of the regulation of calcium in the SR.

Although the specific binding of myotoxin *a* to two SR proteins is conclusively shown in this investigation, it is important to emphasize that the experiment was done with an isolated cell component in vitro. Since the path of myotoxin *a*'s entry into the muscle cell has not been investigated either in vivo or in tissue culture, it is premature to say that the binding of myotoxin *a* to SR protein may also take place in the intact cell system.

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Registry No. ATPase, 9000-83-3; YKQCHKKGGHCFPKEK, 134882-41-0; PKEKICIPPSSDLGKM, 134882-42-1.

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Intramolecular Semiquinone Disproportionation in DNA. Pulse Radiolysis Study of the One-Electron Reduction of Daunorubicin Intercalated in DNA†

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ABSTRACT: The one-electron reduction of daunorubicin, a quinonic antitumor antibiotic, intercalated in DNA was studied by pulse radiolysis using carboxyl radicals as reductants. The reaction's first stage is the daunorubicin semiquinone formation ($k = 1.9 \times 10^8$ mol^{-1}·dm^3·s^{-1}) in a way entirely consistent with a simple competition between $^{\bullet}COO^-$ disproportionation and the drug reduction. The semiquinone drug disappears by a first-order reaction ($k = 1340$ s^{-1}) producing the hydroquinone form. This reaction leads to an equilibrium similar to the one without DNA and the equilibrium constant is very close to its value free in water ($K_c \sim 25$). In addition, the stoichiometry of the first-order reaction is the one of a dismutation process. Therefore, it appears that the disproportionation occurs along an intramolecular path across DNA. This migration takes place under our experimental conditions, over a distance of ca. 100 base pairs, with a mobility of ca. 4.4×10^{-11} m^2·V^{-1}·s^{-1}, similar in magnitude to an excess electron mobility in doped organic polymers.

There is a great interest in long-range electron transfer across biological macromolecules (Isied, 1984; Marcus & Sutin, 1985; Gray & Malstrom, 1989). More generally speaking, the conductivity of organic polymers is being investigated and a considerable amount of literature has been accumulated on the subject of electronic properties at the molecular level (Frommer, 1986; Hopfield et al., 1989). In all kinds of polymer assemblies, electron transfer can occur over large distances (>10-20 Å) (Isied, 1984). Theories indicate that donor-acceptor distance, thermodynamic driving force, and the nature of the intervening medium are critical in determining rates of electron transfer (Marcus & Sutin, 1985; Closs & Miller, 1988).

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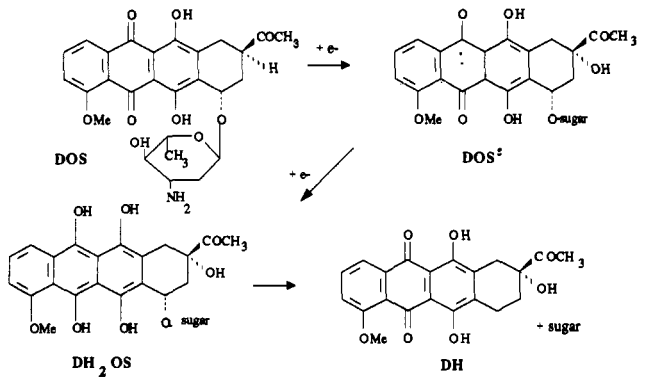
Scheme I



Long-range electron transfer has been much less studied in DNA than in proteins, probably because no fundamental role in the vital functions of DNA has yet been discovered for such a phenomenon. However, it has been reported that the double-stranded DNA macromolecule not only mediates but also accelerates electron transfer between donor–acceptor pairs (Purugganan et al., 1988). Indeed, theories indicate that the linear structure of this polymer should render electron transfer efficient and nondispersive (Pethig, 1979; Purugganan et al., 1988; Baverstock & Cundall, 1988).

Many antitumor, mutagenic, and/or cytotoxic agents can be complexed in DNA. Anthracyclines are known to intercalate in DNA and to have powerful redox properties in aqueous solution. They are supposed to undergo oxido-reductive activation in vivo. Numerous studies have demonstrated that daunorubicin (DOS),[1] one of the anthracycline antitumor drugs, was activated in vivo by reduction (Bachur et al., 1977). The intercalative binding of the drug would have numerous consequences, such as inhibition of the replication and transcription processes (Snyder, 1987) and of enzymatic repair (Nelson et al., 1984; Snyder, 1987) possibly due to alterations of the catalytic activity of topoisomerase II (Nelson et al., 1984; Theologides et al., 1968; Drlicka & Franco, 1988). During reductive activation, daunorubicin accepts one or two electrons to provide semiquinone ($^{\bullet}DOS^{-}$) or hydroquinone (DH_2OS) reduced transients (Houée-Levin et al., 1984, 1985). Structures of these compounds are given in Scheme I. The hydroquinone form undergoes glycosidic loss to give 7-deoxydaunomycinone (DH) at the end of a complex process (Houée-Levin et al., 1985). We have demonstrated that the antibiotic intercalated in DNA (Rouscilles et al., 1989; Houée-Levin et al., 1990) can be reduced by $^{\bullet}COO^{-}$ free radicals and that the final product is the same as without DNA, i.e., 7-deoxydaunomycinone.

