PHOTOINACTIVATION OF HUMAN ERYTHROCYTE ENZYMES BY α-TERTHIENYL AND PHENYLHEPTATRIYNE, NATURALLY OCCURRING COMPOUNDS IN THE ASTERACEAE

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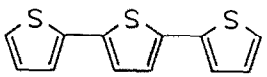
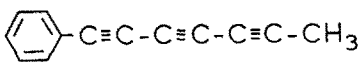
1. Introduction

In our laboratory, naturally occurring polyacetylenes and thiophene derivatives of the Asteraceae (Compositae) have been found to evoke antibiotic activities in light in the region 312–400 nm (UV-A) [1–5]. Phenylheptatriyne and α-terthienyl are two examples of those compounds, which display lethal photosensitization towards various kinds of uni- and multicellular organisms [3–6]. The primary and major target of these photo-induced reactions has not been determined. Experiments with calf thymus DNA indicate that, unlike photoactive furano coumarins, PHT does not form interstrand crosslinkages with DNA in ultraviolet light [4]. Cell respiration of



α-Terthienyl



Phenylheptatriyne

Abbreviations: AChE, acetylcholinesterase (EC 3.1.1.7); GOT, glutamate-oxaloacetate transaminase (EC 2.6.1.1); LDH, lactate dehydrogenase (EC 1.1.1.27); G6P DH, glucose 6-phosphate dehydrogenase (EC 1.1.1.49); 6-PGA DH, 6-phosphogloconic dehydrogenase (EC 1.1.1.43); PK, pyruvate kinase (EC 2.7.1.40); GAP DH, glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12); MB, methylene blue; PHT, phenylheptatriyne; α-T, α-terthienyl

Escherichia coli was found to be rapidly inhibited by mild treatments of PHT and ultraviolet radiation [6]. Recently we also observed K^+ loss from human erythrocytes, followed by hemolysis, when the erythrocytes were exposed to some polyacetylenic compounds and UV-A [7]. Prior to hemolysis, AChE, which is located on the outer surface of the membrane, was found to be inactivated [7]. The inactivation of G6P DH by α-T and black light has been reported [8]. The erythrocyte is a good system for photosensitization studies because it has no intracellular organelles and the characteristics of its enzymes are well known.

Here we tested the phototoxic effects of PHT and α-T on several enzymes in human erythrocytes. We have observed a remarkable inactivation of AChE by PHT and α-T in the prelytic period, in contrast to a lack of inactivation of cytoplasmic enzymes during this period. We have also tested the effect of methylene blue, a well-known photodynamic compound, on these enzymes and its action in comparison to that of PHT and α-T is discussed.

2. Materials and methods

2.1. *Preparation of erythrocytes*

Whole blood was obtained by venipuncture from W.D.M. It was washed 3 times with NaCl–PO_4 buffer (0.139 M NaCl, 0.088 M Na_2HPO_4) [9] to avoid contamination by serum and leucocyte enzymes, which

cause large errors in assays of erythrocyte enzymes, such as PK. A 1:20 dilution (v/v) of packed cells was prepared and used in the irradiation experiment.

2.2. *Irradiation experiment*

To test tubes (13 X 100 mm) containing 300 μl NaCl–PO_4 buffer-dye mixture, 300 μl of a 1:20 diluted erythrocyte suspension were added. The test tubes were sealed with Parafilm and laid on a tray under four black light blue fluorescent lamps (Sylvania F20T12-BLB). In the case of methylene blue, cool white lamps (F20T12-CW) were used instead of ultraviolet lamps. The light intensities of the ultraviolet and white lamp, measured with TSI-Kettering, were 0.6 mW/cm^2 and 1.7 mW/cm^2, respectively. The test tubes were irradiated for 60 min under controlled temperature (34°C) with continuous shaking (100 strokes/min).

2.3. *Enzyme assay*

At the desired time, the tubes were taken from the incubator, 100 μl aliquots were removed and % hemolysis determined [7]. The remaining erythrocytes (500 μl) were lysed by adding 1 ml 2.7 mM EDTA solution containing 0.72 mM 2-mercaptoethanol (pH 7.4) and put on ice.

Assays were performed on a Unicam SP 1750 ultraviolet recording spectrophotometer at 37°C. All enzyme assays, except GOT, followed Beulter's text book [10]. The assay of GOT was carried out using Sigma's kit (no.55-5) with the further addition of pyridoxal-phosphate (0.02 mM).

2.4. *Chemicals*

PHT and α-T were obtained by the methods in [7].

3. Results and discussion

Irradiation of erythrocyte suspensions with appropriate concentrations of dyes caused hemolysis [7]. Usually, the onset of hemolysis, caused by 300 μM PHT, 6 μM α-T and 50 μM MB, occurred after 20 min, 30 min and 40 min irradiation, respectively. The activity of several enzymes in erythrocytes was measured before and after the onset of hemolysis.

