Radioprotection (UV- and Gamma-rays) of DNA Molecule by Indole and Indole-derivatives

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DNA radioprotection, indole, radioprotective action

The radioprotective ability of L-tryptophan, tryptamine, 3-indoleacetic and indole (10^{-4} M), on UV and gamma irradiated DNA (10^{-4} M) is studied.

UV radioprotection is accomplished in the following order of efficiency: L-Tryptophan and 3-indoleacetic acid > indole and tryptamine. The corresponding DRF measured as absorbancy loss at 260 nm, were: L-Tryptophan and 3-indoleacetic acid around 3 and tryptamine and indole around 1. When absorbancy loss plus changes in the patterns of the absorbancy curve were considered the DRF was: L-Tryptophan 2.53, 3-indoleacetic acid 1.54, indole 1.22 and tryptamine 0.75, respectively.

For gamma radiation damage the order of radioprotection found was: Indole, L-tryptophan and tryptamine > 3-indoleacetic acid. The DRF when compairing the absorbancy loss at 260 nm, were: L-Tryptophan, Indole and tryptamine around 11 and 3-indoleacetic Acid around 4. When evaluating total changes in absorbancy the DRF were: Indole and L-tryptophan around 6.5, tryptamine 3.7 and 3-indoleacetic 2.1.

These data were correlated with measurement of the degree of molecular interactions of DNA and protectors, studied by: Spectrophotometry, m.p. determinations, circular dichroism and oriented circular dichroism, viscosity, and rate of dialysis. Also data of fluorescence and phosphorescense were considered.

For gamma radioprotection a mechanism based in radical trapping, local radical trapping and partial recovery of radioprotectors is proposed.

UV radioprotection is discussed considering: The close interaction of L-tryptophan with DNA which might impede dimer formation, its proton and electron donor ability. A possible (triplet-triplet) energy transfer from excited DNA to indole derivative is discussed on the basis of acetone competition and a dimer excision process considered.

In previous work the interaction of L-tryptophan with DNA, ribonucleotides, nucleosides and nitrogen bases in aqueous solutions, was shown by measurement of: UV absorbancy, circular dichroism, viscosimetry, equilibrium dialysis, and nuclear magnetic resonance spectroscopy [1–3]. Moreover, the high UV gamma radioprotector ability of tryptophan has been communicated [4]. In accordance with these studies an intercalation model of tryptophan-DNA bases has been proposed permitting, for the excited molecular state, an energy transfer process (triplet-triplet) from DNA to tryptophan [5].

In the present work we analyze the UV and gamma radioprotection of the DNA molecule by different indole derivatives (L-tryptophan, tryptamine, indoleacetic and indole), its molecular interaction, and radioprotective ability by means of spectrophotometric and m. p. determinations of measurements circular dichroism, of viscosity, and of equilibrium dialysis in irradiated (UV and gamma radiation) and non irradiated samples of DNA plus indole derivatives. The radioprotectors showing the highest DRF (dose reduction factor) were tryptophan and indoleacetic, when DNA was irradiated with UV radiation. A mechanism for this action is proposed based on the nature of its molecular interaction, its proton and electron donor ability, energy transfer processes, and dimer excision ability. In the case of DNA irradiated with gamma rays and protected with indole derivatives the best radioprotectors were tryptophan and indole. A mechanism based on radical trapping, local radical trapping, and partial recovery of the damaged protector is suggested.

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Material and Methods

DNA from Calbiochem, ex-Salmon sperm highly polymerized was used. The radioprotectors studied were: L-Tryptophan, tryptamine, indole and 3-in-

doleacetic acid all purchased from Calbiochem. E. Merck AG. Acetona and celluphane dialyzing tubes from A. H. Thomas (ϕ: 48 Å) were employed. Molecular interactions and molecular changes were measured in the following way: UV Absorbancy and Tm in a Beckman DU spectrophotometer, with temperature regulator. Circular dichroism and oriented circular dichroism in a Shimatzu QV (50) with a CD 1 attachment. Viscosimetry in a Beckman Rotatory Viscosimeter. Oriented DNA samples with or without indole and indole derivatives (mole/mole) were prepared in a flat quarz support, after stroke, until the material had air dried. For irradiation we used a UV lamp GE 30 W, G 30 T 8 (dose rate of $5 \cdot 10^3$ ergs/mm^2/min) and a Gamma Cs^{137} source of 100 Ci (dose rate of $5 \cdot 10^4$ ergs/cm^3/min, max. dose of $5 \cdot 10^6$ ergs/cm^3).