Our aim is now to provide additional information about the role of DNA on the mechanism of electron transfer in a daunorubicin–DNA complex. We report here the one-electron reduction mechanism of daunorubicin intercalated in DNA (DOS_{DNA}) by the method of pulse radiolysis using carboxyl radicals as reductants. This species is selective of the drug since it does not react with DNA (Nabben et al., 1983; verified by us).

Materials and Methods

Daunorubicin hydrochloride was a generous gift of Rhône-Poulenc. Calf thymus DNA was provided by Sigma. It was dissolved by magnetic stirring during 24 h. Other reagents were from Rhône-Poulenc (Normapur). Water was triply distilled (resistivity > 6 MΩ·cm) or purified through a Millipore MilliQ system. All glassware was thoroughly cleaned up and rinsed with triply distilled water. After being washed, it was heated at 400 °C for several hours to burn off impurities.

The DOS_{DNA} complex was made up by mixing equal volumes of DOS and DNA solutions. The structure of the complex is well-known (Neidle, 1979; Arcamone, 1981) and strongly depends on the ratio r = [DOS]/[DNA]. For ratios lower than 1/10, intercalation is mostly of only one type, that is, the antibiotic cycles are stacked between two base pairs (Patel et al., 1981; Barcelo et al., 1988; Malatesta & Andreoni, 1988). We have worked in such conditions and have obtained the well-known absorption spectrum of the antibiotic intercalated in DNA. We have verified that Beer's law was followed by [DOS] between 2×10^{-5} and 4×10^{-4} mol·dm^{-3} and r = 1/10 or 1/20, between 350 and 600 nm, where only the antibiotic has a nonnegligible absorption spectrum.

Pulse radiolysis experiments were performed with a Febetron 707 whose set-up has been described (Marignier & Hickel, 1984).

Aqueous solutions of complex were irradiated in the presence of formate ions (0.1 mol·dm^{-3}). The pH was equal to 7 (phosphate buffer 6×10^{-2} mol·dm^{-3}). Solutions were deoxygenated and saturated by bubbling slowly nitrous oxide (CFPO, France, purity >99.9%) prior to irradiation (ca. 1 h for a volume of 20 cm^3). It is known that, under these conditions, the free radicals produced by water radiolysis, $^{\bullet}OH$, $^{\bullet}H$, and e_{aq}^{-}, are transformed quantitatively into $^{\bullet}COO^{-}$ free radicals:

$$^{\bullet}OH + HCOO^{-} \rightarrow {}^{\bullet}COO^{-} + H_2O$$

$$^{\bullet}H + HCOO^{-} \rightarrow {}^{\bullet}COO^{-} + H_2$$

$$e_{aq}^{-} + N_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2$$

e_{aq}^{-} free radicals are scavenged by N_2O giving $^{\bullet}OH$ radicals. $^{\bullet}OH$ and $^{\bullet}H$ radicals react with formate to give $^{\bullet}COO^{-}$ anions with a well-known yield of 0.62 μmol·J^{-1}.[2] The average dose, delivered in less than 20 ns, was between 10 and 100 Gy/pulse ($[^{\bullet}COO^{-}]$ between 6.2×10^{-6} and 6.2×10^{-5} mol·dm^{-3}). Each point of the absorption spectra is the average of 3–5 independent measurements.

Results

The chosen concentration range was a compromise between the need to have a ratio $[DOS_{DNA}]/[^{\bullet}COO^{-}]$ large enough to ensure good kinetic conditions and the need for a signal of good quality. Thus aqueous solutions of complex ([DOS] between 10^{-4} and 4×10^{-4} mol·dm^{-3}, r = 1/10 or 1/20) were irradiated with doses of ca. 32 Gy ($[^{\bullet}COO^{-}]_0 = 2.05 \times 10^{-5}$ mol·dm^{-3}) for recording absorption spectra. Raising the drug concentration led to too large an absorbance of unirradiated solutions, and lowering it gave a signal too weak. For similar reasons, it was impossible to irradiate with doses lower than 10 Gy. For doses higher than 100 Gy, the probability for two $^{\bullet}COO^{-}$ radicals to react with the same antibiotic molecule was not negligible; this dosage was therefore avoided.

Daunorubicin Semiquinone Formation. At all wavelengths between 320 and 750 nm, a biphasic process is observed, with

[1] Abbreviations: DOS, daunorubicin; $^{\bullet}DOS^{-}$, daunorubicin semiquinone; DH_2OS, daunorubicin hydroquinone; DH, 7 deoxydaunomycinone. DNA complexes are noted by symbols such as DOS_{DNA}, $^{\bullet}DOS^{-}_{DNA}$, DH_2OS_{DNA}.