The activity of some erythrocyte enzymes decreased in the presence of PHT (300 μM), α-T (6 μM) or MB (50 μM) during 60 min irradiation of UV-A (table 1). The sensitivity of these enzymes to each compound was found to vary. For example, catalase (EC 1.11.1.6) and GOT were rather insensitive to the compounds compared with other enzymes tested, and LDH was highly sensitive to MB in contrast to PHT and α-T. No inactivation of the enzymes was observed either in the absence of the above compounds or in dark. The results indicate that protein molecules can be attacked by these compounds as in the case of certain other

Table 1
The effect of PHT and α-T on human erythrocyte enzymes in UV-A

Enzymes	Residual activity after irradiation (%)					
	PHT		α-T		MB	
	20 min	60 min	30 min	60 min	30 min	60 min
Catalase	98	97	108	97	–	–
GOT	–	95	–	88	102	69
LDH	97	89	97	93	81	13
AChE	53	31	36	21	76	41
G6P DH	72	–	93	–	–	–
6-PGA DH	105	–	91	–	–	–
PK	98	78	100	92	–	–
GAP DH	90	84	94	89	82	35

The concentration of compounds are 300 μM, 6.04 μM and 50 μM, respectively. The onset of hemolysis, caused by PHT, α-T and MB occurs after 20 min, 30 min and 40 min irradiation, respectively

photosensitizers [11]. Acetylcholinesterase, which is located on the outer surface of the membrane [12], was strongly inactivated in the prelytic period by the above compounds and light [7]. The inactivation of this enzyme may be caused by damage to its structure or a modification of the membrane environment in which it is located. The cytoplasmic enzymes, LDH and GAP DH, but not GOT, were slightly inactivated by 50 μM MB in the prelytic period and strongly inactivated after the onset of hemolysis. As MB is a non-penetrating dye, it probably exerts its toxic effect inside the cell via diffusible activated oxygen [13]. In contrast to the inactivation by MB, all other cytoplasmic enzymes with the exception of G6P DH, did not lose their activity after treatment with PHT (300 μM) and α-T (6 μM) before the onset of hemolysis: GAP DH, which is known to bind weakly to the inner surface of the membrane [12], was also not inactivated. Since some of the enzymes, such as PK and GAP DH, started to lose their activity after the onset of hemolysis, the photosensitization mechanism of these compounds would appear to depend on the degree of membrane intactness: the inactivation of these enzymes was enhanced by erythrocytes being lysed by osmotic shock prior to irradiation or by a higher dose of PHT (500 μM) (data not shown). The insensitivity of the cytoplasmic enzymes to PHT and α-T in the prelytic period may represent the inaccessibility of these compounds to the inside of the cell. The simplest explanation of these results is that diffusible activated oxygen is not involved. More or less, direct interaction between the activated compounds, which may stay in the membrane, and the target molecule(s) may be significant in the photoinactivation process [14]. Alternatively the insensibility may be derived from either interference of the sensitized reaction by an endogenous quencher [13] or protection of the active site of the enzyme against the toxic substance by enzyme cofactors or substrates [15].

Finally, the present experiments show that the primary target of photosensitization of PHT and α-T in the photoreaction can be membrane component and that membrane proteins can be damaged by PHT and α-T [16].

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References

[1] Camm, E. L., Towers, G. H. N. and Mitchell, J. C. (1975) Phytochemistry 14, 2007–2011.
[2] Towers, G. H. N., Wat, C.-K., Graham, E. A., Bandoni, R. J., Chan, J. C., Lam, M. and Lam, J. (1977) Lloydia 40, 487–498.
[3] Towers, G. H. N. and Wat, C.-K. (1978) Rev. Lat. Quim. 9, 162–170.
[4] Wat, C.-K., Biswas, R. K., Graham, E. A., Bohm, L., Towers, G. H. N. and Waygood, E. R. (1978) J. Nat. Prod. 42, 103–111.
[5] Chan, G. F. Q., Towers, G. H. N. and Mitchell, J. C (1975) Phytochemistry 14, 2295–2299.
[6] Towers, G. H. N. (1979) unpublished data.
[7] Wat, C.-K., Yamamoto, E., MacRae, W. D., Chan, G. F. Q. and Towers, G. H. N. (1979) 20th Ann. Meet. Am. Soc. Pharmacognosy, West Lafayette.
[8] Bakker, J. and Gommers, F. J. (1978) Congr. Plant Pathology, Munich.
[9] Parpart, A. K., Lorenz, P. B., Parpart, E. R., Gregg, J. R. and Chase, A. M. (1947) J. Clin. Invest. 636–640.
[10] Beutler, E. (1975) Red Cell Metabolism, a Manual of Biochemical Methods, 2nd edn, Grune and Stratton, New York.
[11] Spikes, J. D. and Macknight, M. L. (1970) Ann. NY Acad. Sci. 171, 149–162.
[12] Wallach, D. F. H. and Weidekamm, E. (1973) in: Erythrocytes, Thrombocytes Leucocytes, Recent Advances in Membrane and Metabolic Research, 2nd Int. Symp., Vienna (Gerlack, E. et al. eds) pp. 2–8, Georg Thieme, Stuttgart.
[13] Ito, T. (1978) Photochem. Photobiol. 28, 493–508.
[14] Spikes, J. D. (1977) in: The Science of Photobiology (Smith, K. C. ed) pp. 87–112, Plenum/Rosetta, New York.
[15] Buchanan, J. D. and Armstrong, D. A. (1978) Int. J. Radiat. Biol. 33, 409–418.
[16] Schothorst, A. A. and Van Steveninck, J. (1972) Clin. Chim. Acta 39, 161–170.