High Protective Action against Gamma Radiation Damage, by a Tryptophan-ethanol Mixture

G. SÁNCHEZ, S. HASBÚN, E. DÍAZ, M. GREZ, F. LYON, M. PIEBER, C. ROMERO, and J. C. TOHÁ

Biofísica, Departamento de Física Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile and Comisión Nacional de Energía Nuclear

In this research we have investigated the high radio-protective ability of a mixture of tryptophan plus ethanol, on a Gamma irradiated water solution of purine, pyrimidine bases, DNA and even on irradiated seeds (Lens Sculentum).

From a group of non sulphydryl radioprotectors (methanol, formaldehyde, acetaldehyde, formiate, acetate, ethanol and tryptophan); ethanol and tryptophan were selected because their high protector action on irradiated purine and pyrimidine bases. At molarities of the order of $10^{-4}$ for tryptophan-ethanol and bases and DNA the respective DRF values were: for guanine: 20.58; for adenine: 3.75; for thymine: 4.94; for cytosine: 5.52; for uracil: 5.43; and for DNA: 18.95. For irradiated seeds protected with tryptophan-ethanol ($1.5 \times 10^{-3} \text{ M}; 2.6 \times 10^{-3} \text{ M}$) the DRF value was 3.33 and radiation damage becomes evident only after a total dose of $2 \times 10^{6}$ rads.

A partial different mechanisms for radioprotection with ethanol and tryptophan is postulated; this is supported by the fact that when tryptophan molarities attains that of protected DNA, there are a rapid increase in its values of DRF, crossing up the ethanol protection curve. Actually tryptophan could act not only as a radical scavenger, but also, as localized radical quencher and/or energy trapper.

In the study of the radioprotective action of the mixture, the different mechanisms of tryptophan and ethanol protection, were evidenced.

Materials and Methods

Adenine and uracil bases were purchased from Mann Research Lab. Inc.; cytosine from Sigma Chemical Co.; guanine * from Pabst Laboratories; thymine, l-tryptophan and DNA (ex. Salmon Sperm) from Cal-biochem; methanol, ethanol, formaldehyde, acetaldehyde, acetic acid and sodium hydroxide, from E. Merck AG, and formic acid from Riedel de Haen AG.

Irradiation experiments were carried out in a 137 Cs source of $10^{5}$ Ci at a dose rate of 5.376 to 8.922 rads/min. Spectrophotometric determinations were done in a Cary 16, double beam spectrophotometer.

The development of seeds, on moistened filter paper, were followed by measuring daily the growth of epicotyledons.

* Guanine was used as chlorhydrate.
Results

1. Study of radioprotection at the molecular level

a) Radioprotection of irradiated purine and pyrimidine bases by: methanol, ethanol, formaldehyde, sodium formiate, sodium acetate and tryptophan solutions

Table 1 shows the "dose reduction factor" (DRF), calculated as the ratio of the $D_{37}$ of irradiated nitrogen bases with and without protector; being the $D_{37}$, the dose at which after irradiation, it remains 37% of the original bases U.V. absorbancy.

For each one of the studied chemical protectors an index of its radioprotective action was calculated, considering in this index, the sum of all the DRF of the different bases treated with it.

Since tryptophan and ethanol had the highest DRF values (see Table 1), they were therefore selected to be used as a mixture.

It is worth while to point out that guanine which is the base that interacts more strongly with tryptophan, presents the highest DRF value (32.80) when at concentration of $0.38 \times 10^{-4}$ M, was irradiated in the presence of a $0.76 \times 10^{-4}$ M solution of tryptophan.

b) Radioprotection of irradiated purine and pyrimidine bases by the combined action of tryptophan and ethanol

Figs. 1 to 3 show the U.V. absorbancy pattern of adenine at $0.71 \times 10^{-4}$ M, guanine ($0.38 \times 10^{-4}$ M) and a typical pyrimidine base, (thymine, $1.6 \times 10^{-4}$ M), after irradiation. In each figure the curves corresponds respectively to protected and no protected nitrogen bases. Ethanol was used at a concentration of 2.5 mole/mole of base and tryptophan at 0.5 mole/mole of base. The same molarities of both were used in the mixture, obtaining a high degree of radioprotection (Fig. 1 to 3 and Table 2).

