Bifunctional Antitumor Compounds: Synthesis and Characterization of a Au(III)-Streptonigrin Complex with Thiol-Modulating Properties

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In order to synthesize bifunctional antitumor compounds, the interaction of streptonigrin with $[AuCl_4]^-$ has been studied. Using absorption, circular dichroism, and fluorescence measurements, we have shown that streptonigrin forms with Au(III) a 1:1 Au(III)-streptonigrin complex. This complex is very stable as long as gold is in the trivalent state and is able to inhibit glutathione reductase activity. In the presence of biological agents such as NADH and reduced glutathione, Au(III) is slowly reduced to Au(I) and removed from its binding site to streptonigrin. Original streptonigrin is thus recovered. This complex exhibits antitumor activity against P-388 leukemia which compares with that of the free drug.

All cells have a certain natural capacity for protection against cytotoxic insults, either by detoxifying the toxic agent before the damaging event or by repairing the damage after it has occurred. It has become increasingly clear that glutathione plays a vital role in protecting cells from a variety of agents, including X-rays and a wide variety of chemotherapeutic drugs,[1-4] and also against cytolysis by granulocytes and activated macrophages, which is an oxygen-dependent process.[5-8] On the other hand, several groups have recently shown that human tumor cells contain an extremely high concentration of glutathione, and it has been suggested that the tumor glutathione cycle can be a rate-limiting factor in cytotoxicity.

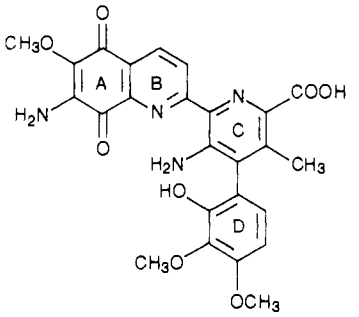
The mechanisms by which glutathione protects cells are still poorly understood. The following observations have been reported: (i) interruption of the glutathione redox cycle by inhibition of glutathione reductase or glutathione peroxidase markedly sensitized tumor cells to peroxide-mediated lysis,[8] and glutathione depletion sensitizes tumor cells to oxidative cytolysis;[9] (ii) an inverse relationship appears to exist, in some tumor cells, between the extent of cellular damage brought about by the intracellular formation of superoxide anion occurring on reaction of O_2 with free drug radical and the efficiency of the glutathione-mediated H_2O_2-detoxyfying system.[10]

These observations prompt us to report the synthesis of a bifunctional compound obtained by interaction of streptonigrin with Au(III). Streptonigrin (SN) (Chart I), a highly substituted and highly functionalized 7-aminoquinoline-5,8-dione, first isolated from *Streptomyces floculus*,[11] is an antitumor agent that has seen only limited use as an anticancer agent because of its toxicity[12-14] but continues to receive attention because of interest in its ability, common to a number of quinone antibiotics, to degrade DNA.[15]

Single-strand cleavage of CCC-DNA (covalently closed circular DNA) in vitro has been demonstrated, and the reaction, which was shown to be oxygen dependent, also requires the presence of a reducing agent.[16] The facts that the free-radical scavengers catalase and superoxide dismutase both inhibit the DNA degradation led Lown et al.[16] to propose that free radicals, generated from reduced streptonigrin and oxygen, are the principal species that initiate attack on the DNA.

In this paper we report the synthesis of a bifunctional compound by association of streptonigrin with Au(III). This compound exhibits thiol-modulating properties: (i) it inhibits glutathione reductase; (ii) in the presence of reducing agents such as NADH or reduced glutathione, Au(III) is slowly reduced to Au(I) and removed from its binding site to streptonigrin. Free streptonigrin is then recovered and able to exert its cytotoxic action.

Chart I



streptonigrin

Materials and Methods

Purified streptonigrin was a gift from Rhône-Poulenc Laboratory and National Cancer Institute. Contrarily to what has been previously reported, streptonigrin is soluble in water. However, it is necessary to first increase the pH up to about 7 to ensure dissolution; the pH of the solution can then be decreased down to 3.8. At the lower pH value a precipitate appears. Solution

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concentrations were currently determined by using $\epsilon_{365} = 14\,200$ at pH 7.2. The value of ϵ at 365 nm was first of all determined from 10 solutions prepared from accurately weighed amounts of streptonigrin.

