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Journal of Photochemistry and Photobiology B: Biology 40 (1997) 299–304

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY B:BIOLOGY

RNA-protein cross-links induced by sensitization with a pyrroloquinolinone derivative, a furocoumarin analogue

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Received 21 March 1997; accepted 14 July 1997

Abstract

The capacity of 2,6-dimethyl-9-methoxy-4H-pyrrolo [3,2,1-ij] quinolin-4-one (PQ), a furocoumarin analogue, of inhibiting protein synthesis in Ehrlich cells upon UVA irradiation was investigated. Using 8-methoxypsoralen (8-MOP) as a reference, we observed that in our short-term test the block of RNA synthesis do not affect protein synthesis, which is driven by pre-synthesised molecules of m-RNA; actually 8-MOP, studied at 100 μM concentration, practically abolished RNA synthesis without affecting significantly protein synthesis. Studying PQ sensitization in HL60 cells by alkaline elution and protein precipitation, the formation of covalent RNA-protein cross-links was observed. 8-MOP, assayed in severe experimental conditions, induced only moderate amounts of such lesion. On the basis of the data obtained in experiments carried out using various scavengers or exposing cells to UVA light in a nitrogen atmosphere, this damage appeared to be due to singlet oxygen formation, which is generated by PQ to a large extent. These results are consistent with the data obtained by H. Singh and J.A. Vadasz (Singlet oxygen: a major reactive species in the furocoumarin photosensitized inactivation of *E.coli* ribosomes. Photochem. Photobiol., 28 (1978) 539–545) on *E.coli* ribosomes. The lower activity we observed with 8-MOP might be attributed to a different sensitivity of whole mammalian cells in comparison with isolated ribosomes. © 1997 Elsevier Science S.A.

Keywords: Protein synthesis; RNA-protein cross-links; Singlet oxygen; Pyrroloquinolinones; Furocoumarins

1. Introduction

The unusual photosensitizing properties of 2,6-dimethyl-9-methoxy-4H-pyrrolo [3,2,1-ij] quinolin-4-one (PQ), a furocoumarin analogue, have been already described [1–3]. Upon UVA irradiation, this compound damages DNA, inducing single-strand breaks and DNA-protein cross-links [1]; in addition, it produces high levels of singlet oxygen and photobinds to a significant extent to sieroalbumin [3]. Contrary to the well-known behaviour of classic furocoumarins, PQ is practically unable of photobinding to DNA and RNA [3], and it cannot induce inter-strand cross-links into DNA at all [1]. However, PQ produces strong antiproliferative effects in mammalian cells, with an evident inhibition of the syntheses of the main cellular macromolecules, DNA, RNA and proteins [1,2]. In particular, PQ affects protein synthesis in mild experimental conditions, similar to that known furocoumarins generally required to reduce DNA or RNA synthesis; on the other hand, the inhibition of protein synthesis after sensitization with furocoumarins occurs only at large drug concentrations and after high UVA doses [4].

In this paper we describe some experiments carried out to obtain information on PQ capacity to inhibit protein synthesis and to explain its mechanism. We studied the relationships between the inhibition of RNA and protein syntheses in Ehrlich ascites cells and the formation of RNA damage in HL60 cells cultivated in vitro. As a reference we chose a well-known linear furocoumarin, 8-methoxypsoralen (8-MOP).

2. Materials and methods

2.1. Chemicals

2,6-Dimethyl-9-methoxy-4H-pyrrolo [3,2,1-ij] quinolin-4-one (PQ) was prepared by chemical synthesis [2]; 8-

Abbreviations: PQ: 2,6-Dimethyl-9-methoxy-4H-pyrrolo [3,2,1-ij] quinolin-4-one; 8-MOP: 8-methoxypsoralen; DMSO: dimethyl sulfoxide; RPC: RNA-protein cross-links; CC: cross-linking coefficient; PPO: 2,5-diphenyloxazole; POPOP: 2,2′-p-phenylene-bis-(5-phenyloxazole); DABCO: 1,4-diazabicyclo [2.2.2] octane