The inserts of Figs. 1 and 3 display, in the case of irradiated and protected adenine (Fig. 1), an hyperchromic effect in the region of 230 and 300 nm which is also evidenced at 240 nm for thymine (Fig. 3).

![U.V. absorbancy at 260.5 nm of water solutions of adenine after irradiation in the presence or absence of radio-protectors. (Semilog plot.) Adenine solution ($0.71 \times 10^{-4}$ M): --; Adenine solution ($0.71 \times 10^{-4}$ M) plus ethanol ($1.77 \times 10^{-4}$ M): --; Adenine solution ($0.71 \times 10^{-4}$ M) plus tryptophan ($0.35 \times 10^{-4}$ M): --; Adenine solution ($0.71 \times 10^{-4}$ M) plus ethanol ($1.77 \times 10^{-4}$ M) plus tryptophan ($0.35 \times 10^{-4}$ M): -- Insert: Curves of U.V. absorbancy of water solutions of adenine, irradiated with a total dose of 1.18 $\times 10^{5}$ rads (D50) in the presence or absence of radioprotectors (same molarities mentioned above). Adenine solution: --, Irradiated adenine solution: --, Irradiated adenine solution in the presence of ethanol plus tryptophan: --.

Table 1. Radioprotection of nitrogen bases with: tryptophan, ethanol, methanol, formaldehyde, acetaldehyde, formic or acetic acid. * Radioprotectors were used at molarities 10 times greater than that of bases, with the exception of tryptophan employed only twice. ** $D_{37}$ Ratio = $D_{37}$ of protected base / $D_{37}$ of non protected base. Standard deviation of measurements 0.01.

<table>
<thead>
<tr>
<th>Radioprotectors*</th>
<th>Nitrogen bases</th>
<th>Adenine $0.71 \times 10^{-4}$ M</th>
<th>Guanine $0.38 \times 10^{-4}$ M</th>
<th>Thymine $1.6 \times 10^{-4}$ M</th>
<th>Cytosine $1.8 \times 10^{-4}$ M</th>
<th>Uracil $1.8 \times 10^{-4}$ M</th>
<th>Sum of $D_{37}$ Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryptophan</td>
<td>$D_{37}$ ratio**</td>
<td>5.48</td>
<td>32.80</td>
<td>5.70</td>
<td>12.40</td>
<td>8.10</td>
<td>64.48</td>
</tr>
<tr>
<td>ethanol</td>
<td>$D_{37}$ ratio</td>
<td>8.60</td>
<td>8.75</td>
<td>5.28</td>
<td>6.90</td>
<td>14.80</td>
<td>44.33</td>
</tr>
<tr>
<td>formaldehyde</td>
<td>$D_{37}$ ratio</td>
<td>5.96</td>
<td>5.00</td>
<td>3.44</td>
<td>17.10</td>
<td>3.59</td>
<td>35.09</td>
</tr>
<tr>
<td>formic acid</td>
<td>$D_{37}$ ratio</td>
<td>2.42</td>
<td>2.84</td>
<td>3.25</td>
<td>6.15</td>
<td>3.46</td>
<td>18.12</td>
</tr>
<tr>
<td>methanol</td>
<td>$D_{37}$ ratio</td>
<td>1.54</td>
<td>7.50</td>
<td>3.05</td>
<td>3.74</td>
<td>3.31</td>
<td>19.44</td>
</tr>
<tr>
<td>acetaldehyde</td>
<td>$D_{37}$ ratio</td>
<td>1.04</td>
<td>1.25</td>
<td>1.68</td>
<td>7.25</td>
<td>3.34</td>
<td>14.56</td>
</tr>
<tr>
<td>acetic acid</td>
<td>$D_{37}$ ratio</td>
<td>1.14</td>
<td>1.50</td>
<td>1.03</td>
<td>1.21</td>
<td>1.05</td>
<td>5.93</td>
</tr>
</tbody>
</table>

* Radioprotectors were used at molarities 10 times greater than that of bases, with the exception of tryptophan employed only twice. ** $D_{37}$ Ratio = $D_{37}$ of protected base / $D_{37}$ of non protected base. Standard deviation of measurements 0.01.
c) Radioprotection of irradiated DNA by tryptophan-ethanol mixture

In Table 3, the radioprotective effect of tryptophan-ethanol mixture on irradiated DNA at a mol ratio of 1, is expressed as the DRF<sub>260nm</sub> of 37% of remaining absorbancy of DNA irradiated in the presence or absence of the protective molecules; this ratio attains a value as high as 18.95.