Results

1. *Radioprotective action of indole and indole derivatives on UV and gamma irradiated DNA solutions*

Fig. 1 and Table I show the different UV radioprotective efficiency of indole and indole derivatives. The best radioprotectors are tryptophan and indoleacetic acid with DRF of around 3 as evidenced by the DNA absorbancy loss at 260 nm or with DRF of 2.53 and 1.54 respectively when evaluation of the molecular damage is based on the absorbancy loss at 260 nm plus the degree of changes in the pattern of DNA absorbancy curve, between 230 – 290 nm (250/260, 280/260 and 290/260 ratios). Fig. 2 and Table II show the loss of DNA absorbancy after gamma irradiation, in the presence or in the abscence of the studied radioprotectors. The curves corresponding to loss of absorbancy at 260 nm as well as those showing the changes of the pattern of absorbancy curve (230 – 290 nm), Fig. 3, indicate that the best radioprotectors are tryptophan and indole. The DRF for both substances amounts to 11 for absorbancy loss at 260 nm and to 6.5 when considering the loss of absorbancy at 260 nm and the pattern changes of curves (230 – 290 nm).

2. *Molecular interactions between DNA indole and indole derivatives*

DNA complexes with indole and indole derivatives induce different changes in the values of the rate of dialysis, *Tm*, specific viscosity, circular dichroism and oriented circular dichroism (see Table III and Figs. 4 and 5).

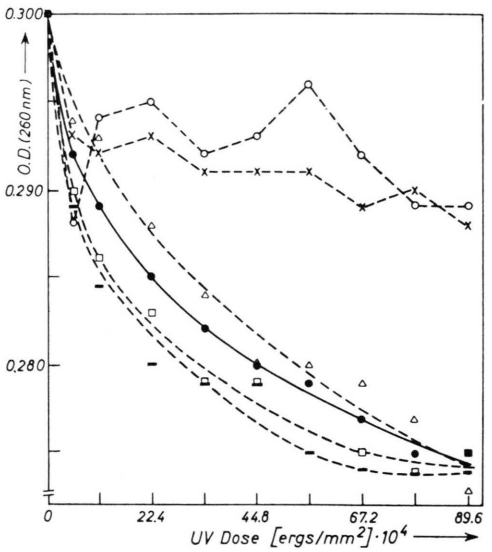


Fig. 1. Absorbancy loss at 260 nm of DNA solution, after radiation (280 nm), with or without protectors. Water solution of DNA (10^{-5} M): ●—●—●; water solution of DNA (10^{-5} M) protected with: 1. Tryptophan (10^{-5} M): ×--×--×; 2. indoleacetic acid (10^{-5} M): ○--○--○; 3. indole (10^{-5} M): □--□--□; 4. tryptamine (10^{-5} M): △--△--△; 5. alanine (10^{-5} M): □--□--□.

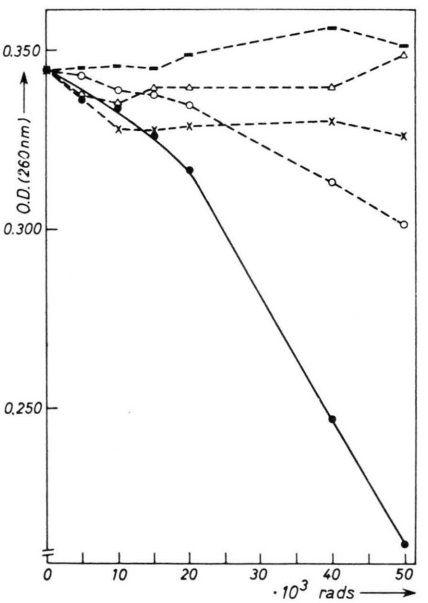


Fig. 2. Absorbancy loss at 260 nm of DNA solution after gamma radiation (Cs^{137}), with or without protectors. Water solution of DNA (10^{-5} M): ●—●—●. Water solution of DNA (10^{-5} M) protected with: 1. Tryptophan (10^{-5} M): ×--×--×; 2. indoleacetic acid (10^{-5} M): ○--○--○; 3. indole (10^{-5} M): ■--■--■; 4. tryptamine (10^{-5} M): △--△--△.

