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Spectrophotometric and dielectric studies of an interaction between a new aminothiol, DNA and dipalmitoylphosphatidylcholine*

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Introduction

Sulphydryl compounds have been shown to reduce radiation-induced injury. Chemical modification of radiation response has received a renewed interest as a possible adjunct to radiation in the treatment of cancer [1]. To prevent radiation injury to normal tissue, many radioprotective agents have been developed and some of them used clinically [2–8]. The maximum tolerated dose and the side effects observed are a limitation for therapic utilisation of these drugs. The most widely studied of this group of agents are derivatives of cysteamine which act through their free sulphydryl aminothiol moiety which is liberated *in vivo* [9–15].

New aminothiols, associating cysteamine with an amino acid, were recently synthesised by Imbach *et al.* [16]. One, known by the code number I-143 (personal communication),

$$\text{I-143:HS-(CH}_2)_2\text{-CO-NH-(CH}_2)_2\text{-SH},$$

was provided by Professor J.L. Imbach (Montpellier University, France).

Electrochemical behaviour of I-143 and its interaction with DNA or lipids were studied in our laboratory. These experiments are very interesting because when the drug penetrates the cell nucleus it appears that interaction between the active molecule and DNA as a target is an important factor for protection. The rate of transport of I-143 across cell membranes is also a very interesting point: we chose synthetic lecithin as a model membrane.

Experimental

Materials

Dipalmitoylphosphatidylcholine (DPPC) (DL-3-synthetic lecithin from Koch-Light

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Laboratories) was dissolved in bidistilled water. The lipid–water phase was prepared following the Hill procedure [17]. The solutions, which were stirred at 45°C by a vortex for 20 min, had an average concentration of 8×10^{-4}M.

Solutions of DNA–sodium salt from calf thymus (Sigma) were prepared. The DNA was dissolved in a solution of sodium chloride (3×10^{-3}M). The concentration (P) of phosphate sites was about 2.5×10^{-3}M.

I-143 which had been synthesised in powder form was dissolved in bidistilled water for studies of interaction with lipid or in sodium chloride solution (3×10^{-3}M) for experiments on the interaction with DNA.

Methods

Our studies were done by spectrophotometry and dielectric measurements.

Spectrophotometric measurements were made with a Gilford 2400-2 spectrophotometer connected to a 2527 thermoprogrammer. We could then determine thermal transitions of the lipid phase assessing lipid turbidity variations with temperature over a range of 25°–50°C in increments of 0.5°C at a wavelength (λ) of 450 nm [18].

We can also determine the melting point (T_m) of DNA at 260 nm and its phosphate sites concentration. For practical reasons the determined DNA concentration (P′) by spectrophotometry is ten times inferior to dielectric measurements.

Dielectric measurements were performed using a Hewlett–Packard impedance bridge (HP 4192 A) with which it is possible to measure the parallel capacitance (C_p) and the parallel conductance (G_p) of an impedance. This instrument is equipped by a fast automatic frequency scan and driven by the computer HP 9826. All results are stored and visualised on the HP Think Jet printer and graphics recorder HP 7470.

The solutions were enclosed in a cylindrical condenser thermostated at 25°C by a Haake thermostat [19]. We varied the frequency between 1 and 10 mHz in steps of 0.5 MHz so that there were no polarisation problems. The relative complex permittivity of the solution is given by:

$$\epsilon^*_{rel} = \epsilon'_{rel} - i\,\epsilon''_{rel}, \quad \begin{cases} \epsilon'_{rel} = \text{real part of } \epsilon^*_{rel} \\ \epsilon''_{rel} = \text{imaginary part of } \epsilon''_{rel} \end{cases}$$

with

$$\epsilon'_{rel} = (C_p - C_o)/g \text{ and } \epsilon''_{rel} = G_p/g\,\omega, \quad \begin{cases} C_p = \text{parallel capacitance} \\ G_p = \text{parallel conductance} \\ \omega = \text{pulsation of the alternating field} \end{cases}$$

where C_o is the capacitance of the empty cell and $C_o = 2.05$ pF, ν is the frequency of the alternating field, g is the geometrical constant for the sample cell and $g = 0.65$ p.

The conductivity σ of the liquid sample is given by:

$$\sigma = \epsilon_o\,\epsilon''_{rel}\,\omega = \frac{\epsilon_o\,G_p}{g}$$

where ϵ_o is the permittivity of free space.