[2] The radiation dose is the amount of energy absorbed per matter unit and is expressed in Gray units (1 Gy = 1 J·kg^{-1}). The radiolysis yield is the number of moles formed in the solution per unit of absorbed energy (mol·J^{-1}).

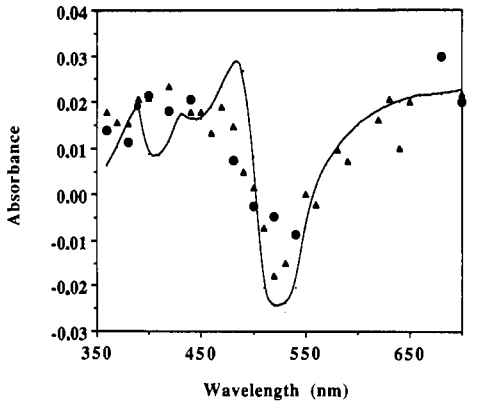


FIGURE 1: Difference absorption spectrum of daunorubicin semiquinone in DNA, recorded 160 μs after the pulse and compared to the one of the same species free in solution (Houée-Levin et al., 1985): $[HCOO^-]$ = 0.1 mol·dm^{-3}, phosphate buffer 6 × 10^{-2} mol·dm^{-3}, pH 7, N_2O atmosphere (1 atm), 32 Gy, optical path 2.5 cm. Each experimental point it an average of 3–5 independent measurements. ▲, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/10; ●, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/20; (—), DOS free in solution, spectrum normalized to the former one (●).

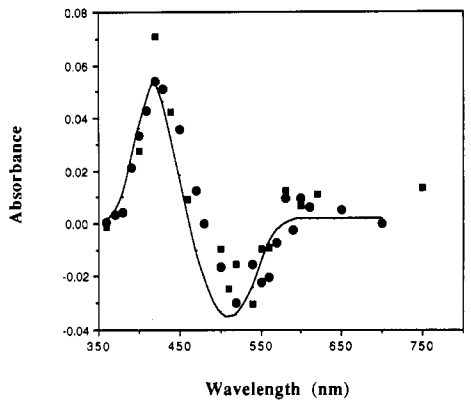


FIGURE 2: Difference absorption spectrum of daunorubicin hydroquinone in DNA, recorded 3–10 ms after the pulse and compared to the one of the same species free in solution: $[HCOO^-]$ = 0.1 mol·dm^{-3}, phosphate buffer 6 × 10^{-2} mol·dm^{-3}, pH 7, N_2O atmosphere (1 atm), 32 Gy, optical path 2.5 cm. Each experimental point is an average of 3–5 independent measurements. ●, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/10; ■, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/20; (—), DOS free in solution, spectrum normalized to the former one (●), at 420 nm.

the formation of a single transient characterized by an absorption culminating around 150 μs ([DOS] = 10^{-4} mol·dm^{-3}) or earlier for higher initial concentrations, together with the subsequent formation of a second species at a slower rate (around 5 ms).

The difference absorption spectrum (reference, unirradiated solution) recorded 160 μs after the pulse is given in Figure 1 ([DOS] = 10^{-4} mol·dm^{-3}, r = 1/10 and 1/20). Similar spectra were obtained with higher initial concentrations. This spectrum is compared to the one of daunorubicin semiquinone alone in solution normalized at 380 nm. Both have a broad band from 550 nm to the infrared region and a minimum around 520 nm, corresponding to the maximum absorbance of the initial solution. The maxima at 380 and 420 nm are replaced by a large band, as it is for the semiquinone daunorubicin–protein complex (Houée-Levin et al., 1989). Consequently the spectrum of Figure 1 is attributed to daunorubicin semiquinone intercalated in DNA. The absorbances at all wavelengths between 350 and 700 nm are the same for r = 1/10 or 1/20, showing that they depend only on the drug concentration and on the irradiation dose and not on r. Thus, it appears that, in the limit of our experimental conditions, the reduction kinetics is governed only by the daunorubicin concentration and not by that of the DNA. Furthermore, at the microsecond time scale, there was no spectral or kinetic evidence of another radical intermediate formation at any time of the reaction.