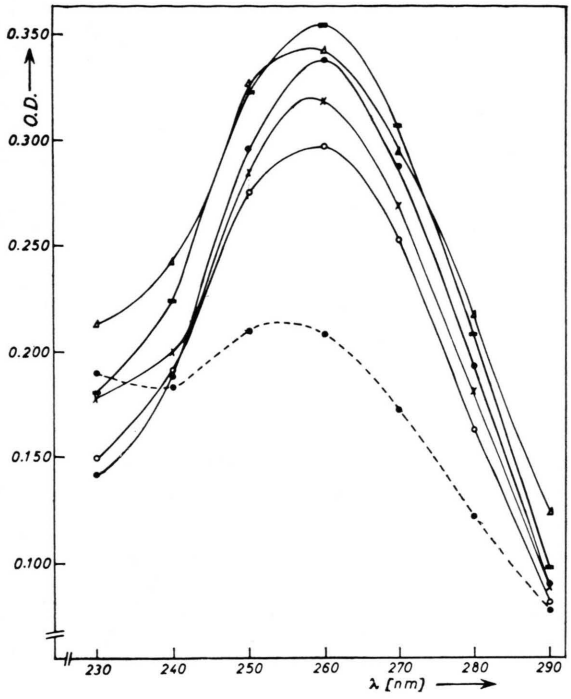
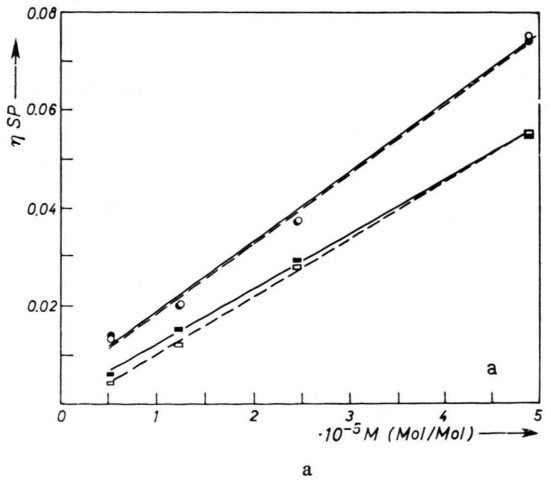
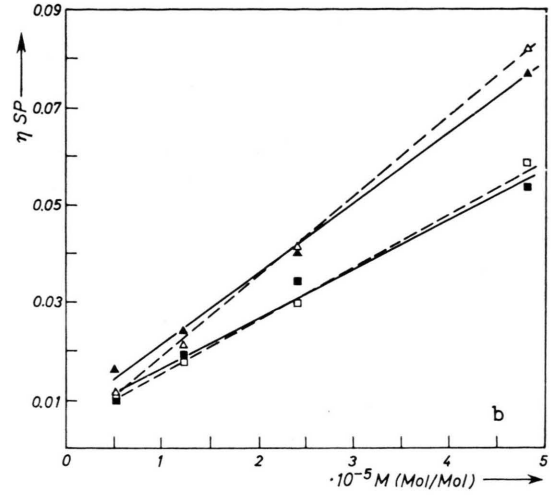


Fig. 3. Absorbancy pattern (between 230 to 290 nm) of DNA solution after gamma radiation, with or without protection (total dose: 50 kR). Water solution of DNA (10^{-5} M) (control): ●—●—●; water solution of DNA (10^{-5} M) protected with: 1. tryptophan (10^{-5} M): ×—×—×; 2. indoleacetic acid (10^{-5} M): ○—○—○; 3. indole (10^{-5} M): ■—■—■; 4. tryptamine (10^{-5} M): △—△—△. Water solution of irradiated DNA (10^{-5} M): ● - - ● - - ●.