Aqueous stock solutions of Au(III) in the presence of 0.2 M KCl were prepared from $HAuCl_4 \cdot 3H_2O$ supplied by Strem Laboratories. Metal contents of solutions were analyzed on a Perkin-Elmer 360 atomic absorption flame spectrophotometer. All samples were assayed against serial dilutions of reference standards.

Unless otherwise stated, sodium acetate/acetic acid buffer (0.01 M) was used to buffer the systems at pH between 4.8 and 5.5 as desired, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was used at pH 7.2.

Cytochrome *c* (type VI from horse heart), NADH (grade III), cardiac NADH dehydrogenase, superoxide dismutase (SOD), NADPH (type I), reduced glutathione (GSH) and oxidized glutathione (GSSG) (grade IV), and glutathione reductase (type IV) from Bakers yeast, as a suspension in 3.6 M $(NH_4)_2SO_4$ at pH 7.0 (200 units/mg of protein), were purchased from Sigma Chemical Co. All other reagents were of the highest quality available, and deionized bidistilled water was used throughout these experiments.

Absorption spectra were recorded on a Cary 219 spectrophotometer and circular dichroism (CD) spectra on a Jobin Yvon dichrograph Model Mark V. 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 $\Delta\epsilon$ are expressed in terms of [SN], the molar concentration of streptonigrin. Uncorrected fluorescence spectra were recorded at 20 °C on a Jobin Yvon JY3C spectrofluorometer. Potentiometric measurements were obtained with a Metrohm pH meter, Model E603, at 25 °C using a Metrohm EA 147 combined glass electrode.

NADH Dehydrogenase Assay. NADH dehydrogenase activity was determined at 25 °C by modification of a method described previously[17] 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 equal to 19600. The reaction mixture contained 0.05 M HEPES buffer at pH 7.2, 120 μM cytochrome *c*, 50 μM NADH, 12 units/L NADH dehydrogenase, and either 0 or *C* μM free or complexed streptonigrin (*C* was varied from 0 to 75 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 of 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 calculated from the rate of cytochrome *c* reduction inhibited by SOD (20 μg/mL).

Glutathione Reductase Assay. Glutathione reductase activity was determined at 25 °C by the classical procedure[18] using NADPH as electron donor. Streptonigrin and its complex were assayed for their ability to inhibit glutathione reductase by following NADPH oxidation at 340 nm. The molar extinction coefficient of reduced NADPH was taken equal to 6600. The reaction mixture contained 3 mM GSSG, 0.1 mM NADPH, about 25 units/L glutathione reductase, and either 0 or *C* μM free or complexed streptonigrin (*C* was varied from 0 to 60 μM). The reaction was initiated by addition of the enzyme. Enzymatic activity is the amount of enzyme that reduces 1 μmol of GSSG/min at pH 7.2 and 25 °C under the reaction conditions specified above.

In Vitro Inhibition of P-388 Leukemia Cell Growth. P-388 cells can be 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.

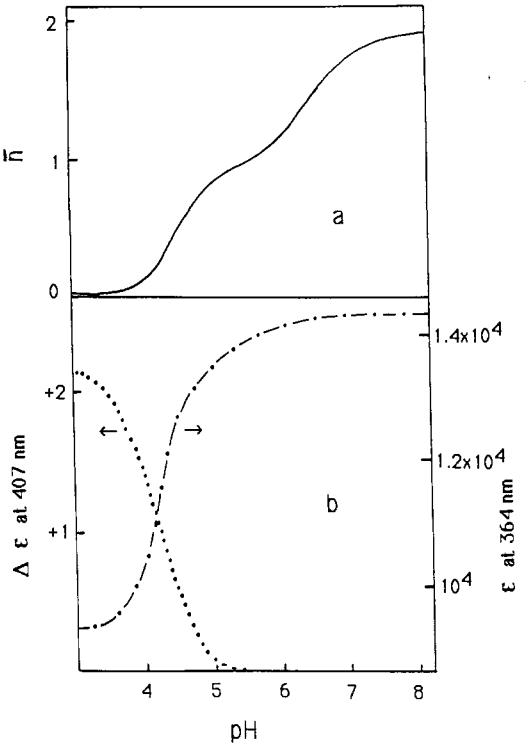

Figure 1. Potentiometric and spectroscopic titration of streptonigrin. (a) $\bar{n}$, the number of protons released per streptonigrin, has been plotted as a function of pH. (b) ϵ at 364 nm and $\Delta\epsilon$ at 407 nm have been plotted as a function of pH. Experimental conditions: 100 μM streptonigrin in 0.15 M KCl aqueous solution at 25 °C.