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PII S1011-1344(97)00073-0

methoxypsoralen (8-MOP), used as a reference, was obtained from Chinoin, Milan, Italy. The molecular structure of these compounds is reported in Fig. 1. Both compounds were dissolved in dimethyl-sulfoxide (1 mg ml^{-1}); these solutions were frozen and kept in the dark. Just before the experiment, a calculated amount of the drug solution was added to growth medium, to a solvent final concentration of 0.5%. ^{3}H-uridine (1.1 TBq mM^{-1}) and ^{3}H-leucine (2.37 TBq mM^{-1}) were both obtained from Amersham International plc, UK. Tetrapropylammonium hydroxide (1 M aqueous solution) and ribonuclease type III-B from bovine pancreas (cat. R-5750) were both obtained from Sigma Chemie, Deisenhofen, Germany.

2.2. UVA irradiations

Cell suspensions containing PQ were incubated at room temperature for 15 min in the dark and then put into Petri dishes (5 cm in diameter, 3 ml) placed in an ice-bath. The samples were then exposed to UVA light. Irradiations were carried out by Philips HPW 125 lamps, provided with a built-in Philips filter; emission in the range of 320–400 nm, with a maximum, over 90% of the total, at 365 nm; the irradiation intensity, determined by a UV-X radiometer (Ultraviolet Products Inc., Cambridge, UK), was 5.5 J s^{-1} m^{-2}.

Some irradiations were carried out in a nitrogen atmosphere. Cell suspensions were putted in culture flasks with caps provided with two tygon tubes which allowed to equilibrate with nitrogen the atmosphere and the liquid inside the flasks. To avoid damage to the cells, the tubes were collocated above the liquid surface so that nitrogen was not bubbling through the liquid. Before irradiations, we saturated the environment with nitrogen at a flow rate of 500–800 ml min^{-1} for 10 min with occasional shaking, maintaining this flow rate during the entire irradiation time.

2.3. RNA and protein syntheses

RNA and protein syntheses were assayed in Ehrlich ascites tumor cells (Lettré strain, weekly transplanted into Swiss mice) as already described [1,2]; briefly, just after irradiation 10^6 cells were incubated in growth medium for 30 min in the presence of 40 kBq ml^{-1} of the tritiated precursor (uridine or leucine). The acid-insoluble fraction was precipitated by adding ice-cold trichloroacetic acid to a final concentration of 5%, and then filtering on Whatman GF/C filters (2.5 cm in diameter) using a Sample Manifold apparatus (Millipore Corporation, Bedford, MA, USA). After several washing, the filters were dried and counted. The results were expressed as the percent of the radioactivity incorporated into untreated cells (about 3–6 kBq); the data were then computed by probit analysis.

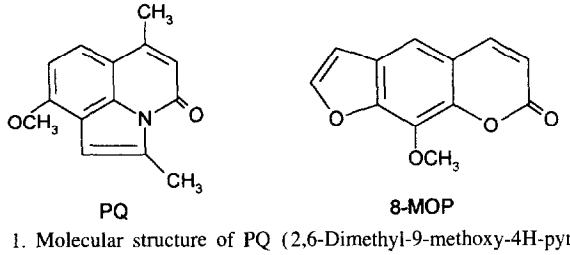

Fig. 1. Molecular structure of PQ (2,6-Dimethyl-9-methoxy-4H-pyrrolo [3,2,1-ij] quinolin-4-one) and 8-MOP (8-methoxypsoralen).

2.4. Experiments with mammalian cells cultivated in vitro

HL60 cells were grown in RPMI-1640 medium (Whitiaker Bioproduct, Walkersville, MD, USA) containing 10% foetal calf serum (Biological Industries, Kibbutz Beth Haemek, Israel), supplemented with 25 mM HEPES buffer and l-glutamine. For UVA irradiations, HL60 cells were seeded in Petri dishes in growth medium without foetal calf serum containing PQ, and incubated in the dark for 15 min to allow uptake of the drug into the cells. Samples were then exposed to UVA. In any case, 0.5% dimethyl sulfoxide, the solvent used to dissolve PQ, was added to untreated controls. Each experiment was carried out at least three times.