a

Tryptophan does not induce appreciable changes in the UV absorbancy and *Tm* of the mixture with DNA (mole/mole) nor in the specific viscosity. The



b

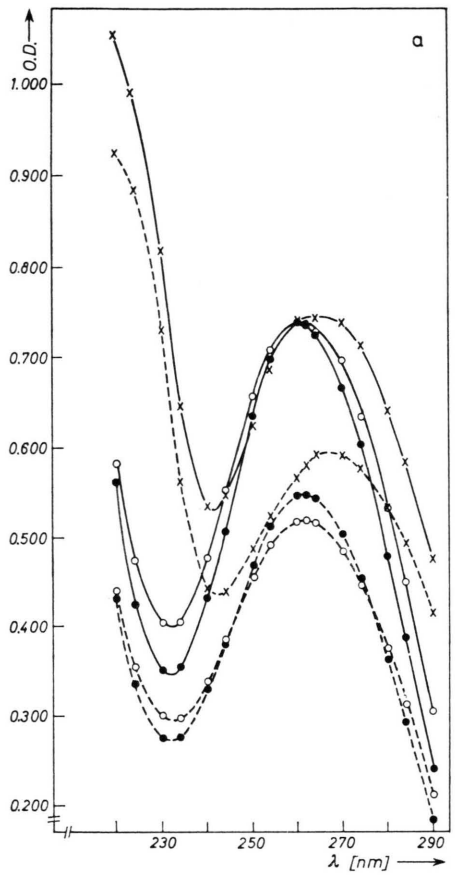
Fig. 4. Comparison of experimental data of specific viscosity of DNA radioprotector mixtures and calculated values. a. DNA water solution plus L-tryptophan (mole/mole), experimental: ●—●—● and calculated: ○ - - ○ - - ○. DNA water solution plus indole (mole/mole), experimental: ■—■—■ and calculated: □ - - □ - - □. b. DNA water solution plus tryptamine (mole/mole), experimental: ▲—▲—▲ and calculated: △ - - △ - - △. DNA water solution plus 3 indoleacetic acid (mole/mole), experimental: ■—■—■ and calculated: □ - - □ - - □. Differences between experimental and calculated value were not statistically significant.

Table I. Indexes of absorbancy changes induced by UV radiation of DNA plus protectors.

	Percentage of variation in the amplitude of peak (260 nm)	Percentage of variation of the absorbancy pattern *	Total [%]
L-tryptophan	4.11	0.46	4.57
3-indoleacetic acid	3.89	3.61	7.50
indole	8.34	1.07	9.41
tryptamine	8.29	7.12	15.41

* Variations in the absorbancy pattern were valorated, as the mean value of variations in the ratios 250/260 nm, 280/260 nm, and 290/260 nm when compairing protected samples *versus* control. Each value corresponds to a mean value of 3—4 samples being the standard deviation lower than 5%.

values of circular dichroism and of oriented circular dichroism change in the mixture by about 20% compared to those of DNA solutions. The strengh of binding of L-tryptophan with DNA is greater than the binding force of indole or tryptamine as follows from measurements of the rate of dialysis (Table



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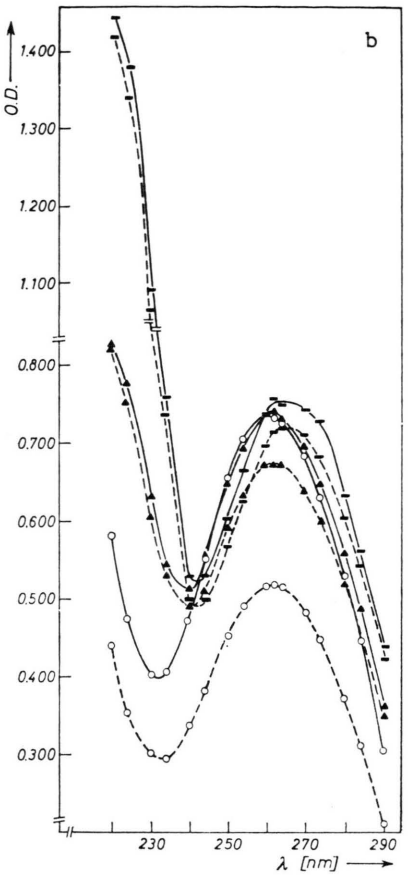


b

Fig. 5. Oriented circular dichroism of (⊥) and (‖) DNA samples and (⊥) and (‖) DNA plus indole or indole derivatives mixtures. a. Film of oriented DNA: ⊥: ○—○—○; ‖: ○ - - ○ - - ○. Film of oriented plus L-tryptophan (mole/mole): ⊥: ×—×—×; ‖: ×--×--×. Film of oriented DNA plus indole (mole/mole): ⊥: ●—●—●; ‖: ● - - ● - - ●. b. Film of oriented DNA: ⊥: ○—○—○; ‖: ○ - - ○ - - ○. Film of oriented DNA plus 3-indoleacetic acid (mole/mole): ⊥: ▲—▲—▲; ‖: ▲ - - ▲ - - ▲. Film of oriented DNA plus tryptamine (mole/mole): ⊥: ■—■—■; ‖: ■ - - ■ - - ■.