Results

Spectroscopic and Potentiometric Characteristics of Streptonigrin. In order to accurately characterize the physicochemical properties of streptonigrin, we first performed potentiometric and spectroscopic titrations of an aqueous solution of 100 μM SN in the presence of 0.15 M KCl. As can be seen in Figure 1a, in the pH range 3–8 two protons were released with pK_a equal to 4.7 and 6.6, respectively. Previous data have reported the pK_a of free ligand equal to 6.5 in 1:1 dioxane–water mixture.[19] Spectroscopic titrations performed under similar conditions using absorption and circular dichroism are shown in Figures 1b and 2. As can be seen in Figure 2, at pH 3.5 when streptonigrin was fully protonated, the absorption spectrum exhibited a band at 375 nm ($\epsilon_M = 8200$); the CD spectrum, in the same wavelength range, exhibited a weak signal of the couplet type centered at 380 nm. When the pH was increased up to 5.5, a blue shift of the absorption band to 364 nm ($\epsilon = 14\,200$) was observed. Concomitantly, the CD signal of the couplet type changed to a negative band at 370 nm. A further increase of the pH up to 8 did not give rise to modification either in the absorption or in the CD spectrum of streptonigrin. ϵ at 364 nm and $\Delta\epsilon$ at 407 nm have been plotted as a function of pH (Figure 1b). In both cases a pK_a equal to 4.3 was observed which can be assigned to deprotonation of the carboxyl group. Modifications of the CD signal were also observed as a function of the concentration. In HEPES buffer at pH 7.2, a 750 μM solution of streptonigrin exhibited a CD

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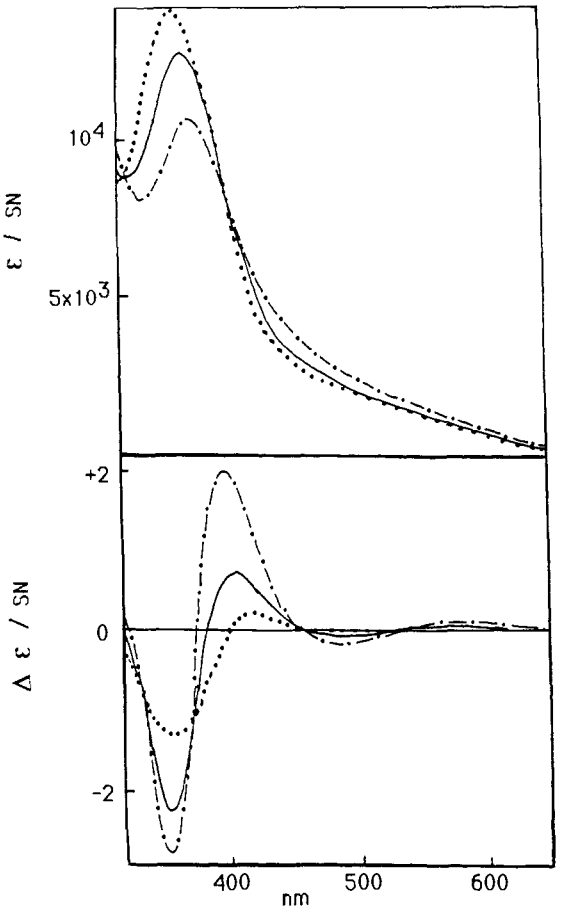

Figure 2. Absorption (upper) and circular dichroism (lower) spectra of streptonigrin at various pH values: 3.5 (-·-); 4.5 (—); 5.2 (···). Experimental conditions: 100 μM streptonigrin in 0.15 M KCl aqueous solution at 25 °C.

signal of the couplet type centered at 380 nm. When the concentration was decreased down to 25 μM, the CD signal of the couplet type changed to a negative band at 370 nm.

According to Sidman,[20] three regions of absorption are common to nearly all of the *p*-quinones. They are around 450, 340, and 250 nm and are assigned to $^1U \leftarrow {}^1A$, $^1L_b \leftarrow {}^1A$, and $^1B_b \leftarrow {}^1A$ transitions, respectively. The band observed at 364 nm in the absorption spectrum of free streptonigrin can thus be assigned to a $^1L_b \leftarrow {}^1A$ transition of the quinoid ring (A ring).

