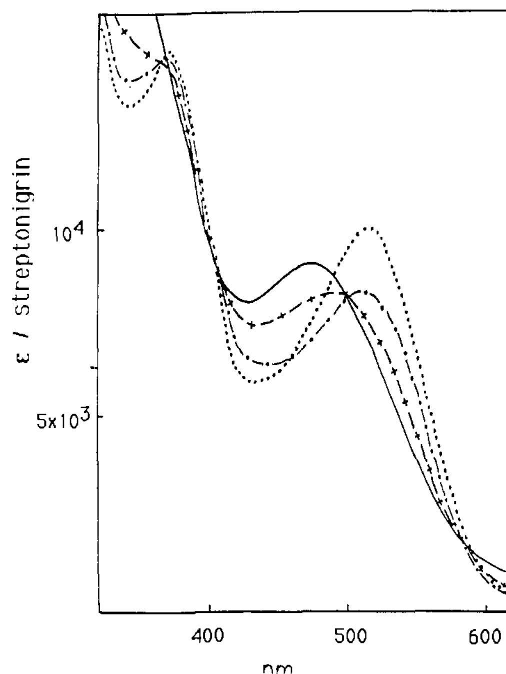


Figure 4. Reaction of Pd-SN complex I with NADH monitored by absorption spectroscopy. Experimental conditions: 60 μM Pd-SN in 0.1 M KCl, 0.01 M HEPES buffer at pH 7.2; in absence of NADH, complex I (···); in presence of 100 μM NADH and O_2, Δt (time after addition of O_2) = 1 min (—), 3 min (-+-), 11 min (-·-). The spectrum of complex I′ obtained by addition of NADH to Pd-SN in the absence of O_2 is similar to that of complex I″ obtained by addition of NADH to Pd-SN in the presence of O_2.

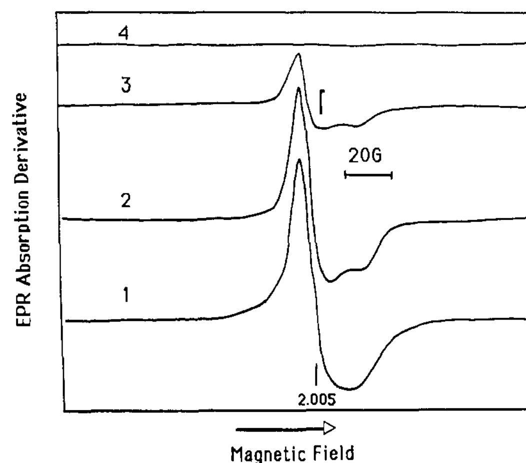


Figure 5. Reaction of Pd-SN complex I with NADH monitored by EPR spectroscopy. Experimental conditions: 200 μM Pd-SN in 0.1 M KCl, 0.01 M HEPES buffer at pH 7.2; in the presence of 600 μM NADH and absence of O_2 (complex I′, line 1). The color of the solution was brown. The spectrum was recorded at liquid-nitrogen temperature. The temperature of the tube was then increased to 25 °C, O_2 was allowed to enter the solution, and the temperature was immediately decrease to that of liquid nitrogen (complex I″, line 2). The solution was then allowed to stand at room temperature in the presence of O_2 for 3 min (line 3) and 10 min (line 4). The color of the solution was red. The apparatus was calibrated by using DPPH as the standard.

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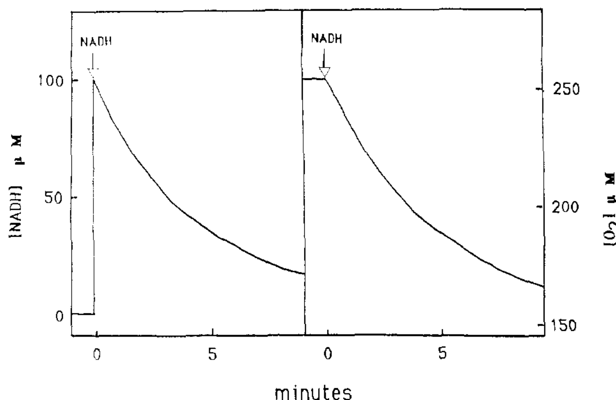

Figure 6. Pd-SN complex I, NADH, O_2 system evolution monitored by NADH oxidation and O_2 consumption. The oxidation of NADH to NAD^+ was monitored spectroscopically at 340 nm (ϵ_{NADH} = 6600, ϵ_{NAD^+} = 0), and the consumption of O_2 was measured as a function of time. Experimental conditions: 100 μM NADH, 67 μM SN-Pd in HEPES buffer pH 7.2 at 25 °C.