Table II. Indexes of absorbancy changes induced by gamma radiation of DNA plus protectors.

	Percentage of variation in the amplitude of peak (260 nm)	Percentage of variation of the absorbancy pattern *	Total [%]
indole	5.10	2.54	7.64
L-tryptophan	5.49	2.44	7.93
tryptamine	1.20	12.51	13.71
3-indoleacetic acid	11.79	12.31	24.21

* Variations in the absorbancy pattern were valorated, as the mean value of variations in the ratios 250/260 nm, 280/260 nm and 290/260 nm when compairing protected samples *versus* control. Each value corresponds to a mean value of two samples, being the standard deviation of the measurement lower than 5%.

III). At variance with these findings tryptamine shows, in its complex with DNA molecules big changes in the *Tm* value, specific viscosity, circular dichroism and oriented circular dichroism. Possibly in this interaction the secondary and tertiary structures of the macromolecules were modified. Also the rate of dialysis is low. Intermediate results were found for indole and 3-indoleacetic acid interactions with DNA.

Discussion

The high radioprotecting efficiency given by indole and indole derivatives is remarkable at mole-

Table III. Indexes of molecular interaction of DNA with indole or indole derivatives.

	A	B [%]	C [%]	D
L-tryptophan	2.9	23.86	0.00	1.24
indole	3.4	13.00	8.46	1.62
3-indoleacetic acid	29.2	70.34	20.58	0.78
tryptamine	57.8	80.00	54.45	1.5

A: Specific viscosity ($\cdot 10^4$) at infinite dilution. B: Circular Dichroism of oriented molecular. (Expressed as percentage of deviation from control values.) C: *Tm* (m.p. of DNA radioprotector mixture) (expressed as percentage of deviation from control values). D: Rate of dialysis measured by the indole or indole derivatives out put against time. Standard deviation of measurement lower than 5%.

cular level as well as in cell cultures (DRF up to 10 in the case of gamma irradiated samples and of 500, for instance, in UV or gamma irradiated erythrocytes maintained in a solution of L-tryptophan 10^{-4} M [6]). Having in mind that the prevailing mechanisms for UV or gamma radioprotection are possibly different, it is understable that while L-tryptophan is one of the best radioprotectors for both kinds of radiation damage, indoleacetic acid follows the tryptophan index for UV radioprotection and on the other hand indole is a good gamma radioprotector as tryptophan (see Table II).

Actually the radioprotecting ability for gamma radiation at the molecular level of indole and tryptophan can be ascribed principally to their radical trapping capacity for OH [7] and to their possible local radical quenching, (proton and electron donor ability), favored by the close interaction of these molecules with DNA as described above (Table II). Moreover the proposed self quenching and [7, 8] recombination processes of radicals of indole and indole derivatives permit to understand the high protective action specially at relatively low concentrations of L-tryptophan ($\cong 10^{-4}$ M) solutions.

For UV radiation damage tryptophan has the high protecting ability which could be correlated with its strong binding to the DNA molecule (low diffusion rate of tryptophan in the dialyzed complex) and with the nature of this close interaction which induces only small changes in the viscosity and molecular oriented circular dichroism (Table III). In discussing the mechanism of this action the nature of the tryptophan-DNA interaction should also be considered (which could hinder the formation of dimers), as well as the proton and electron donor ability of UV excited indole and indole derivatives facilitated in tryptophan and indole acetic acid by an easier quenching at pH 7 [8] and the feasibility of energy transfer from a triplet state of an excited DNA to tryptophan molecule. Dimer excision [9] induced by indole and indole derivatives radicals could play a role. In fact, tryptamine shows a lower protective efficiency than tryptophan, as well in simultaneously irradiated and protected DNA, as in experiments in which DNA was irradiated first alone and thereafter reirradiated in the presence of tryptamine or tryptophan. As the radicals formed in indole and indole derivatives after flash irradiations are probably the same [10] it is possible that the easier quenching of tryptophan, mentioned above could explain the differences of tryptophan and tryptamine protection maintained in reirradiated DNA (Fig. 6).