X-ray diffraction study of streptonigrin cocrystallized with ethyl acetate has shown that rings A–C, including the carboxyl group on ring C, are very nearly coplanar.[21] This configuration is partially maintained by a short, probably bent, hydrogen bond between the NH_2 of ring C and the N atom within ring B. Some intermolecular contacts are suggestive of n···π interactions. Ring A is near the carboxyl group of a symmetry-related molecule. A carboxyl oxygen is almost at the midpoint between quinone rings of different molecules related by the *c* axis translation and is 3.4 Å from the ring. If this is an n···π interaction, it is surely weak.

These molecular and crystal structures can be related to our spectroscopic observations. At low pH value (i.e., 3.5) the carboxyl group is protonated, the streptonigrin molecule is then neutral, and it is very likely that intermolecular associations, similar to that observed in the crystal state, are present and responsible for the CD

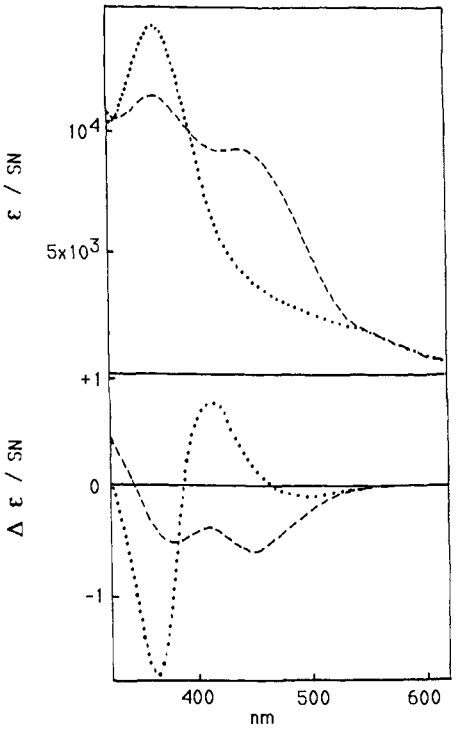

Figure 3. Absorption (upper) and circular dichroism (lower) spectra of Au–SN complex (--). 100 μM $[AuCl_4]^-$ was added to 100 μM streptonigrin in 0.01 M acetate buffer/0.15 M KCl at pH 4.7. Spectra were recorded 1 h after mixing. For comparison, absorption and circular dichroism spectra of free streptonigrin (···), recorded under the same conditions, are shown.

spectrum of the couplet type associated to the transition of the quinoid (A) ring. An increase of the pH from 3.5 to 5.5 gives rise to the deprotonation of the carboxyl function; the association state of the molecules is thus destroyed, and the molecules are in monomeric state. The CD signal of the couplet type at 370 nm thus disappears. This can also explain the blue shift of the absorption band. Such intermolecular associations are also responsible for the modification of the CD spectrum as a function of concentration.

Interaction of SN with $[AuCl_4]^-$. The addition of an aqueous solution of $[AuCl_4]^-$ to streptonigrin in acetate buffer in the pH range 4.7–5 gave rise to complex formation. Buffer solution was used because, $[AuCl_4]^-$ solution being very acid (pH = 3), its addition to an unbuffered solution of streptonigrin yielded a strong decrease of the pH. Under those conditions, no complex formation could be observed. On the other hand, we observed that if the reaction was performed in pH 7 HEPES buffer, formation of gold hydroxide took place and partly prevented complex formation. Moreover, according to the dependence of the distribution of hydrolyzed $[AuCl_4]^-$ complexes on the concentrations of H^+ and Cl^- ions[22] at pH 4.7 in the presence of 0.15 M KCl (pH + pCl = 5.4) about 90% of Au(III) is present as $[AuCl_4]^-$ and 10% as $[AuCl_3OH]^-$. The best data concerning complex formation were obtained by using 0.01 M acetate buffer in the pH range 4.7–5, in the presence of 0.15 M KCl. Complex formation was attested by the appearance of a new band at 450 nm in the absorption and CD spectra. In order to determine the stoichiometry of the complex formed, the molar ratio of $[AuCl_4]^-$ 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 ob-

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served when the molar ratio was varied from 1 to 2. Isosbestic points were present at 400 and 334 nm, and an isodichroic point was present at 385 nm. We observed that complex formation did not occur at once after mixing but within about 30 min. From these data we inferred that a complex of 1:1 Au:SN stoichiometry was formed. Figure 3 shows the absorption and CD spectra of the complex obtained by addition of 100 μM $[AuCl_4]^-$ to 100 μM streptonigrin in 0.15 M KCl/0.02 M acetate buffer. Spectra were recorded 1 h after mixing.

