Inhibition by Trioxalen (Psoralen) plus Near-Ultraviolet Light of the Induction of Ornithine Decarboxylase in Chinese-Hamster Cells

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Trioxalen (trimethylpsoralen) plus near-u.v. light, a potent inhibitor of DNA and RNA synthesis, inhibits the induction of ornithine decarboxylase in stationary-phase V79 fibroblasts. It does not affect the translation of pre-existing mRNA. The method, in view of its high degree of specificity and precise timing, is a better choice for inhibiting RNA synthesis than the commonly used chemical inhibitors and precursor analogues.

Furocoumarins (psoralens) photosensitize biological systems to near-u.v. light (340–400 nm) (Scott *et al.*, 1976). They specifically react with pyrimidine bases in DNA to form monoadducts and cross-links, thereby leading to immediate inactivation of the DNA as a template for DNA and RNA synthesis (Trosko & Isoun, 1971; Epstein & Fukuyama, 1970).

Using the XD line of tobacco cells, Heimer *et al.* (1977, 1978) suggested the use of psoralen plus near-u.v. light as a probe for the study of control of protein synthesis, to replace the commonly used chemical inhibitors and precursor analogues of RNA synthesis. The present paper describes the application of this method to the study of enzyme induction in mammalian cells.

Materials and Methods

Chinese-hamster V79 fibroblasts were grown as monolayers with Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) foetal calf serum, at 37°C in an humidified CO₂ incubator. Ornithine decarboxylase in stationary-phase cells (15×10^6 – 20×10^6 cells/9 cm plate) was induced and assayed as described previously (Bachrach, 1976). Enzyme activity is expressed as pmol of CO₂ released/h per mg of protein. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Trioxalen (4,5',8-trimethylpsoralen) (Poul B. Elder Co., Bryan, OH, U.S.A.) was dissolved in ethanol (2 mm) and diluted into phosphate-buffered saline (Dulbecco & Vogt, 1954) to give a final concentration of 5 μ M. Cells were washed twice with this solution and then 5 ml of it was added to each plate. The plates were incubated in the dark for 5 min and irradiated for 5–7 min with two tubular black fluorescent lamps at room temperature. The incident flux was 20 J·m⁻²·s⁻¹. At the end of the irradiation period the trioxalen solution was removed and replaced with new

medium. The plates were then returned to 37°C for the indicated period of time.

Results and Discussion

Ornithine decarboxylase can be induced in stationary-phase Chinese-hamster cells by replacing the old medium with a new one, similarly to the induction reported for other cell cultures (Bachrach, 1976). The development of the enzyme activity as a function of time and the effect of trioxalen plus near-u.v. light on the induction are summarized in Fig. 1. After replacement of the old medium with a new one, there is a 1 h lag period, during which there is a very little increase in enzyme activity. Thereafter the enzyme activity increases rapidly. By the third to the fourth hour, the activity is 100–400-fold higher than that in the non-induced cells. The activity then declines and reaches the zero time value by the sixth hour. As in other systems (Bachrach, 1976), the induction is very sensitive to inhibitors of RNA synthesis commonly used, actinomycin D and 6-methylpurine (results not shown) when applied at zero time. Trioxalen plus near-u.v. light, when applied to the cells just before the start of the induction, strongly inhibits the development of the enzyme activity (Fig. 1). The induction, however, is much less sensitive to trioxalen plus near-u.v. light when applied to cells at the end of the lag period, 1 h after the start of the induction. Thus, when RNA synthesis is inhibited before the start of the induction, no development of ornithine decarboxylase activity occurs. On the other hand, if RNA synthesis is allowed to proceed for a period of time and is then inhibited, enzyme activity can develop. It was previously shown (Heimer *et al.*, 1977) that trioxalen plus near-u.v. light does not affect synthesis of total protein, since pre-existing RNA species and the synthesizing machinery are used. It is suggested that the 50% ornithine decarboxylase activity retained in cells treated at 1 h with

Fig. 1. Effect of trioxalen plus near-u.v. light on the induction of ornithine decarboxylase in stationary-phase Chinese-hamster cells

Cells were induced to develop ornithine decarboxylase as described in the Materials and Methods section. ●, Control cells; ○, cells exposed to trioxalen plus near-u.v. light just before induction; □, cells exposed to trioxalen plus near-u.v. light 1 h later.

trioxalen plus near-u.v. light reflects the availability of the various RNA species required for induction, and a longer period of RNA synthesis is required to obtain the value in the control cells.

The effect of trioxalen plus near-u.v. light was further tested on the development of ornithine decarboxylase activity in exponential-phase cells. It was shown (E. Ben-Hur & E. Riklis, unpublished work) that, upon shifting exponential-phase cells from 37°C to 42°C, protein synthesis is arrested. This cellular process is apparently the only one impaired during the first 60 min at 42°C. Ornithine decarboxylase, which has a half-life of 15 min (Russell & Synder, 1969), started to decrease, and at the end of 60 min of incubation at 42°C there was very little enzyme left in the cells. On returning the cells to 37°C, protein synthesis resumed as well as development of ornithine decarboxylase activity. Within 90 min at 37°C the activity had returned to the value found in the preheating period. Trioxalen plus near-u.v. light, given just before the cells were returned to 37°C, did not inhibit the development of the enzyme activity (Table 1). The activity in the treated cells was 70% of that in cells not treated with trioxalen plus near-u.v. light. The increase in ornithine decarboxylase activity in treated cells was inhibited by cycloheximide (4 µg/ml) (results not

Table 1. Effect of trioxalen plus near-u.v. light on the development of ornithine decarboxylase activity in preheated cells

Exponential-phase V79 cells were transferred to 42°C for 70 min. At this point some of the cells were returned to 37°C for 90 min or were first exposed to near-u.v. light before being returned to 37°C for 90 min.

Treatment	Ornithine decarboxylase activity	
	(c.p.m./h per mg of protein)	(% of control)
70 min at 42°C	9680	8.3
Return to 37°C for 90 min	174300	100
Treated with trioxalen+ near-u.v. light and returned to 37°C for 90 min	120000	70

shown). This indicates that the enzyme that developed in the treated cells was synthesized *de novo* and does not represent activation of old enzyme molecules. Thus the various RNA species required for the development of ornithine decarboxylase are present in the cells at the end of the heating period. Trioxalen plus near-u.v. light does not interfere therefore with the translation process.

We propose that the combination of trioxalen plus near-u.v. light can indeed be used to inhibit RNA synthesis, thereby preventing enzyme induction in mammalian cells in a manner similar to that reported for plant cells (Heimer *et al.*, 1977, 1978). The easy penetration into the cells, the precise timing of action and the high degree of specificity (Scott *et al.*, 1976; Trosko & Isoun, 1971; Epstein & Fukuyama, 1970; Heimer *et al.*, 1977, 1978) makes it a better choice for inhibition of RNA synthesis than the commonly used chemical inhibitors and precursor analogues.

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