The kinetics of the free radical formation was studied at all wavelengths between 350 and 700 nm. The formation was not pure first order except for the highest drug concentration and for low doses (<40 Gy). Thus the rate constant k_1 of the reaction

$$^{\bullet}COO^- + DOS_{DNA} \rightarrow {}^{\bullet}DOS^-_{DNA} + CO_2 \qquad (1)$$

was estimated at these pseudo-first-order conditions:

$$k_1 = 1.9 \times 10^8 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$$

To check this rate constant value, we performed a computation based upon the competition between reaction 1 and

$$2\,{}^{\bullet}COO^- \rightarrow C_2O_4^{2-} \qquad (2)$$

$$2k_2 = 1.3 \times 10^9 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$$

(verified by us and in agreement with numerous other determinations). The corresponding differential equation system was solved analytically. The maximal $^{\bullet}DOS^-_{DNA}$ concentration was calculated as a function of the initial $^{\bullet}COO^-$ concentration and for several initial drug concentrations (between 1 × 10^{-4} and 4 × 10^{-4} mol·dm^{-3}) and r values (20 independent experiments). k_1 was considered as an adjustable parameter, and the difference absorbances at 400 and 650 nm were fitted to the experimental results, with extinction coefficients used as parameters. We found

$$k_1 = (1.9 \pm 0.1) \times 10^8 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$$

which is in excellent agreement with previous estimation, and

$$\Delta\epsilon = 1100 \pm 100 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{cm}^{-1}$$

at both wavelengths. The absorptivities are hence deduced at all wavelengths. They are very similar to the ones of daunorubicin semiquinone alone in solution without DNA (Houée-Levin et al., 1985). It seems that intercalation has little effect on the transition energies and on the oscillator strengths of $^{\bullet}DOS^-$, which is not surprising since it is the same for the quinone parent compound.

Daunorubicin Hydroquinone Formation. The semiquinone daunorubicin disappears at the millisecond time scale. The resulting different absorption spectrum (between 3 and 10 ms after the pulse) is reported in Figure 2 ([DOS] = 10^{-4} mol·dm^{-3}, r = 1/10 or 1/20), with the one of daunorubicin hydroquinone alone in water (Houée-Levin et al., 1985) normalized at 420 nm to the one obtained with [DOS] = 10^{-4} mol·dm^{-3}, r = 1/10. The chromophore of daunorubicin hydroquinone has the same absorption spectrum either free in water (Houée-Levin et al., 1985) or intercalated in a protein (Houée-Levin et al., 1989) or without its amino sugar moiety in DNA (Rouscilles et al., 1989), and the agreement between the molar absorptivities in all these conditions is strikingly good. It is evident that this second spectrum belongs mostly to daunorubicin hydroquinone intercalated in DNA (DH_2OS_{DNA}), despite the fact that the peak at 420–430 nm is broader in DNA and the absorbances at wavelengths >600 nm are higher than in water, but it seems that some semiquinone drug is still present, since the absorbances at regions in which the free radical species absorbs more than the hydroquinone (460–500 and >600 nm) look higher than for the pure species intercalated in a protein. Indeed, alone in water,