Once formed, the Au–SN complex was stable in acetate buffer, pH 5, for about 2 h, and then, as time elapsed, decomposition of the complex was observed that was characterized by a decrease of the absorption band at 450 nm. However, this decomposition did not occur when the complex, once formed in acetate buffer, pH 5, was subsequently put in HEPES buffer, pH 7.2. Such solutions were stable for more than a week.

As can be seen in Figure 3, the absorption band at 370 nm is only slightly modified through complexation of streptonigrin to gold. On the other hand, the CD-negative band at 370 nm, characteristic of streptonigrin in the monomeric state, is present in the CD spectrum of the complex.

Moreover, the weak fluorescence emission of streptonigrin obtained through excitation at 370 nm is not modified through complexation. All these data strongly suggested that the A quinoid ring is not involved in the complexation to gold.

It is well-known that Au(III) is a strong oxidizing agent and that in numerous cases the addition of Au(III) to a ligand yields Au(I) complex; in the absence of reducing agent the reaction involves the oxidation of the ligand. The following experiments were performed in order to determine (i) the oxidation state of gold in the Au–SN complex and (ii) whether the ligand streptonigrin has been chemically modified or not through reaction with $[AuCl_4]^-$.

Reaction of CN^- with Au–SN Complex. For testing the reversibility of the reaction, small amounts of KCN solution were added to Au–SN in pH 5 acetate buffer solution. Aqueous solution of 1 M KCN was used; the pH of this solution was adjusted to the pH of the reaction mixture with HCl. Addition of a large excess of KCN to Au–SN, with the molar ratio of CN^- to Au–SN equal to 300, reversed the spectrum and gave rise to the original spectrum of streptonigrin. This was checked by the decrease of the absorption band at 450 nm and the increase of that at 370 nm. About 80% of the absorption spectrum characteristic of free streptonigrin was thus recovered. Analogous data were obtained when the reaction was performed at pH 9.

Reaction of Au–SN with Reduced Glutathione. The addition of glutathione in the oxidized form (GSSG) to Au–SN in HEPES buffer did not yield any modification of the Au–SN spectra. However, the addition of glutathione in the reduced form (GSH) yielded very interesting data. In a typical experiment GSH was added to 60 μM Au–SN in 0.1 M HEPES buffer/0.15 M KCl at pH 7.2. The molar ratio of GSH to Au–SN was varied from 0 to 4. The reaction of GSH with Au–SN was characterized by a decrease of the absorption band at 450 nm and an increase of that at 370 nm. The plot of the ratio $\epsilon_{360}/\epsilon_{450}$, as a function of the molar ratio of GSH to Au–SN, reached a plateau at a molar ratio of GSH to Au–SN equal to 3:1. About 80% of the absorption spectrum characteristic of free streptonigrin was thus recovered. The reaction was time dependent, and at GSH:Au–SN equal to 3:1, 50% of the reaction was achieved within 20 min.

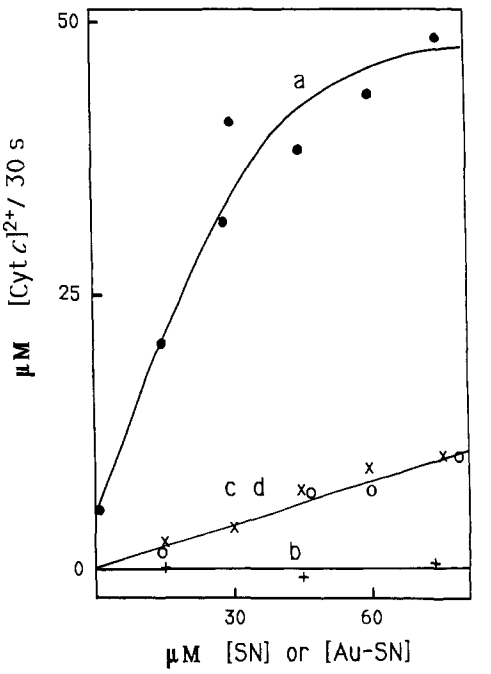

Figure 4. Reduction of streptonigrin and Au–SN by NADH. The concentration of cytrochrome *c* reduced during the first 30 s of the reaction has been plotted as a function of streptonigrin concentration in the presence (curve a) or in the absence (curve b) of enzyme, and as a function of Au–SN concentration in the presence (×) and in the absence (○) of enzyme. Experimental conditions: 0.1 M HEPES buffer, pH 7.2, 50 μM NADH, 120 μM cyt *c*, and 12 units/mL NADH dehydrogenase.