Fig. 4 corresponds to the decay of the absorbancy of DNA at 260 nm after irradiation with and without the protectors, and Fig. 5 clearly shows the differences in the U.V. absorbancy curves of protected and non protected irradiated DNA. The same hyperchromic effect above described for some irradiated bases at 230 and 300 nm is more evident for irradiated DNA molecule and besides, a blue shift of the peak of absorbancy is present.

The results of plotting the DRF values of DNA samples against increasing amount of tryptophan, ethanol, and tryptophan-ethanol mixture are shown in Fig. 6. Above a tryptophan molarity of 1 × 10<sup>-4</sup> the DRF curve, crosses the ethanol one, showing a rapid increase, so that a better radioprotection of tryptophan, begins now to be point out.

Even the high DRF values found for the curve of tryptophan-ethanol mixture, they are not, nevertheless, enough high to reach the values of the product of the independent DRF figures of ethanol and tryptophan.

At higher molarities the efficiency of the mixture comes down.

2. Radioprotection of “Lens Sculentum” seeds irradiated in the presence of tryptophan-ethanol solution

These “in vivo” experiments of radioprotection were carried out in “Lens Sculentum” seeds, irradiated with a total dose up to 10<sup>5</sup> rads, in the

Fig. 3. U.V. absorbancy at 264.5 nm of water solution of thymine after irradiation in the presence or absence of radioprotectors. (Semilog plot.) Thymine solution (1.6×10<sup>-4</sup> M) —— —— ——, Thymine solution (1.6×10<sup>-4</sup> M) plus ethanol 4×10<sup>-4</sup> M) —— —— ——, Thymine solution (1.6×10<sup>-4</sup> M) plus tryptophan (0.8×10<sup>-4</sup> M) —— —— ——, Thymine solution (1.6×10<sup>-4</sup> M) plus ethanol 4×10<sup>-4</sup> M plus Tryptophan 0.8×10<sup>-4</sup> M) —— —— ——. Insert: Curves of U.V. absorbancy of water solutions of thymine, irradiated with a total dose of 25×10<sup>3</sup> rads (D50), in the presence or absence of radioprotectors. (Same molarities mentioned above.) Thymine solution: —— —— ——, Irradiated thymine solution: —— —— ——, Irradiated thymine solution in the presence of ethanol plus tryptophan: • ◦ ◦ ◦ ◦ .
Adenine 0.71 \times 10^{-4} M
Guanine 0.38 \times 10^{-4} M
Thymine 1.6 \times 10^{-4} M
Cytosine 1.8 \times 10^{-4} M
Uracil 1.8 \times 10^{-4} M

Table 2. Radioprotection of nitrogen bases by tryptophan, ethanol and tryptophan-ethanol mixture.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>D_{37} of irradiated base (1)</th>
<th>D_{37} of irradiated base in the presence of Tryptophan (2)</th>
<th>D_{37} Ratio (2)/(1)</th>
<th>D_{37} of irradiated base in the presence of ethanol (3)</th>
<th>D_{37} Ratio (3)/(1)</th>
<th>D_{37} of irradiated base in the presence of tryptophan-ethanol mixture (4)</th>
<th>D_{37} Ratio (4)/(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>165 \times 10^3 rads</td>
<td>2.09</td>
<td>1.46</td>
<td>604 \times 10^3 rads</td>
<td>3.75</td>
<td>38 \times 10^3 rads</td>
<td>11.65</td>
</tr>
<tr>
<td>Guanine</td>
<td>9 \times 10^3 rads</td>
<td>236 \times 10^3 rads</td>
<td>3.89</td>
<td>185 \times 10^3 rads</td>
<td>4.94</td>
<td>443 \times 10^3 rads</td>
<td>11.65</td>
</tr>
<tr>
<td>Thymine</td>
<td>34 \times 10^3 rads</td>
<td>33 \times 10^3 rads</td>
<td>1.88</td>
<td>168 \times 10^3 rads</td>
<td>5.52</td>
<td>159 \times 10^3 rads</td>
<td>4.18</td>
</tr>
<tr>
<td>Cytosine</td>
<td>29 \times 10^3 rads</td>
<td>64 \times 10^3 rads</td>
<td>2.76</td>
<td>160 \times 10^3 rads</td>
<td>5.43</td>
<td>104 \times 10^3 rads</td>
<td>3.75</td>
</tr>
<tr>
<td>Uracil</td>
<td>35 \times 10^3 rads</td>
<td>80 \times 10^3 rads</td>
<td>2.37</td>
<td>190 \times 10^3 rads</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The molarities of tryptophan and ethanol solutions were: 0.5 and 2.5 times the base molarity respectively. (Same molarities in the tryptophan-ethanol mixture.) Standard deviation of measurement: 0.01.