O_2 to I′. As time elapsed, the EPR signal characteristic of I″ completely vanished (Figure 5) and the absorption spectrum characteristic of complex I was recovered. The color of the solution was red. At 25 °C these changes occurred within about 10 min. isosbestic points were present at 500 and 410 nm. The original intensity of the absorption spectrum was not fully recovered, and about 15% of the initial intensity was lost during the reaction (Figure 4). This reaction was repeated several times by addition of new amounts of NADH to the same initial solution.

In order to obtain quantitative information on the oxidation of NADH by O_2 catalyzed by SN-Pd complex I, the reaction was monitored by the decrease of the optical density at 340 nm, the consumption of O_2, and the reduction of cyt c^{3+} to cyt c^{2+}.

(C) Pd-SN Complex I, NADH, O_2 System Evolution Monitored by NADH Oxidation and O_2 Consumption. NADH exhibits an absorption band at 340 nm (ϵ = 6600) whereas NAD^+ has no absorption in that region. In a typical experiment 100 μM NADH was added to 67 μM of Pd-SN in HEPES buffer at pH 7.2 in the presence of O_2 at 25 °C. The oxidation of NADH to NAD^+ was monitored spectroscopically at 340 nm, and in a parallel experiment, the O_2 consumption was measured as a function of time. As can be seen in Figure 6, 1 mol of NADH was oxidized when 1 mol of O_2 was consumed. The reaction was not inhibited by the presence of superoxide dismutase.

(D) Pd-SN Complex I, NADH-O_2, cyt c^{3+} System Evolution Monitored by NADH Oxidation, cyt c^{3+} Reduction, and Oxygen Consumption. The reduction of cyt c^{3+} was monitored at 550 nm with the relation $\epsilon(\text{cyt}c^{2+}) - \epsilon(\text{cyt } c^{3+}) = 19600$. In a typical experiment, 77 μM NADH was added to 54 μM Pd-SN in HEPES buffer at pH 7.2 in the presence of 42 μM cyt c^{3+} and O_2 at 25 °C. As can be seen in Figure 7, a two-step process was observed. In the first step, 1 mol of NADH was oxidized/2 mol of cyt c^{3+} reduced. No consumption of O_2 was observed. Once all cyt c^{3+} present was reduced, O_2 consumption was observed and 1 mol of NADH was oxidized when 1 mol of O_2 was consummed.

Antitumor Activity of Pd-SN. The in vitro inhibition of P-388 leukemia cell growth by Pd-SN was compared with that induced by the free drug. An ID_{50} value equal to 0.05 μg/mL was found for both compounds.

Discussion

Our data show that, depending on the conditions, Pd(II) forms several complexes of 1:1 stoichiometry with streptonigrin. Let us first focus on complexes I and II, which are obtained by addition of Pd(II) to streptonigrin at pH 7.2 and 5, respectively. Potentiometric titrations have shown that free streptonigrin exhibits two titrable functions in the pH range 3-9.[18] The first one has a pK_a equal to 4.4 and can be assigned to the carboxylic function on the C ring. This deprotonotation is accompanied by spectral changes, which have been assigned to a transition of the molecular state from an associated to a monomeric one.[18] This assignment

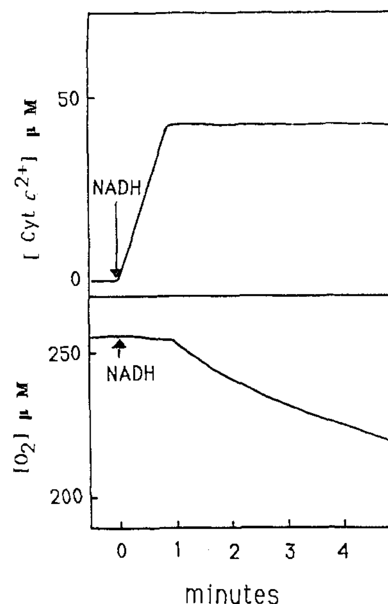

Figure 7. Pd-SN complex I, NADH, cyt c^{3+}, O_2 system evolution monitored by cytochrome *c* reduction and O_2 consumption. The reduction of cyt c^{3+} to cyt c^{2+}, monitored spectroscopically at 550 nm, and the consumption of O_2 were measured as a function of time. Experimental conditions: 77 μM NADH, 54 μM Pd-SN, 42 μM cyt c^{3+} in HEPES buffer pH 7.2 at 25 °C.