In the reduction of Au(III) with cysteine, the conventional reaction requires 3 mol of cysteine/mol of Au(III):[23]

$$3RSH + Au(III) \rightarrow RS\text{–}Au(I) + RS\text{–}SR + 3H^+$$

The observations that (i) Au–SN did not react with oxidized glutathione and (ii) three molecules of GSH were required to ensure the decomplexation of gold to its binding site to streptonigrin strongly suggest that gold is complexed to streptonigrin as Au(III). We thus proposed the occurrence of the reaction

$$Au(III)\text{–}SN + 3GSH \rightarrow SN + Au(I)\text{–}GS + GS\text{–}SG + 3H^+$$

The presence of Au(III) in the Au–SN complex was corroborated by the observation that Au(I) did not react with streptonigrin.

Reaction of Au–SN with NADH. The reduction of streptonigrin by NADH is catalyzed by NADH dehydrogenase. Streptonigrin reduced to semiquinone by the enzyme is reoxidized by molecular oxygen, yielding the formation of the radical superoxide. Figure 4 shows the amount of cyt c^{3+} reduced during the first 30 s of the reaction, as a function of streptonigrin concentration. As can be seen, streptonigrin increased superoxide formation by NADH dehydrogenase in a dose-dependent fashion that appeared to follow saturation kinetics. The reaction was inhibited by SOD. Similar experiments were performed in the presence of Au–SN complex (Figure 4). As can be seen, the kinetics of reduction of cyt c^{3+} was about 7 times slower in the presence of Au–SN than in the presence of free streptonigrin. Moreover, the kinetics of reduction of cyt c^{3+} in the presence of Au–SN did not depend on the presence of NADH dehydrogenase. This reaction was not

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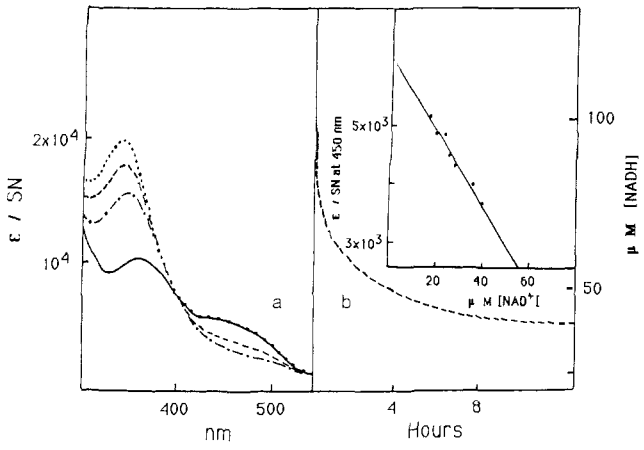


Figure 5. Reduction of Au-SN by NADH. (a) Absorption spectra of 60 μM Au-SN in 0.15 M KCl/HEPES buffer at pH 7.2 in the absence (—) and in the presence of 100 μM NADH; the time elapsed after the addition of NADH was 6 min (···), 2 h (- - -), and 5 h (-·-). (b) The concentration of NADH has been plotted as a function of time. (b, inset) ε/SN at 450 nm has been plotted as a function of the concentration of NAD^+ present.

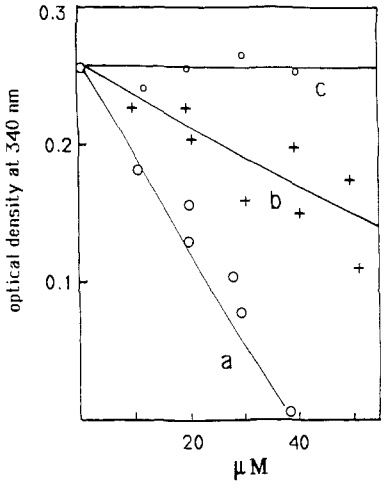


Figure 6. Inhibition of glutathione reductase activity by SN, Au-SN, and Au-GS. The variation of the optical density at 340 nm has been plotted as a function of Au-SN (curve a), SN (curve b), and Au-GS (curve c) concentrations. Experimental conditions: 100 μM NADPH, and 3 mM GSSG, 25 units/L GR in 0.15 M KCl/HEPES buffer, pH 7.2 at 25 °C. The lines drawn have been squares fitted to the data. The correlation coefficients are −0.98 and −0.91 for curves a and b, respectively.