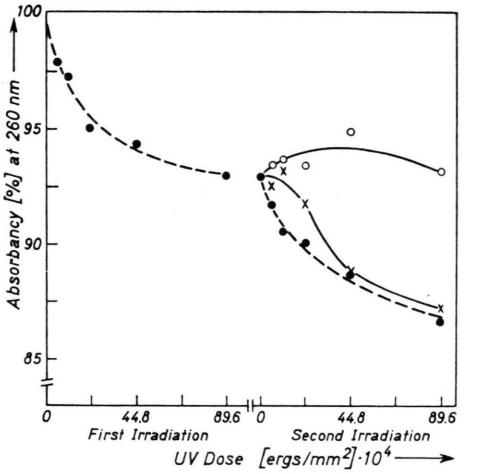
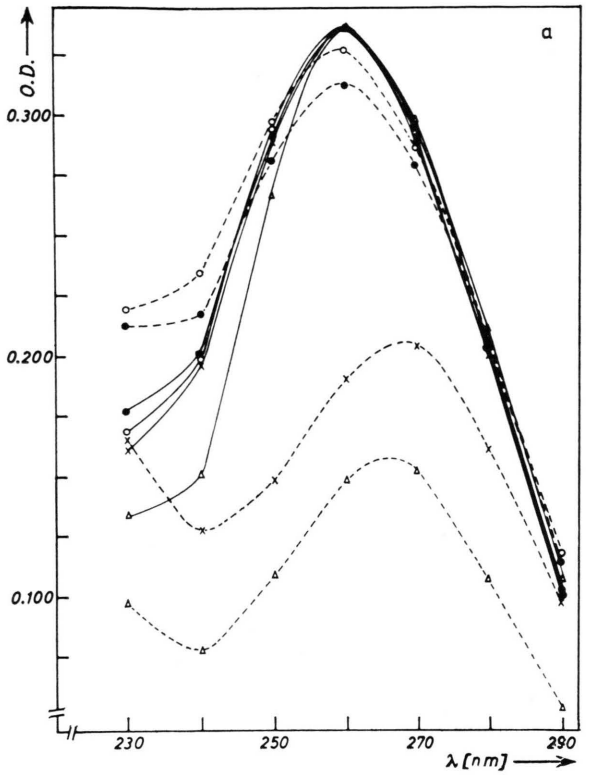


Fig. 6. UV irradiated DNA solution protected with indole and indole derivatives (mole/mole) during second irradiation. DNA water solution: ●--●--●. Irradiated DNA solution plus L-tryptophan ($5 \cdot 10^{-5}$ M): ○—○—○. Irradiated DNA solution plus tryptamine ($5 \cdot 10^{-5}$ M): ×—×—×.

We have not seen at the molarity (10^{-4} M) of solutions studied evidence of charge transfer process, which, however does not contradict data of literature based on observations of samples with higher molarities (10^{-2} to 10 M) [11].

UV irradiation of DNA-tryptophan solutions in the presence of acetone 0.34 M (2%) induces greater damage than irradiation of DNA plus acetone (2%) (Fig. 7), but at similar concentration (10^{-4} M) of