2.4.1. Alkaline elution

In order to detect the formation of RNA-protein cross-links (RPC), the classic method of alkaline elution described by Kohn for DNA-protein cross-links [5] was modified. HL60 cells in exponential growth were incubated overnight in the presence of ^{3}H-uridine (7.4 KBq ml^{-1}) to label RNA. The radioactive medium was removed and replaced by fresh growth medium and the cells were further incubated for 3 hours. Then, the calculated amount of DMSO solution of the compound to be studied was added and the cells were exposed to UVA. The cells were then washed with PBS. Contrary to the method for DNA-protein cross-links, the samples were not exposed to gamma rays. About $0.5–1.0 \times 10^6$ ^{3}H-labeled cells were deposited on a polyvinyl chloride filter (pores 5 μm in diameter, Nucleopore Corp., Pleasanton, CA, USA) in a Swinnex-25 filter holder (Millipore Corp., Bedford, MA, USA) and immediately lysed with 2% sodium dodecylsulfate, 0.1 M glycine, 0.025 M Na_2EDTA, pH 10, (5 ml); after this, the solution was allowed to flow out by gravity. 2 ml of the same solution was then gently poured on the filter (in some experiments containing 0.5 mg ml^{-1} of ribonuclease), followed by 40 ml of the eluting solution (tetrapropylammonium hydroxide–EDTA 0.1% sodium dodecyl sulphate, pH 12.1). Elution was carried out with a Gilson Minipuls peristaltic pump, at a flow of 0.03–0.04 ml min^{-1}. The pH of the fractions, collected with a Gilson fraction collector (approximately, 3.5 ml per fraction), were adjusted to about 7, and their radioactivity was determined.

2.4.2. Protein precipitation

The method described by Zhitkovich and Costa [6] for DNA-protein cross-links was modified for the detection of RPC; practically, the main modification is the selective labelling of RNA by overnight incubation in the presence of ^{3}H-uridine, as above described for alkaline elution. Briefly, HL60

cells ($1–2 \times 10^7$ cells per ml, 0.1 ml aliquots) were lysed with 0.1 ml of SDS 2%, 1 mM EDTA, pH 7.5. The samples were energetically mixed for 10 s, warmed for 10 min at 60 °C; 0.5 ml of 200 mM KCl, 20 mM Tris-HCl, pH 7.5 were added and the mixtures were cooled in an ice-bath for 5 min. The formed precipitates were collected at 4 °C by an Eppendorf centrifuge and resuspended in 1 ml of 100 mM KCl. These mixtures were heated again and the above procedure repeated. Finally, the pellets were dissolved in 1 ml aliquots of water and the solutions and corresponding supernatants were counted.

2.4.3. Calculations

The data obtained with alkaline elution were expressed in terms of cross-linking coefficient (CC), a parameter which is proportional to the number of RPC. It is defined as follows:

$$CC = \frac{F_t}{F_c}$$

were F_t and F_c are the fractions of the radioactivity retained on the filters observed with the treated and the control samples, respectively. These fractions were calculated using the method of the extrapolation at the zero fraction, as described by Kohn [5] for DNA-protein cross-links.

The results obtained with protein precipitation were also elaborated in terms of cross-linking coefficients as above. However, in this case F_t and F_c represent the fractions of the radioactivity precipitated together with proteins in the treated and in the control samples, respectively. These data were also expressed as relative activity, assuming the datum obtained with PQ in the most severe conditions as the reference unit. The relative activity was calculated as follows:

$$\text{Relative activity} = \frac{CC_t - 1}{CC_R - 1}$$

where CC_t and CC_R are the cross-linking coefficients obtained with the treated sample and that taken as the reference unit, respectively.

2.5. Radioactivity measurements

The filters from detection of macromolecular synthesis were counted by a toluene based scintillation fluid (PPO 5 g, dimethyl-POPOP 0.25 g, toluene up to 1 l of solution). The fractions from alkaline elution were neutralized with acetic acid and then counted using Instagel (Packard Instruments, Meriden, CT, USA). Samples from protein precipitation were counted with the same scintillation fluid. All determinations were carried out with a Packard A 300 CD spectrometer.