Table 3. Radioprotection of DNA molecule by tryptophan, ethanol and tryptophan-ethanol mixture. Standard deviation of measurements: 0.01—0.02.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>D_{37} of DNA (1.22 \times 10^{-4}) (1)</th>
<th>D_{37} of DNA irradiated in the presence of tryptophan (6 \times 10^{-4} M) (2)</th>
<th>D_{37} of DNA irradiated in the presence of ethanol (3 \times 10^{-4} M) (3)</th>
<th>D_{37} of DNA irradiated in the presence of tryptophan-ethanol mixture (6 \times 10^{-4} M) (4)</th>
<th>D_{37} Ratio (2)/(1)</th>
<th>D_{37} Ratio (3)/(1)</th>
<th>D_{37} Ratio (4)/(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>38 \times 10^3 rads</td>
<td>443 \times 10^3 rads</td>
<td>159 \times 10^3 rads</td>
<td>720 \times 10^3 rads</td>
<td>11.65</td>
<td>4.18</td>
<td>18.95</td>
</tr>
</tbody>
</table>

Discussion

It is timely to emphasize the magnitude of the above described radioprotection displayed by tryptophan-ethanol mixture (10^{-4}) on irradiated nitrogen bases, DNA molecule or seeds. For instance the DRF for DNA is of 18.95 and that of guanine of 20.58.
In spite of the small changes found in the pattern of U.V. absorbancy curve of irradiated and protected DNA and nitrogen bases, an hyperchromic effect appears at 230 and 300 nm in the curve of adenine, thymine and specially in that of DNA; this last curve shows also an small blue shift probably related with the swift radiation damage of the more sensitive tryptophan molecule, which has a U.V. peak of absorbancy around 280 nm.

A different mechanism for the radioprotective action performed by tryptophan and that carried out by ethanol can be postulated considering the behavior of the DRF curve of tryptophan (Fig. 6), which shows a rapid increase in its value just when...
its molarity comes up to that of protected DNA, that in accordance, with kinetics data of molecular interactions of tryptophan and bases before described. Nevertheless, the high DRF values of the tryptophan-ethanol curve, they are lower than the expected values for an independent action of tryptophan and ethanol. Therefore, the different mechanisms postulated above are probably, partially distincts.

Ethanol for instance, could act mainly as a trapper of radicals from the irradiated water solution; and tryptophan, on the other hand, notwithstanding being also a free radical scavenger, could act also as a localized radical trapper or as an energy quencher of the excited DNA molecule; this in agreement with the previously described strong interaction of tryptophan with DNA; and the probable transfer of energy from excited DNA, through a triplet-triplet interaction.

Otherwise, irradiated tryptophan and ethanol could interact between them in such a way, that a different ability for radical trapping could be induced. Nevertheless in the literature an increase of the G value of tryptophan is described, for tryptophan solutions irradiated in the presence of ethanol.

When tryptophan is added to a sample where the high concentration of ethanol determines a saturation effect, lower values of the DRF were obtained due, probably, to the preferential interaction of both protectors present actually at higher molarity than DNA.

Work in progress related with mechanisms of action of tryptophan-ethanol solutions and with its use in higher organisms as radioprotectors and/or anti-aging agents. The probable advantage of using combinations of radioprotectors having different metabolic pathways in the organisms, is brought out.

The growth stimulation found in epicotyledons of protected and irradiated seeds, could be assimilated to that described in the literature for low dose-rates irradiation.

To Prof. J. MARDONES for helpful suggestions. To A. del Río and A. GARRÃO for technical assistance.

This work was partially supported from a Grant of Comisión Nacional de Investigación Científica y Tecnológica.