was based on an X-ray diffraction study of streptonigrin, showing that ring A is near the carboxyl group of a symmetry-related molecule.[23] A carboxyl oxygen is thus almost at the midpoint between quinone rings of different molecules related by the *c*-axis translation. The second titrable function, which exhibits a pK_a equal to 6.6, is a priori more difficult to assign. Moreover, the deprotonation of this function does not give rise to spectral modification. It is thus likely that, in aqueous solution at pH lower than 3.5, streptonigrin is neutral and thus insoluble. At pH 5.5 streptonigrin is most likely once negatively charged, and it is twice negatively charged at pH higher than 7. Formation of complex II at pH 5.5 gives rise to the release of one proton/molecule, and it is most likely that this proton is released by the second titrable function. Taking into account the fact that Pd(II) forms predominantly square-planar complexes and binds strongly to N ligands, we can suggest that the site of coordination of Pd(II) to streptonigrin involves the nitrogen of the pyridine B ring and the amine nitrogen of the pyridine C ring (which loses a proton through coordination). This structure was first proposed by Hajdu and Armstrong[15,24] for a Cu(II)-SN complex. Moreover, such a coordination would explain the presence of a new absorption band at 500 nm ($\epsilon = 5 \times 10^3$), which can be assigned neither to a d-d transition nor to a charge-transfer transition. In fact, such a coordination of the metal ion to streptonigrin can promote π-electronic delocalization over the B and C rings. The band at 500 nm can thus tentatively be assigned to a $\pi \rightarrow \pi^*$ transition. At pH 5.5 the two other positions of the coordination square are most likely occupied by Cl^- and/or H_2O ligands. The transition from complex II to complex I (λ_M = 515 nm, $\epsilon_M = 10^4$), which occurred at pH 6, may involve the deprotonation of a Pd(II)-ligated water molecule. This suggestion is based on the observation that, in a complex such as $[enPd(H_2O)_2]^{2+}$, the deprotonation of a ligated water molecule occurs at a pH value around 6.[25] We have recently reported the preparation and spectroscopic characterization of a 1:1 Au(III)-SN complex.[18] The absorption of this gold complex exhibits a strong absorption band at 450 nm, which compares with that observed in the absorption spectra of the Pd-SN complex. We have suggested the same site of coor-

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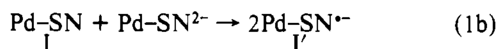
dination for both metal ions to streptonigrin.

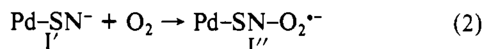
Consider now the redox and catalytic properties of complex I. The addition of NADH to Pd–SN (I), which is EPR silent, in the absence of O_2 yields complex I′. The EPR spectrum of complex I′ is characteristic of the presence of a free radical, indicating that the quinone group of streptonigrin has been reduced by NADH to semiquinone according to eqs 1a and 1b.

$$\underset{\text{I}}{\text{Pd–SN}} + \text{NADH} \rightarrow \text{Pd–SN}^{2-} + \text{NAD}^+ + \text{H}^+ \quad (1a)$$



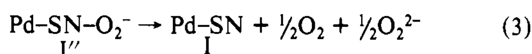
$$\underset{\text{I}}{\text{Pd–SN}} + \text{Pd–SN}^{2-} \rightarrow 2\underset{\text{I}'}{\text{Pd–SN}^{\bullet-}} \quad (1b)$$

In the absence of oxygen, complex I′, involving the semiquinone, is very stable. The addition of molecular oxygen to complex I′ yields a new complex (I″) that we suggest to be a superoxo–Pd(II) complex.



$$\underset{\text{I}'}{\text{Pd–SN}^-} + \text{O}_2 \rightarrow \underset{\text{I}''}{\text{Pd–SN–O}_2^{\bullet-}} \quad (2)$$

Complex I″ is not stable and as time elapses decomposes to give complex I most likely according to eq 3. Equations 1–3 are