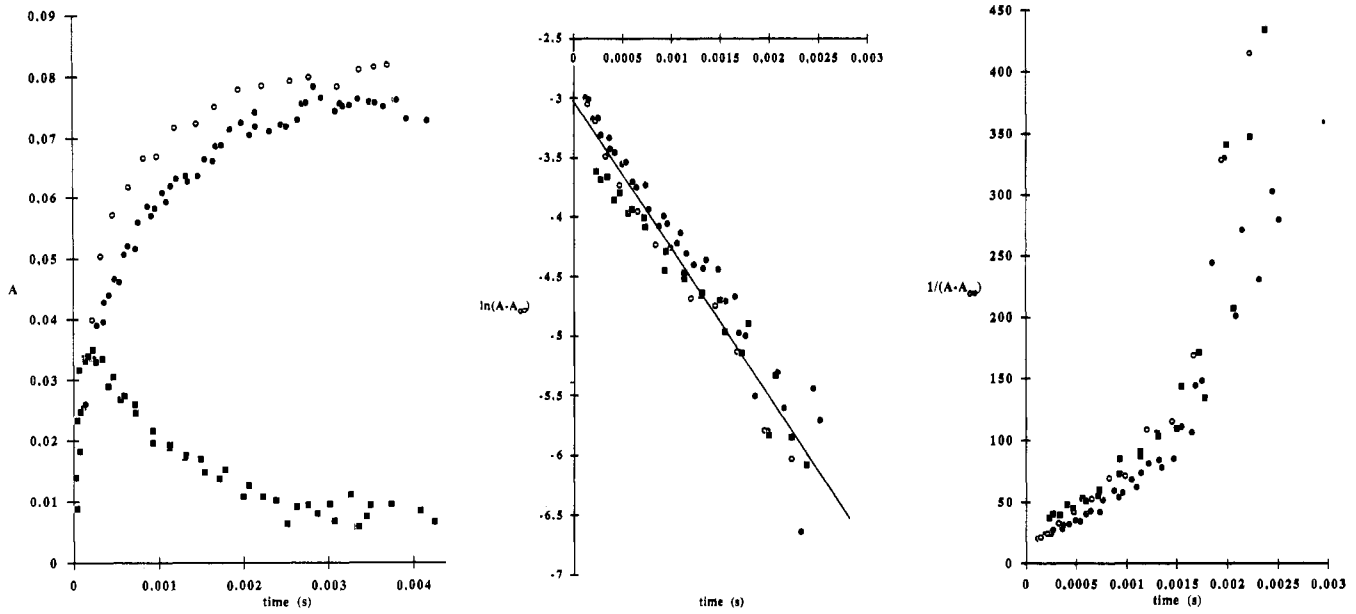


FIGURE 3: Variation of the difference absorbances (left panel) and of their first- and second-order plots (center and right panels) with time corresponding to the semiquinone decay. [$HCOO^-$] = 0.1 mol·dm^{-3}, phosphate buffer 6 × 10^{-2} mol·dm^{-3}, pH 7, N_2O atmosphere (1 atm), optical path 2.5 cm. ●, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/10; 430 nm, 36 Gy; ○, [DOS] = 1.5 × 10^{-4} mol·dm^{-3}, r = 1/20, 420 nm, 39 Gy; ■, [DOS] = 4 × 10^{-4} mol·dm^{-3}, r = 1/10, 620 nm, 43 Gy.

the daunorubicin semiquinone decay leads to an equilibrium ($K_c \cong 25$) (Houée-Levin et al., 1985).

The composition of the solution, evaluated by the absorbances, is the same for r = 1/10 or 1/20 and is thus independent of r, as it is for the semiquinone drug. No spectral or kinetic evidence of the formation of another intermediate at any time of the reaction was observed.

The kinetics of the semiquinone decay leading to the hydroquinone formation are shown in Figure 3 (left panel) for several drug and DNA initial concentrations and for different doses (i.e. different semiquinone form concentrations), at different wavelengths, with their first- and second-order plots (Figure 3, center and right panels). It appears that the reaction is pure first order, with a rate constant k_3 = 1340 ± 325 s^{-1} (obtained over 20 independent measurements). The maximal absorbances at the peak (420 nm) increase with the $^{\bullet}DOS^-_{DNA}$ maximal concentrations, calculated at the end of the free radical formation process (Figure 4). These maximal absorbances are independent of the initial drug and DNA concentrations. It seems to be the same at 650 nm, though absorbances are weak at wavelengths >600 nm (Figure 4).

In order to evaluate the stoichiometry of the transformation corresponding to the kinetics of Figure 3, the concentrations of semiquinone and hydroquinone species at the end of the semiquinone decay were estimated. Considering that the absorptivities of daunorubicin and of its reduced forms (semiquinone or hydroquinone) free in solution (Houée-Levin et al., 1985) and complexed either in DNA or in aporiboflavin binding protein (Houée-Levin et al., 1989) are very close, we can approximate the absorptivities of daunorubicin hydroquinone in DNA by those of the same form free in solution. For instance, using the data of Figure 2, we obtain

$$[DH_2OS_{DNA}] = 2.16 \times 10^{-6} \text{ mol·dm}^{-3}$$

[determined at 420 nm, using $\Delta\epsilon$ = 6700 mol^{-1}·dm^3·cm^{-1}, value belonging to aglycon daunorubicin hydroquinone–DNA complex (Rouscilles et al., 1989)]

$$[^{\bullet}DOS^-_{DNA}] = 2.9 \times 10^{-6} \text{ mol·dm}^{-3}$$

(determined at 650 nm, using $\Delta\epsilon$ = 1100 mol^{-1}·dm^3·cm^{-1}). The

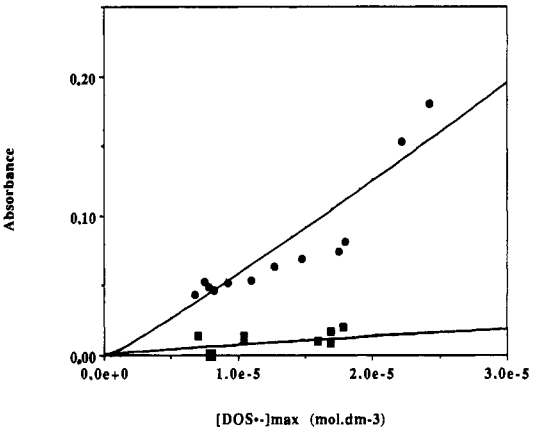


FIGURE 4: Variation of the difference absorbance at 420 and 650 nm, between 3 and 10 ms after the pulse, vs the maximal semiquinone concentration. [$HCOO^-$] = 0.1 mol·dm^{-3}, phosphate buffer 6 × 10^{-2} mol·dm^{-3}, pH 7, N_2O atmosphere (1 atm), optical path 2.5 cm, [DOS] = 10^{-4} mol·dm^{-3}, r = 1/10 or 1/20. Curves are computed (see the text); points are experimental. ●, 420 nm; ■, 650 nm.

maximal amount of semiquinone form was, at the plateau of its formation

$$[^{\bullet}DOS^-_{DNA}]_{max} = 7.36 \times 10^{-6} \text{ mol·dm}^{-3}$$

The stoichiometry coefficient α is thus

$$\alpha = \frac{([^{\bullet}DOS^-_{DNA}]_{max} - [^{\bullet}DOS^-_{DNA}])}{[DH_2OS_{DNA}]} = 2.06$$

The calculation of this ratio was made for several other initial conditions of drug concentration, drug/DNA ratio, and dose. We obtain

$$\alpha = 1.98 \pm 0.1$$

which could correspond to a global dismutation process:

$$2^{\bullet}DOS^-_{DNA} (+2H^+) \rightleftarrows DH_2OS_{DNA} + DOS_{DNA} \quad (3)$$

The equilibrium constant is evaluated

$$K_3 = \frac{[DH_2OS_{DNA}][DOS_{DNA}]}{[^{\bullet}DOS^-_{DNA}]^2} = 24.4$$

which is very close to the value of K_c for the same species alone in water ($K_c = 25$).