3. Results

3.1. RNA and protein synthesis in Ehrlich cells

At first, we checked the capacity of PQ to inhibit the syntheses of RNA and proteins in Ehrlich ascites cells; the results are shown in Fig. 2. In the same figure the data related to 8-MOP, used as a reference, are also reported. PQ (20 μM) is more effective in inhibiting protein than RNA synthesis: at low UVA dose both inhibition curves show a similar slope, but at doses higher than 4 kJ m^{-2} the curves are diverging. This behaviour appeared to be more evident performing a comparison between these two data set by probit analysis and using the method of the parallel dose–response lines. The two straight lines thus obtained were clearly not parallel; the χ^2 test for parallelism gave a value of 0.246002, while for the significance the limit is 0.0158 for $p = 0.90$.

When 8-MOP was employed at 20 μM concentration, small values of inhibition, especially for protein synthesis, were obtained. Therefore, we studied 8-MOP at a higher concentration, 100 μM. In these severe experimental conditions, the inhibition of RNA synthesis increased dramatically, but protein synthesis remained mainly unaffected. Thus, while in mild conditions a correlation between the inhibitions of RNA and protein synthesis seems to exist, at high 8-MOP concentration these two effect are evidently disassociated.

This picture was confirmed by calculating the ID_{50} by probit analysis, i.e., the UVA dose (kJ m^{-2}) required to yield a 50% inhibition of the macromolecular synthesis when delivered in the presence of a selected drug concentration (see Table 1).

3.2. Detection of RNA-protein cross-links in HL60 cells

3.2.1. Alkaline elution

RNA of HL60 cells was labelled with ^{3}H-uridine and RNA-protein cross-links were detected using the classic method of alkaline elution for DNA-protein cross-links, omitting the exposure to gamma rays. Fig. 3 shows the results. According to Kohn [5] the linear part of the elution curves were extrapolated to the fraction zero and the values of y axes thus

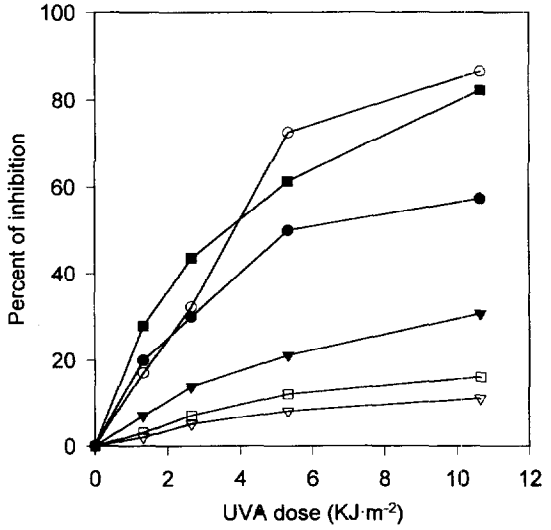

Fig. 2. Inhibition of macromolecular synthesis in Ehrlich cells by sensitization with PQ and 8-MOP. Open symbols (○, □, ▽): Protein synthesis. Filled symbols (●, ■, ▼): RNA synthesis. PQ 20 μM: ○●. 8-MOP 20 μM: ▽, ▼. 8-MOP 100 μM: ■, □.

Table 1
Inhibition of RNA and protein synthesis in Ehrlich cells. Data were computed by probit analysis and expressed as $ID_{50} \pm$ S.D.

Compound	μM	RNA	Proteins
PQ	20	3.14 ± 0.42	1.63 ± 0.18
8-MOP	20	24 ± 1.2	> 30
	100	3.2 ± 0.2	> 30

The ID_{50} is the UVA dose (kJ m^{-2}) which induces a 50% inhibition of the macromolecular synthesis in the presence of the indicated drug concentration.
PQ was not studied at high concentration because of its low water-solubility.

obtained were used to compute the cross-linking coefficients. While the untreated cells gave a fast elution profile, the radioactivity from the sample exposed to UVA light (5.3 kJ m^{-2}) in the presence of PQ (20 μM) was largely retained on the filter. Other samples submitted to PQ sensitization as above were treated with ribonuclease before elution: in this case, elution profiles, superimposable to that of untreated control cells, were obtained. This results clearly tell us that the radioactivity associated to the filters was due to labelled RNA, which was retained by the formation of covalent RNA-protein cross-links. ^{3}H-labelled HL60 cells were then exposed to increasing doses of UVA light in the presence of PQ (20 μM) and then submitted to alkaline elution for detection of RNA-protein cross-links. Plotting the UVA dose against the values of the cross-linking coefficients thus obtained, we obtained a straight line, which is shown in the insert of the Fig. 3.