Results and Discussion

Spectral and electrostatic properties of I-143 in a solution of sodium chloride as vehicle

Spectrophotometric studies of I-143 dissolved in a solution of sodium chloride (3×10^{-3}M) were performed at 25°C for different concentrations of the drug. We observed an absorption band of which the wavelength of maximum absorption increases slowly with drug concentration from 195 to 205 nm. We then measured variations of absorbance (A) versus concentration of I-143 at mean absorption peak wavelength of 200 nm (Fig. 1). We obtained a linear increase in absorbance up to $A = 2$. The molar absorptivity which is valid only for $A < 2$ was found to be 6700 l mol^{-1} cm^{-1}.

Our results indicate that the sulphydryl group is in a non-ionised state. This is confirmed by dielectric measurements: we observed no alteration in permittivity or conductivity frequency spectra. This was not surprising considering the neutrality of the molecule. Variations in concentration has no influence on permittivity value: there was no dielectric increment. On the contrary, we observed a small increase in conductivity with concentration. We can determine the conductivity of I-143 ($\sigma_{I\text{-}143}$) at the concentration of solvent. It is given by:

$$\sigma_{I\text{-}143} = \sigma_{solution} - \sigma_{NaCl}$$

We found $\sigma_{I\text{-}143} = 1.25 \times 10^{-3}\ \Omega^{-1}$ mol l^{-1} which corresponds to:

$$\sigma_{I\text{-}143} = 4.7\%\ \sigma_{NaCl}$$

Interaction between I-143 and solutions of Na-DNA dissolved in a solution of sodium chloride

Spectrophotometric measurements at 25°C show that there is no alteration in the DNA absorption maximum at 260 nm by drug additions. However, DNA melting point (at $\lambda = 260$ nm) varies with concentration of I-143 until the phosphate sites become saturated (Fig. 2). Dielectric measurements do not give such positive results.

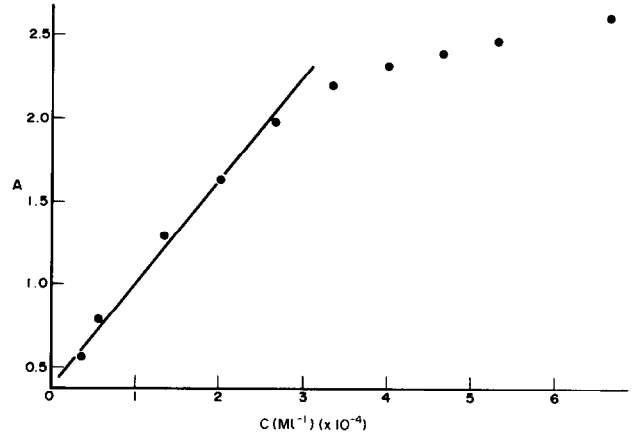

Figure 1
Absorbance (A) versus concentration (in mol l^{-1}) of I-143 (at fixed temperature 25°C) at absorption band maximum (≈200 nm). A linear increase of A up to ≈2 was observed.

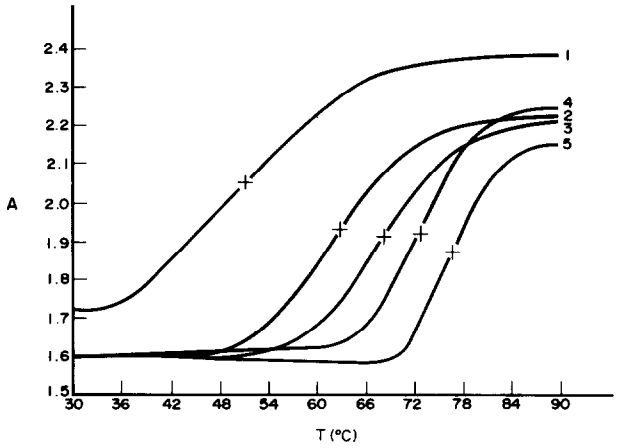

Figure 2
Absorbance at 260 nm versus temperature T for DNA alone and for the complex DNA–I-143 for several concentrations of I-143. Concentration of DNA phosphate sites (P′) is 2.5×10^{-4}M. I-143 concentration is expressed as a multiple of P′. (1) = DNA alone; (2) = DNA + I-143 (c = P′/3); (3) = DNA + I-143 (c = P′/2); (4) = DNA + I-143 (c = 2P′/3); (5) = DNA + I-143 (c = P′).