Discussion

The following kinetic scheme summarizes the set of reactions that are proposed in order to explain the data presently reported:

$$^{\bullet}COO^- + DOS_{DNA} \rightarrow {}^{\bullet}DOS^-_{DNA} + CO_2 \quad (1)$$

$$k_1 = 1.9 \times 10^8 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$$

$$2^{\bullet}COO^- \rightarrow C_2O_4^{2-} \quad (2)$$

$$2^{\bullet}DOS^-_{DNA} (+2H^+) \rightleftarrows DH_2OS_{DNA} + DOS_{DNA} \quad (3)$$

The daunorubicin semiquinone radical is the only radical intermediate formed upon reaction 1 of daunorubicin, intercalated or not, with $^{\bullet}COO^-$. This reaction is in competition with the $^{\bullet}COO^-$ dimerization (reaction 2). Surprisingly, the rate constant k_1 has a similar value if daunorubicin is intercalated in DNA or in the aporiboflavin binding protein (Houée-Levin et al., 1989) and is only one order of magnitude lower in DNA than without DNA. The relative amount of DNA does not influence the reaction 1 kinetics.

The spectral analysis (Figure 2) establishes that the daunorubicin semiquinone decay ends in the equilibrium formation of the corresponding hydroquinone form (reaction 3), like without DNA, whereas the kinetic analysis shows that the rate of this process is independent of the quinone, semiquinone, and DNA concentrations.

In order to assess the proposed equilibrium constant K_3 and the extinction coefficients of the hydroquinone drug, a fit of the absorbances at 420 and 650 nm, as a function of the maximal semiquinone drug concentration, was performed. The adjustable parameters were the equilibrium constant

$$K_3 = \frac{[DOS_{DNA}]_{eq}[DH_2OS_{DNA}]_{eq}}{[DOS^-_{DNA}]_{eq}^2}$$

where the subscripts eq denote the equilibrium concentrations, and the hydroquinone absorptivities. Different initial conditions of drug, r values, and doses were considered. The result is shown in Figure 4 and is compared to the experimental points. We obtain

$$10 \leq K_3 \leq 60$$

$$\Delta\epsilon^{420}_{DH_2OS} = 6000 \pm 500 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{cm}^{-1}$$

$$\Delta\epsilon^{650}_{DH_2OS} \leq 500 \text{ mol}^{-1}\cdot\text{dm}^3\cdot\text{cm}^{-1}$$

These absorptivity values are very close to the ones of daunorubicin hydroquinone in water ($\Delta\epsilon^{420}_{DH_2OS}$ = 7000 mol^{-1}·dm^3·cm^{-1} and $\Delta\epsilon^{650}_{DH_2OS}$ < 200 mol^{-1}·dm^3·cm^{-1}) (Houée-Levin et al., 1985) and of aglycone daunorubicin hydroquinone in DNA (respectively 6700 mol^{-1}·dm^3·cm^{-1} and <200 mol^{-1}·dm^3·cm^{-1}) (Rouscilles et al., 1989). Such a similarity is observed for the parent drug either intercalated or free in water and also for the semiquinone form. Consequently we consider these values as a proof of the stoichiometry of reaction 3. Daunorubicin semiquinone intercalated in aporiboflavin binding protein withdraws an electron and two protons from the protein matrix (Houée-Levin et al., 1989). Thus, it appears that, in DNA, such an oxidation does not happen and that the radical decays by disproportionation like in water.

By use of the equilibrium constant K_3, the standard free enthalpy and the redox potential at pH 7 and 298 K, ΔE°_7, associated with the disproportionation can be calculated

$$\Delta G^\circ = -RT \ln K_3 = -F\Delta E^{\circ\prime}$$

where F is the Faraday constant (96 500 Cb). This leads to

$$\Delta G^\circ = -8.8 \pm 1.7 \text{ kJ}\cdot\text{mol}^{-1}$$

and

$$\Delta E^{\circ\prime} = 0.09 \pm 0.02 \text{ V}$$

which is very close to the corresponding value in water ($\Delta E^{\circ\prime}$ = 0.04 V) (Houée-Levin et al., 1985). The thermodynamic parameters of this electron transfer are thus slightly affected by intercalation in DNA.