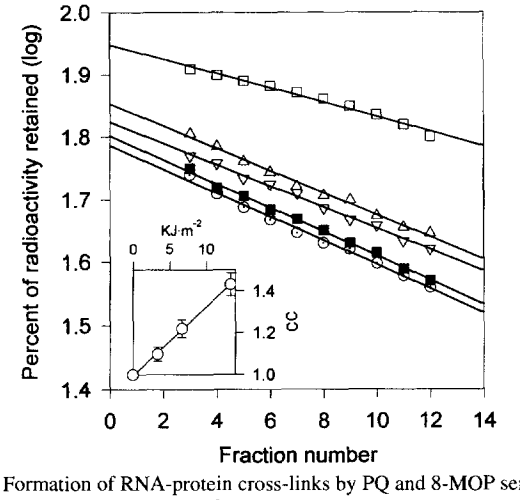

Fig. 3. Formation of RNA-protein cross-links by PQ and 8-MOP sensitization. HL60 cells, labelled with ^{3}H-uridine, were exposed to UVA light in the presence of PQ and 8-MOP and then submitted to alkaline elution. The symbols and the treatment are as follows: untreated controls: ○; PQ (20 μM plus 5.3 kJ m^{-2}): □; PQ (20 μM plus 5.3 kJ m^{-2} followed by ribonuclease treatment): ■; 8-MOP (20 μM plus 10.6 kJ m^{-2}): ▽; 8-MOP (100 μM plus 13.3 kJ m^{-2}): △. The linear part of the elution curves were extrapolated to the fraction zero [5]; for clarity, the data related to the first two fractions were omitted. Insert: Kinetic of RNA-protein cross-link formation by PQ sensitization. HL60 cells, labelled with ^{3}H-uridine, were exposed to increasing doses of UVA light in the presence of 20 μM PQ and then submitted to alkaline elution. The values of cross-linking coefficient were calculated.

Table 2
Protein precipitation in HL60 cells[a] after PQ sensitization

Quencher (μM)	Compound		UVA kJ m^{-2}	CC[b] ± S.D.	Relative activity[c]
	name	μM			
None	None	0	0	1	0
		0	10.6	1	0
	8-MOP	10	0	1	0
			5.3	1	0
			10.6	1.2	0.06
	PQ	10	0	1	
		10	2.6	2.1 ± 0.18	0.31
		10	5.3	3.52 ± 0.21	1
Histidine					
5		10	5.3	2.06 ± 0.27	0.30
10				1.83 ± 0.14	0.17
Mannitol					
5		10	5.3	3.24 ± 0.26	0.64
DABCO					
0.6		10	5.3	2.5 ± 0.38	0.43
1.2		10		2.04 ± 0.25	0.29

[a] HL60 cells were incubated overnight on the presence of ^{3}H-uridine in order to label RNA; after PQ sensitization the cells were processed for protein precipitation as described [6].
[b] Cross-linking coefficient (see Section 2.4.3.).
[c] The relative activity is calculated assuming as a reference unit the coefficients obtained with PQ used in the most severe experimental conditions, as described in Section 2.4.3.

For a comparison, two elution profiles obtained treating cells with 8-MOP are also reported in the same Fig. 3. Cells were exposed to a higher UVA dose (13.3 kJ m^{-2}) but at different concentrations. At 20 μM the effect on radioactivity retention is poor; when 8-MOP was employed at 100 μM concentration the radioactivity retained increased but only to a moderate extent.