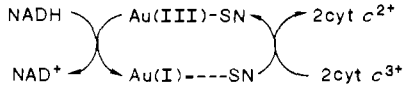
inhibited by the presence of superoxide dismutase, suggesting that O_2^- was not involved in that reaction. We have checked that Au-SN was not able to reduce cyt c^{3+} in the absence of NADH.

We then followed the reduction of Au-SN by NADH in the absence of NADH dehydrogenase. In a typical experiment 60 μM Au-SN in HEPES buffer was added to 100 μM NADH. The reaction was monitored spectroscopically (Figure 5a) by using the observations that (i) the absorption spectra of NADH exhibits a band at 336 nm (ϵ_M = 6600) whereas NAD^+ has no absorption band in that region and (ii) the absorption spectra of streptonigrin and Au-SN exhibit an isosbestic point at 334 nm. The concentration of NADH present in the solution was thus determined as a function of time. At the end of the reaction (50% of the reaction was achieved within 30 min) 60 μM NADH was oxidized to NAD^+ and the spectrum of free streptonigrin was recovered. The concentration of NADH has been plotted as a function of time in Figure 5b. As can be seen, there is a linear relationship between ϵ_{450} and the concentration of NAD^+ (Figure 5b, inset). We can infer that one Au(III) ion is reduced to Au(I) by one NADH molecule and released from its binding site to streptonigrin. The following equation accounts for these observations.

$$\text{Au(III)-SN} + \text{NADH} \rightarrow \text{Au(I)} + \text{SN} + \text{NAD}^+ + \text{H}^+$$

Similar experiments were performed with $[AuCl_4]^-$ instead of Au-SN. One molecule of NADH was required to reduce one Au(III) ion. However, the rate of the reaction was faster than in the presence of Au-SN, and 50% of the reaction was achieved within 1 min.

All these data suggest that when Au(III) is reduced to Au(I), it is released from its binding site to streptonigrin. However, if an oxidizing agent such as cyt c^{3+} is present, Au(I) is reoxidized to Au(III) and remains bound to streptonigrin. The Au-SN complex can thus act as a catalyzing agent according to the following reaction scheme.



Inhibition of Glutathione Reductase by Au-SN. Glutathione reductase catalyzes the reduction of oxidized glutathione by reduced nicotinamide adenine dinucleotide phosphate (NADPH). The rate of absorbance decrease at 340 nm provides a measure of enzyme activity. Figure 6 shows the variation of enzyme activity as a function of Au-SN, Au-GS, and free SN concentrations, respectively. As can be seen, Au-SN inhibited the reaction, and streptonigrin also had an inhibitor effect, but to a smaller extent, whereas Au-GS had no effect at all.

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

Interaction of Au-SN with Albumin. In the blood serum, gold is bound principally to albumin. More precisely, the gold(I) is bound to a cysteine residue (Cys-34) located in a crevice.[24] In order to determine whether Au-SN could be injected into plasma without releasing the metal ion, we incubated for several hours 60 μM Au-SN in the presence of 640 μM albumin in pH 7.2 HEPES buffer. No modification of the 450-nm absorption band characteristic of Au-SN complex was observed.

Discussion

Our data show that streptonigrin forms with Au(III) a 1:1 Au-SN complex. The complex appears to be very stable as long as gold is in the trivalent state: thus a 300-fold excess of CN^- over Au-SN is required to remove gold from its binding site to streptonigrin.

Concerning the binding site of Au(III) to streptonigrin, we can make the following remarks: (i) It is well-known that Au(III) forms predominantly square-planar complexes and binds strongly to N ligands. (ii) Our spectroscopic data strongly suggest that the quinoid A ring is not involved in the coordination to Au(III). (iii) In the solid state, rings A–C, including the carboxyl group on ring C, are very nearly coplanar while ring D is tilted out of the plane; this conformation is more likely still present in solution and at the origin of the optical activity; the observation that the complex is optically active strongly suggests that the D ring is still lying out of the plane of the A–C rings and is not involved in coordination to metal ion and that Au(III) complexation does not alter the conformation of the