The kinetic analysis of reaction 3 shows that it is not a bimolecular reaction, since (a) it appears as a pure first-order reaction and (b) the experimental rate constant and the period are independent of the concentrations of all the species involved. It should be recalled that we have already demonstrated that the whole reduction process took place in DNA (Rouscilles et al., 1989) and that reactions 1 and 3 are considerably faster than the dissociation of the daunorubicin–DNA complex [k_{diss} = 2 s^{-1} (Gandescha et al., 1985), $k_{assoc} \cong 2 \times 10^6$ mol^{-1}·dm^3·s^{-1}, and $K_c \cong 10^6$ (Byrn & Dolsch, 1978)]. We must then conclude that the electron transfer occurs via an intramolecular path. The velocity of this transfer seems to be slightly dependent on the relative amount of DNA. Either this velocity is not very sensitive to the donor–acceptor distance, which should differ by a factor of 2 in our experiments, or the distance between the two reacting semiquinones does not vary much with the DNA amount. This might be the case if conduction would be through space and not through bond.

Conclusion

The aim of this work was to provide better knowledge about the role of DNA on the reduction mechanism of an antitumor drug, daunorubicin, intercalated in it. Such a mechanism may play a role in the drug's cytotoxicity.

It appears that the same intermediates as without DNA are formed. The semiquinone drug is the result of the reduction of daunorubicin by carboxyl free radicals in the presence or in the absence of DNA. The rate constant of this reaction does not vary much upon intercalation [2×10^9 mol^{-1}·dm^3·s^{-1} in water without DNA (Houée-Levin et al., 1985) versus 1.9×10^8 mol^{-1}·dm^3·s^{-1} for the drug–DNA complex]. The value of k_1 would be consistent with a nearly diffusion-controlled estimate predicted for a rigid rod of the dimensions of DNA, similarly to the findings of Whillans (1975) for the reduction by e_{aq}^- of proflavine or ethidium intercalated in DNA. In this step of the reaction no evidence of free electron migration in DNA was obtained.

On the opposite, intramolecular electron migration is the only way to explain the complexed semiquinone free radical decay. The meaning of k_3 does not seem clear. It should be an addition of the forward and the backward rate constants but we have no experimental indication of it, since k_3 is independent of the concentration conditions.

However another approach can be used to the interpretation of k_3. The characteristic time of this conduction can be evaluated by the half-life of the semiquinone form and is of the order of $\tau \cong 5 \times 10^{-4}$ s. Considering average semiquinone and DNA concentrations in our conditions of pulse radiolysis, the average separation distance between two reacting species is ca. 100 base pairs, i.e., $r \cong 34$ nm, assuming a random organization, but we do not know the distribution of these distances. This value corresponds to the limits proposed for the migration of electrons (Van Lith et al., 1986, Cullis et al. 1990) and for the delocalization of energy in DNA (Fielden et al., 1971).

It is interesting to attempt to evaluate orders of magnitude of the diffusion coefficient and the mobility corresponding to the electron migration

$$D = r^2/2\tau = 1.15 \times 10^{-12}\ m^2 \cdot s^{-1}$$

$$\mu = De/k_B T = 4.4 \times 10^{-11}\ m^2 \cdot V^{-1} \cdot s^{-1}$$

where e is the electron charge, k_B is the Boltzmann constant, and T is the absolute temperature ($\cong$ 300 K). These values are much lower than those of hydrated electron and of the excess electron in frozen hydrated DNA (Van Lith et al., 1986). However it corresponds to mobilities observed in molecularly doped organic polymers (Holstein, 1979). The apparent potential difference V corresponding to this mobility would be

$$V = r^2/\tau\mu = 0.05\ V$$

which is very close to the experimental value of the redox potential $\Delta E'^{\circ}$ (0.09 V) of this electron transfer. This result suggests that this reaction might be modeled by a simple electron conduction driven by an electrostatic potential difference, which is the redox potential difference between the two redox couples (here the DOS/$^{\bullet}$DOS$^-$ and the $^{\bullet}$DOS$^-$/DH_2OS couples), also equal to the difference of the Fermi levels of both sites (Reiss, 1985). To our knowledge, this is the first experimental demonstration of this fact in DNA. However, this hypothesis should be ascertained by other experiments of intramolecular electron transfer with various thermodynamic driving forces.

Such a low mobility value excludes a freely delocalized electron conduction, and a hopping mechanism can be envisaged. A very simple approach of this transfer can be proposed following standard diffusion theory (Kittel, 1976). The diffusion coefficient is of the form

$$D = \nu l^2 e^{-\Delta E/RT}$$

where ν is the characteristic hopping frequency, l is the step distance along the chain, and ΔE is the activation energy of the process. Let us assume that the electron "hops" by steps of the length of a base pair (l = 3.4 Å). We have no possibility of measuring the activation energy of this reaction, but it has been shown that, for electron transfer between metal complexes intercalated in DNA, the activation energy is very low (Pullman et al., 1988). The exponential term is thus very close to unity. If it is the same in our system, the characteristic frequency can be evaluated:

$$\nu = 10^7\ s^{-1}$$

which corresponds to global bending movements for nucleic acids (McCammon & Harvey, 1987).