3.2.2. *Protein precipitation*

HL60 cells were labelled with ^{3}H-uridine, submitted to PQ sensitization and then processed for protein precipitation as described [6]. Table 2 shows some data obtained in such experiments. While the single treatment with the drug or with light alone did not increase the amount of ^{3}H-RNA precipitated together with proteins in comparison with untreated controls, the exposure to UVA in the presence of PQ increased significantly the radioactivity associated with the precipitate, thus suggesting the formation of covalent linkages between RNA and proteins. To obtain preliminary information on the mechanism of formation of such lesions, we carried out experiments in the presence of some chemicals known to be singlet oxygen and radical scavengers. As shown in Table 2, the presence during irradiation of histidine and DABCO, two known singlet oxygen quenchers, reduced significantly the value of cross-linking coefficient; on the contrary, mannitol, a radical scavenger, appeared to be ineffective.

Table 3
Oxygen effect on PQ activity

Atmosphere present during irradiation	Inhibition of protein synthesis (%) [a]	Cross-linking coefficient [b]
Air	78.3 ± 0.9	1.4 ± 0.05
Nitrogen	32.63 ± 4.3	1.1 ± 0.01

[a] Ehrlich cells were exposed to 10.7 kJ m^{-2} in the presence of 20 μM PQ in an air or nitrogen atmosphere; the synthesis of proteins was then determined.
[b] HL60 cells were exposed to 13.3 kJ m^{-2} in the presence of 20 μM PQ in an air or nitrogen atmosphere; the cross-linking coefficient was then determined by alkaline elution.

3.3. Experiments carried out in different atmospheres

To obtain information of the role played by oxygen in the activity of PQ, we carried out some experiments by irradiating cells in air or in a nitrogen atmosphere. In these experimental conditions we checked the effect of PQ sensitization on protein synthesis in Ehrlich cells and on RNA-protein cross-linking in HL60 cells. These data are summarised in Table 3. The effect of the presence of oxygen during irradiation is evident: a strong decrease of the PQ activity is observed when irradiation was carried out in a nitrogen atmosphere.

4. Discussion

PQ is a photosensitizer showing singular properties for a furocoumarin homologue being capable of damaging DNA without forming the well-known furocoumarin C_4-cycloadducts [1,3]. One particular feature is its capacity of inhibiting protein synthesis even in gentle experimental conditions [1,2], while linear furocoumarins require larger concentrations and higher UVA doses [4].

PQ might be more active than 8-MOP on protein synthesis because it blocks more efficiently RNA-synthesis, thus reducing the amount of m-RNA molecules available for protein synthesis. Because PQ inhibits both RNA and protein synthesis efficiently, we checked this hypothesis using 8-MOP, which was chosen as a reference to check the response of our experimental system. In fact, studying the effect induced by 8-MOP on RNA and protein syntheses at reduced drug concentration (20 μM), we obtained low values of inhibition, with a certain correlation between these two effects. To obtain more significant values, we raised the 8-MOP concentrations to 100 μM; in these experimental conditions, RNA synthesis was strongly inhibited, but a corresponding increase of the effect on protein synthesis was not observed. We can conclude that in our short-term tests carried out in Ehrlich cells, we detected protein synthesis which very probably is driven by pre-synthesised molecules of m-RNA. In other words, in our experimental conditions the block of RNA synthesis does not affect the results we can obtain studying protein synthesis. Therefore, the inhibition of protein synthesis observed with PQ should be related to another mechanism. Unfortunately, PQ could not be studied at a such high concentration because its low water solubility.

Considering the PQ capacity of inducing covalent linkages between DNA and proteins, we investigated whether it also could form cross-links involving RNA. For this purpose, we modified the well-known methods of alkaline elution and protein precipitation for the detection of DNA-protein cross-links. Both are based on the principle that the nucleic acid follows proteins if it is covalently linked to them; thus, the main modification introduced consists of labelling RNA instead of DNA. Because RNA is small in comparison with DNA, the cell treatment with gamma radiation to break down DNA is not necessary and therefore it was omitted. With both methods we obtained evidence for the formation of covalent cross-links between RNA and proteins by PQ sensitization. Their amount increased with the delivered UVA dose. Actually, the radioactivity associated with proteins is certainly due to RNA because treating the lysed cells with ribonuclease it disappeared from the filter being quickly and completely eluted as for the untreated controls.