Dielectric increment ($\Delta\epsilon' = \epsilon'_{DNA} - \epsilon'_{NaCl}$) of DNA with respect to solvent is not altered by addition of the I-143: there is no depolarisation of DNA. There is no variation of conductivity with I-143 addition for DNA solutions, but variation is significant for sodium chloride solutions (Fig. 3).

There is a very small electrostatic interaction. The authors think that the substance I-143 interacts principally with DNA phosphate groups by means of its sulphydryl groups but other possibilities of interaction cannot be excluded.

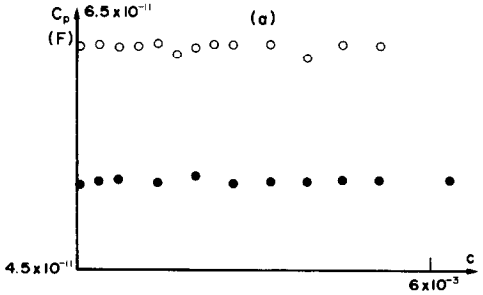

Figure 3a
Capacity variation (in Farad) versus I-143 concentration (in mol l^{-1}) measured at 25°C and fixed frequency 2 MHz. ● I-143 in NaCl (3×10^{-3}M); ○ I-143 in DNA dissolved in NaCl (3×10^{-3}M). Concentration of DNA phosphate sites (P) is 2.5×10^{-3}M.

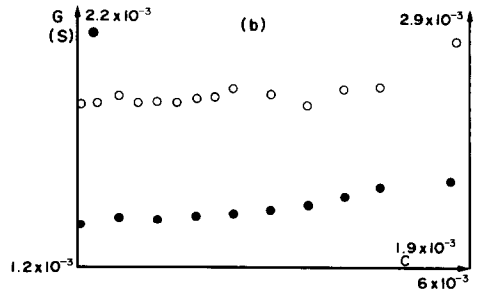

Figure 3b
Conductance variation (in Ω^{-1}) versus I-143 concentration (in mol l^{-1}) measured at 25°C and fixed frequency 2 MHz. ● I-143 in NaCl (3×10^{-3}M); ○ I-143 in DNA dissolved in NaCl (3×10^{-3}M). Concentration of DNA phosphate sites (P) is 2.5×10^{-3}M. We observe a horizontal plateau for DNA and a slight increase for NaCl.

Interaction between I-143 and lipid aqueous dispersions

No interaction was detected either by spectrophotometry or by dielectric measurements. Figure 4 shows that there is no displacement and no modification of pretransition and principal transition characteristics of the Hill phase. This result is opposite to those of the other radioprotectors we have studied [18, 20].

Dielectric measurements show that there is no modification of permittivity or conductivity with radioprotector addition.

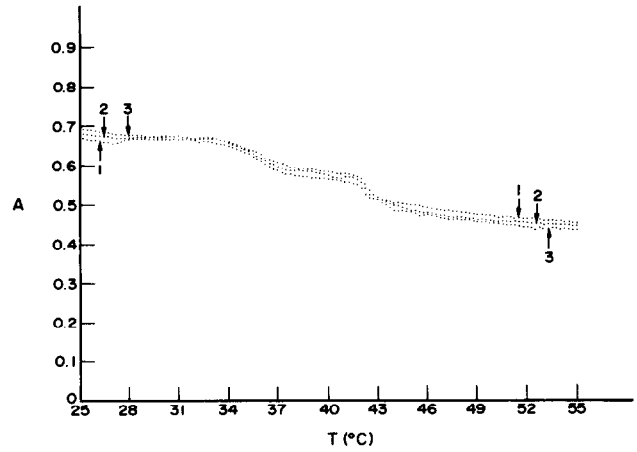

Figure 4
Absorbance at 450 nm versus temperature T for lipid alone and for the lipid–I-143 complex for several concentrations of I-143. Concentration of DPPC is C = 8×10^{-4}M. (1) = DPPC alone. (2) = DPPC + I-143 (c = 3C/4). (3) = DPPC + I-143 (c = C).

Conclusion

This study is a first step in the comprehension of intrinsic properties of the new drug I-143. Experiments on DNA–I-143 interaction show that this drug, like other aminothiols, can play a prominent part in protection from radiation or chemotherapy. To discuss the mechanism of interaction it would be very interesting to know the charge distribution of the neutral molecule: so we would be able to determine the DNA sites involved in this interaction.

The drug is ineffective on lipid phases. It is also thought to be ineffective on cell membranes and this can explain the rapid transfer of I-143 from extracellular medium to nucleus.

An extension of this work will be a pharmacological and clinical investigation.

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