Several mechanisms of such long-range migrations have been proposed (Bredas & Street, 1985), including solitonic excitations arising from nonlinear deformations or excitations and propagating without deformation (Davydoff, 1979). The Davydoff soliton model has been developed in nucleic acids [Englander et al., 1980; Zhang (1987) and references therein] and suggested for energy transfer in DNA (Baverstock & Cundall, 1988). However, there is still controversy whether such solitons do exist. The conduction process that we describe here might be based on such a mechanism or be due to disorder-induced band-tail effects, like in amorphous media [see for example Pethig (1979)].

Finally, we can postulate that one of the effects of intercalating drugs might be the transformation of DNA into a doped polymer with the apparition of conduction properties. The consequences of the modification of biological macromolecule conductivity are not known, but their possible importance in major life problems has been questioned by Szent-Györgi 50 years ago (Szent-Györgi, 1941). In this work we show a new consequence of conduction process, intramolecular free radical disproportionation. In biological conditions this might concern free radicals coming from intercalating drugs as well as nucleosides. It may be an important way of eliminating unpaired electrons, in addition to the transfer to proximal proteins. It may also represent a protection against damages that would have occurred otherwise by one-electron oxidoreduction of DNA. In this sense, the nonradical nature of daunorubicin lesions in cells would not be a proof of the absence of free radical processes but only of their innocuity.

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Disposition of the Phenylalanine B25 Side Chain during Insulin–Receptor and Insulin–Insulin Interactions†

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ABSTRACT: By the semisynthesis of both full-length insulin analogues and their des-pentapeptide-(B26–B30)-α-carboxamide counterparts, we have examined the importance of the electronic character and bulk of the position B25 side chain both in directing insulin interaction with its receptor on isolated canine hepatocytes and in determining the ability of insulin to self-associate in solution. Analogues include those in which Phe^{B25} was replaced by cyclohexyl-Ala; Tyr; *p*-nitro-, *p*-fluoro-, *p*-iodo-, or *p*-amino-Phe; or *p*-amino-Phe in which the aromatic amino function had been acylated by the acetyl, hexanoyl, decanoyl, or 1-adamantanoyl group. Our findings identify that (a) the β-aromatic side chain at position B25 is indeed critical for high-affinity ligand–receptor interactions, (b) neither electron withdrawal from nor electron donation to the β-aromatic ring perturbs ligand–receptor interactions in major ways, (c) considerable lattitude is allowed the placement of linear or polycyclic apolar mass at the para position in *p*-amino-Phe^{B25}-substituted analogues with respect both to receptor binding affinity and to biological activity in vivo, and (d) para apolar mass at position B25 is readily accommodated during the self-association of insulin monomers, as assessed by analytical tyrosine radioiodination and spectroscopic analysis of analogue complexes with Co^{2+} and Co^{3+}. These findings are discussed in terms of a model for insulin–receptor interactions at the cell membrane in which the position B25 side chain defines the edge of intermolecular contact.

The COOH-terminal region of the insulin B-chain and invariant residue Phe^{B25} are known to be important in the high-affinity interactions of insulin with its plasma membrane receptor (Nakagawa & Tager, 1986, 1987; Mirmira & Tager, 1989; Inouye et al., 1981; Wollmer et al., 1981), as well as in the formation of the β-sheet structure between insulin monomers in each of the three identical dimers of the 2-Zn insulin hexamer (Blundell et al., 1972; Baker et al., 1988). Although we have proposed that the position B25 side chain functions in part to induce favorable main-chain adjustments in the COOH-terminal region of the insulin B-chain (rather than to confer direct binding energy), and although deletion of residues B26–B30 from the carboxyl terminus of the B-chain ameliorates to one degree or another the negative effect that attends replacement of Phe^{B25} by various amino acids (Nakagawa & Tager, 1986, 1987), the importance of Phe^{B25} in insulin–receptor interactions has yet to be completely resolved. That is, side-chain structure at position B25 can influence significantly the affinity of receptor for ligand, even in truncated analogues, and the effect of replacement of Phe^{B25} in these shortened analogues can be either beneficial or detrimental. As important examples, des-pentapeptide-(B26–B30)-[His^{B25}-α-carboxamide]insulin and des-pentapeptide-(B26–B30)-[Tyr^{B25}-α-carboxamide]insulin exhibit nearly 300% of the receptor binding potency of insulin (Nakagawa & Tager, 1986; Casaretto et al., 1987), whereas des-pentapeptide-(B26–B30)-[Gly^{B25}-α-carboxamide]insulin and des-pentapeptide-(B26–B30)-[Leu^{B25}-α-carboxamide]insulin exhibit only about 20% of normal binding potency (Nakagawa & Tager, 1986; Mirmira & Tager, 1989).

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