On the contrary, 8-MOP induced only reduced amounts of RNA-protein cross-links, even when it was tested in very severe experimental conditions. This response resembles essentially the data obtained studying protein synthesis; however, raising 8-MOP concentrations and UVA doses, the increase of the RNA-protein cross-links appeared to be slightly higher than that observed in inhibition of protein synthesis. This disagreement might be due to the formation of some lesions in cell structures different from that directly involved in protein synthesis (i.e. ribosomes). This point will be completely clarified working with isolated ribosomes.

Plotting the data obtained studying RNA-protein cross-linking and protein synthesis after PQ sensitization we obtained a straight line, with a very good correlation coefficient (see Fig. 4). Therefore we can reasonably deduce that a relationship between these two effects exists and that the inhibition of protein synthesis by PQ activity depends from

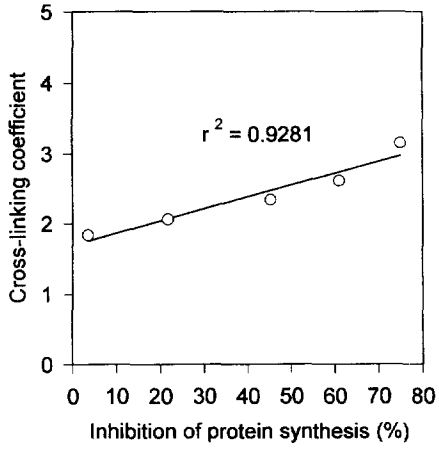

Fig. 4. Relationship between the formation of RNA-protein cross-links by PQ sensitization and its capacity to inhibit protein synthesis. Cross-links were detected in HL60 cells by protein precipitation and protein synthesis was assayed in Ehrlich cells.

the formation of such lesion, probably at the levels of ribosomes.

To obtain information on the mechanism of the formation of RNA-protein cross-links by PQ sensitization we performed some experiments using certain chemical scavengers. Histidine and DABCO, two well-known quenchers of singlet oxygen, appeared to be capable of inhibiting significantly the formation of RNA-protein cross-links, while mannitol, a radical scavenger, appeared to be much less effective. These results are too preliminary to draw conclusive deductions, however, we can suggest that singlet oxygen is involved in the formation of RNA-protein cross-links. In this connection, it is important to remember that PQ produces very high levels of singlet oxygen, about one order of magnitude higher than 8-MOP, while the capacity of both compounds of generating radical anions is comparable [3]. This hypothesis was supported by some experiences carried out by irradiating cells in different atmospheres, in air or in nitrogen. In these experimental conditions we studied the effect of PQ sensitization on protein synthesis and on formation of RNA-protein cross-links. In both tests we obtained a strong reduction of PQ activity when the cells were exposed to UVA light in a nitrogen atmosphere. This result is clearly consistent with the hypothesis of singlet oxygen implication in PQ effects. We could observe that the sensitizing activity of PQ is reduced but not completely abolished when irradiation is carried out in nitrogen. This result could suggest that perhaps another secondary mechanism exists.

Finally, it also seems possible that a particular interaction between PQ and ribosomes might play an important role for the inhibition of protein synthesis; in fact, we have observed that other compounds capable of inducing large amounts of singlet oxygen such as 3-carbethoxyangelicin [7] or 4,8-dimethyl-5′-acetylpsoralen [8] are both unable of inhibiting protein synthesis (data not shown).

Our results are consistent with the data obtained by Singh and Vadasz [9], who studied the effect of 8-MOP sensitization on ribosomes isolated from *E. coli*. These authors found a significant protection in the presence of various singlet oxygen quenchers (e.g. histidine) or simply by irradiating ribosomes in a nitrogen atmosphere; on the contrary no protection was observed in the presence of some radical scavengers. We found a similar behaviour for PQ, while 8-MOP appeared to be poorly active in our system. This discrepancy could be due to the very different substrates used. The weak activity we observed for 8-MOP might also depend from the its low capacity of producing singlet oxygen, which is about ten times lower than that of PQ [3]. Finally, the strong inhibition of RNA synthesis observed with 8-MOP at high concentration is very probably due to the formation of C_4-cycloadducts with pyrimidine bases.

In conclusion, on the basis of these data we can say PQ presents another interesting feature, that is the capacity of inducing covalent linkages between RNA and proteins, a reaction which very probably involves the formation of singlet oxygen at the level of the ribosomes.

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