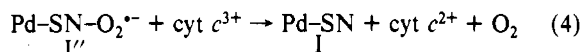


$$\underset{\text{I}''}{\text{Pd–SN–O}_2^-} \rightarrow \underset{\text{I}}{\text{Pd–SN}} + \tfrac{1}{2}\text{O}_2 + \tfrac{1}{2}\text{O}_2^{2-} \quad (3)$$

compatible with the observation that, in the system Pd–SN, NADH, O_2, 1 mol of NADH is reduced/mol of O_2 consumed: the combination of eqs 1–3 yields

$$\text{NADH} + \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}^+ + \text{O}_2^{2-}$$

When the same experiment is performed in the presence of cyt c^{3+}, the first two steps of the reaction still occurred according to eqs 1 and 2. However, in the third step complex I″ reduces cyt c^{3+} according to



$$\underset{\text{I}''}{\text{Pd–SN–O}_2^{\bullet-}} + \text{cyt } c^{3+} \rightarrow \underset{\text{I}}{\text{Pd–SN}} + \text{cyt } c^{2+} + \text{O}_2 \quad (4)$$

Equations 1, 2, and 4 are compatible with the observation that in the system Pd–SN, NADH, cyt c^{3+} no oxygen is consumed overall and 2 mol of cyt c^{3+} is reduced when 1 mol of NADH is oxidized: the combination of eqs 1, 2, and 4 yields

$$\text{NADH} + 2\text{cyt } c^{3+} \rightarrow \text{NAD}^+ + 2\text{cyt } c^{2+} + \text{H}^+$$

These equations perfectly explain our data. Moreover, the reduction of cyt c^{3+} by Pd–SN–$O_2^{\bullet-}$ is in agreement with previous observations that reduction of cyt c^{3+} is not restricted to free O_2^-.[26] We must add that cyt c^{3+} is not reduced by complex I′. On the other hand, the observation that eqs 3 and 4 are not inhibited by superoxide dismutase is also in agreement with the fact that this enzyme is highly selective and is able to dismute free superoxide radical and not superoxide radical bound to the complex.

In this work we have shown that, in the absence of O_2, Pd–SN is able to react with NADH, while there is no reaction of NADH with free streptonigrin. In the absence of O_2, Pd–SN is able to catalyze the oxidation of NADH by O_2 whereas free streptonigrin is unable to do that in the absence of NADH dehydrogenase.

In conclusion, our data provide direct evidence that streptonigrin can form with metal ion complexes that are able to activate oxygen.

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Mechanism of Catalase Activity in Aqueous Solutions of Dimanganese(III,IV) Ethylenediamine-*N*,*N*′-diacetate

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Manganous ions, ligated by ethylenediamine-*N*,*N*′-diacetate (edda = L) decompose hydrogen peroxide with a rate law $-d[H_2O_2]/dt = k_{17}[\text{Mn(edda)}][H_2O_2]$ where $k_{17} = 5.4\ M^{-1}\ s^{-1}$ at pH 7. The reduction of peroxide to water is initiated by the reaction of $Mn^{II}L$ with a dinuclear $Mn^{III,IV}L_2$. A subsequent fast reaction between the transient product of this reaction and hydrogen peroxide or *tert*-butyl hydroperoxide effectively oxidizes Mn(II) to Mn(IV) in a concerted step without formation of the hydroxyl radical. The green mixed-valence complex, which is probably a bis(μ-oxo)-bridged structure, is stable in neutral aqueous solution and exhibits a 16-line ESR signal in frozen solution. The basis of catalase activity is the autocatalytic formation of this complex when hydrogen peroxide is reduced by manganese(II) according to the overall reaction $Mn^{II}L + Mn^{III}L + H_2O_2 \xrightarrow{Mn^{III,IV}L_2} Mn^{III,IV}L_2$. The catalase cycle is independent of the formation of oxy radicals. Mononuclear Mn^{III}edda and Mn^{II}edda react with superoxide radicals, but the decomposition of peroxide is virtually independent of these reactions. In unbuffered solutions, with a moderate excess of hydrogen peroxide, an oscillation in the concentration of the dinuclear complex is detected.

Introduction

A multinuclear manganese unit is well-known to be central to the oxidation of water to O_2 during photosynthesis.[1] A less investigated biological use of multinuclear manganese is in the catalase of certain bacteria, that of *Lactobacillus* plantarum having been the most studied.[2] Many manganese complexes have been investigated for structural[3] as well as functional[4] similarities to these systems. The essential step in an efficient catalase must be the reduction of hydrogen peroxide without the formation of the OH radical, which attacks organic materials at near-diffusion-controlled rates. In general, mononuclear manganous com-

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