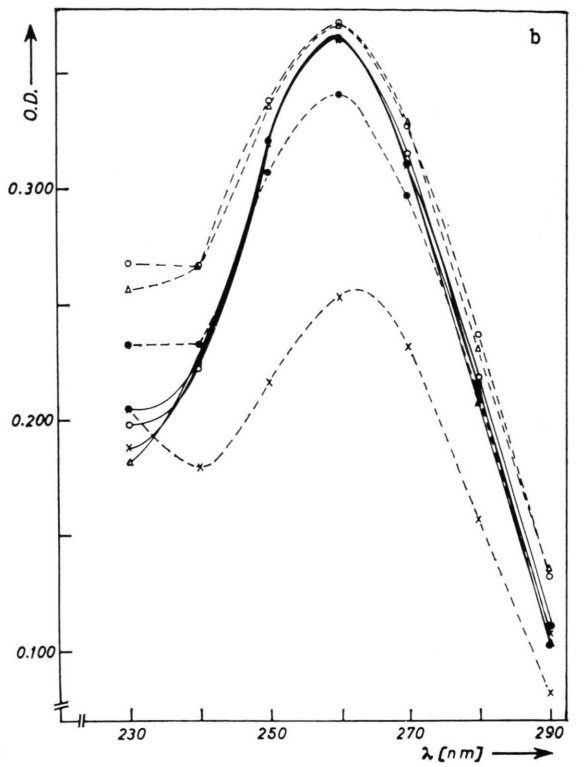


Fig. 7. Radiation damage of UV irradiated solutions of DNA and DNA plus tryptophan (10^{-4} M) in the presence of acetone 0.34 M (a) or 10^{-4} M (b). DNA solution (10^{-4} M): ●—●—●. DNA solution (10^{-4} M) plus: 1. Tryptophan (10^{-4} M): ○—○—○; 2. Acetone 0.34 M (a) (10^{-4} M): ×—×—×; 3. Tryptophan (10^{-4} M) plus acetone 0.34 M (a); (10^{-4} M) (b) △—△—△. UV irradiated ($9 \cdot 10^5$ ergs) DNA solution (10^{-4} M): ●--●--●. UV irradiated ($9 \cdot 10^5$ ergs) DNA solution (10^{-4} M) plus: 1. Tryptophan (10^{-4} M): ○--○--○; 2. acetone 0.34 M (a); 10^{-4} M (b): ×--×--×; 3. tryptophan (10^{-4} M) plus acetone 0.34 M (a); 10^{-4} M (b): △--△--△.

acetone, DNA and tryptophan; DNA is as well protected as when irradiated in the presence of only tryptophan. It is possible that at high molarities the induction of a big number of acetone and OH radicals cooperates with a competitive energy transfer from excited (triplet-states) acetone or DNA molecules to tryptophan [12, 13], increasing the probability of DNA damage, because of its lower probability of energy transfer.

In gamma irradiated DNA-tryptophan acetone (2%) solutions there is a high DNA protection in spite of the radiosensitization described ability of acetone [14]. Probably the energy transfer process from DNA to tryptophan is less important in gamma radioprotection.

Finally indole and indole derivative solutions after UV or gamma irradiation show the formation of a compound ("polymers"), that does not dialyze and shows after radiation increased absorbancy to the far UV region (220 nm). The yield of this compound increases when irradiation is accomplished in *N*-atmosphere or in acetone 2% (w/w).

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Interaction of L-Tryptophan with Inosine and Guanosine

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L-Tryptophan, guanosine, inosine

The equilibrium constants for the complex between L-tryptophan and inosine are established by solubility measurements at different temperatures, the order of magnitude of these constants being about 10^3 times bigger than the corresponding one for the formation of guanosine-tryptophan or any other of the nucleoside-tryptophan complexes. The mode of association of these molecules is governed mainly by stacking interactions as it has been suggested from previous PMR results. The differences in magnitude obtained for the equilibrium constants of (I+T) with respect to (G+T) are discussed in terms of differences in solute-solvent and solvent-solvent interactions. Thermodynamic parameters inferred from the equilibrium constants at different temperatures support this suggestion. Infrared results on I-T complex suggest that changes at the ribose moiety occurs and that possible hydrogen bridges are involved in the mechanism of association besides the stacking interactions.

Introduction

A molecular study of the interaction between amino acids and nucleic acids as well as related molecules may help to understand the chemical specificity of several protein-nucleic acids interactions ocurring at the cell level. In this respect some information on the origin of the genetic code could also arise from these investigations.

An approach to simplify the study of the complex molecular interaction between polymers is to establish first the nature of chemical bondings between the monomeric constituents. The chemical interaction between aromatic amino acids and mononucleosides or derivatives have been measured by different methods [1–6]. The formation of complexes between aromatic amino acids and polynucleotides (single and double stranded) have been also established by means of fluorescence, proton magnetic resonance and temperature studies [7–9].

In general all these studies suggest that the interaction between aromatic amino acids and the different nucleobases in aqueous solutions are governed mainly by stacking forces responsible for the partial overlapping of the aromatic rings. To the authors' knowledge, there is no data concerning the molecular interaction of L-tryptophan with inosine or its derivatives. In this work a quantitative study of the complex formation between inosine and L-tryptophan is reported. The results obtained are based on solubility measurements at different temperatures. A comparison between the chemical nature of this complex and that of guanosine with tryptophan is also established. The much greater binding affinity of L-tryptophan with inosine as compared to guanosine (which has been shown to have the greatest affinity as compared with the rest of the nucleosides [4, 5]) may be indicative of a possible regulation action that this interaction may play in the synthesis of proteins.

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