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streptonigrin molecule. This led us to suggest that nitrogen atoms are involved in the coordination to Au(III). On the other hand, the absorption spectrum of Au–SN exhibits a band at 450 nm (ϵ = 7500) which can be assigned neither to a d–d nor a charge-transfer transition. This strongly suggests that metal complexation occurs at the level of the nitrogen atom or the B ring and either at the nitrogen atom of the C ring or the amino group of the C ring. Such coordination has already been suggested by Hajdu and Armstrong[19] for Cu–SN and Zn–SN complexes. In the present case we can assume that the coordination square is completed by Cl^- and/or OH^- ligands. Such a coordination of the metal ion to streptonigrin can promote π-electronic delocalization over the B and C rings. The band at 450 nm (ϵ = 7500) which appears in the absorption spectrum of Au–SN can thus tentatively be assigned to a $\pi \rightarrow \pi^*$ transition. Such a type of coordination has also been suggested for the complex formed through interaction of streptonigrin with Pd(II).[25]

Glutathione is important in protecting cells against a number of toxic species including H_2O_2 and $^{\bullet}OH$. It accomplishes this protection through a number of independent mechanisms. To accomplish its protective role, it must be maintained in its reduced state. This is accomplished by the enzyme glutathione reductase. In addition to this direct reactivity, glutathione is an important substrate for two enzymes, glutathione peroxidase and glutathione transferase. Glutathione concentration in tissues ranges from 1 to 20 mM, and because of this very high concentration, the direct reactivity of glutathione can be important in modulating the action of commonly used antitumor compounds.

On the other hand, it has been shown that GSH depletion of P-388 leukemia cells markedly enhanced their sensitivity to lysis by a flux of H_2O_2. Such cells could be lysed by stimulated granulocytes and activated macrophages which were ineffective against untreated cells.[9]

In this context Au–SN complex exhibits the following interesting properties: (i) it exhibits antitumor properties which compare with those of the free drug; (ii) it inhibits glutathione reductase, and this can yield glutathione depletion in tumor cells; and (iii) in the presence of GSH, Au(III) is very slowly reduced to Au(I) and removed from its binding site to streptonigrin, releasing free streptonigrin. This strongly suggests that, inside the cell, the complex should be able, in a first step, to modulate the glutathione cycle by inhbiting glutathione reductase and, in a second step, free streptonigrin, which would have been recovered through reduction of Au(III) to Au(I), should be able to exert its cytotoxic action.

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(±)-7-Chloro-8-hydroxy-1-(4′-[^{125}I]iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine: A Potential CNS D-1 Dopamine Receptor Imaging Agent

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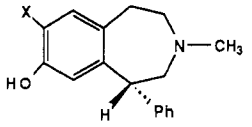
Synthesis, radiolabeling, and in vitro and in vivo properties of an iodinated benzazepine, (±)-7-chloro-8-hydroxy-1-(4′-[^{125}I]iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, [^{125}I]FISCH, as a potential imaging agent for evaluation of central nervous system (CNS) D-1 dopamine receptors in humans, were investigated. After an iv injection, this benzazepine derivative showed good brain uptake in rats (2.27, 1.40, 0.55 % dose/whole brain at 2, 15, and 60 min, respectively). The striatum/cerebellum ratio was high (2.47 at 60 min after the injection). The binding affinity of this agent in rat striatum tissue preparation displayed a K_d of 1.43 ± 0.15 nM. Competition data (in vitro) showed the following rank order of potency: SCH-23390 > (±)-FISCH > (±)-IBZP ≫ apomorphine > WB 4010 > ketanserin ≈ spiperone. The preliminary data suggest that the agent is highly selective for the CNS D-1 receptor.

On the basis of the ability of agonists and antagonists to discriminate between two different distinct dopamine receptors, designated as D-1 and D-2, it is generally accepted that there are two subtypes of dopamine receptors.[1-5] These two subtypes of dopamine receptors exert a synergistic effect on the activity of central nervous system (CNS) dopaminergic neurons in rats.[6,7] More recently, many reports have suggested that D-1 and D-2 agonists invariably exhibit opposite biochemical effects: D-1 agonists stimulate adenyl cyclase activity, while D-2 agonists inhibit the enzyme activity. It is clear that these receptor subtypes influence each other, and yet they display separate and distinct functions on body physiology and biochemistry.[8]

Scheme I. Chemical Structures and in Vitro Binding Constants of Benzazepines



X	Compound	Kd(nM)
H	SKF-83692	197
Cl	SCH-23390	0.36
Br	SKF-83566	2.32
I	IBZP, SKF-103108A (or SCH-23982)	0.7

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