Light-inactivation of submitochondrial adenosine triphosphatase

It has been postulated previously that the diaphorase flavin is involved as an intermediate phosphate acceptor in the mitochondrial ATPase reactions, both the one induced in fresh mitochondria by 2,4-dinitrophenol and the one activated by Mg^{++} and present in mitochondrial preparations the structural integrity of which has been disrupted by means of deoxycholate[1,2]. Recently a comparison has been made[3] of the latter type of ATPase reaction as obtained in deoxycholate-treated mitochondria and in mitochondrial fragments prepared by mechanical means according to KIELLEY AND KIELLEY[4]. No fundamental difference seems to exist between these fragments and the deoxycholate-treated mitochondria with respect to their behavior towards flavin antagonists[3].

If such a mitochondrial-fragment preparation is exposed to ordinary sunlight, made free from most short-wave u.v. light by passing it through several layers of glass, and the temperature during the light exposure is kept at 0°, the ATPase activity in the light-treated preparation is strongly decreased as compared to a dark control (Fig. 1). The inactivation can be enhanced if the light exposure is carried out in the presence of low concentrations of flavin antagonists such as atebrin and chlorpromazine (Fig. 2), whereas inhibitors of other types do not enhance the light

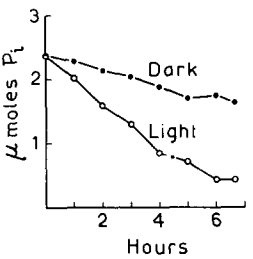

Fig. 1. Effect of illumination with visible light on the ATPase activity of mitochondrial fragments prepared according to KIELLEY AND KIELLEY[4]. The stock suspension of mitochondrial fragments (in 3 m*M* K_2HPO_4, containing about 2 mg protein/ml) was diluted 5-fold with 0.1 *M* Tris buffer (pH, 7.5) and 1-ml aliquots were transferred to small pyrex test tubes standing in a square glass jar at 0° which was exposed to sunlight. Dark control was obtained by placing some tubes coated with aluminium foil in the same glass jar. For ATPase tests, 0.2-ml aliquots were withdrawn from the test tubes and transferred to incubation tubes containing 10 μmoles ATP, 8 μmoles $MgCl_2$ and 10 μmoles Tris buffer in 2.0 ml water (pH, 7.5). The tests were run at 30° for 20 min, after which the reaction was stopped by the addition of 1.0 ml of 1.0 *M* $HClO_4$. Inorganic phosphate was determined according to the modified[5] MARTIN AND DOTY method[6].

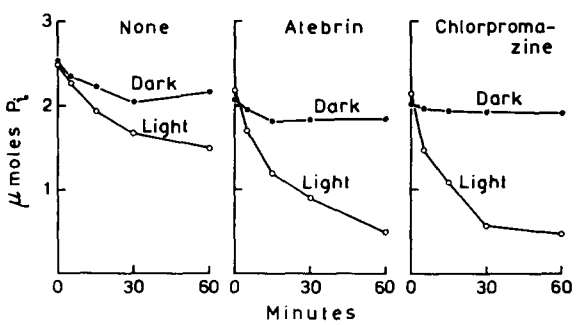

Fig. 2. Promoting effect of atebrin and chlorpromazine on the light-inactivation of ATPase. During preincubation (dark or light), atebrin was present in a concentration of 0.75 m*M*, and chlorpromazine in a concentration of 0.075 m*M*. In the ATPase tests, the concentrations of the agents were 10 times lower.

Abbreviations: ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane; Tetrac, tetraiodothyronoacetic acid; *p*CMB, *p*-chloromercuribenzoate.

inactivation (Table I). When added after the light-exposure, atebrin and chlorpromazine have a relatively weak effect in the concentrations used (Fig. 3).

TABLE I

COMPARISON OF VARIOUS ATPASE INHIBITORS WITH RESPECT TO THEIR ABILITY TO PROMOTE LIGHT INACTIVATION OF ATPASE

The concentrations indicated in the Table refer to the preincubation conditions; in the ATPase tests, the concentrations of the agents were 10 times lower. Time of preincubation, 15 min.

Compound	*Preincubation conditions*		*Light inactivation* %
	Dark	*Light*	
	μmoles P liberated/20 min		
None	2.45	2.25	8
Chlorpromazine (0.5 m*M*)	0.96	0.00	100
Azide (0.3 m*M*)	0.76	0.72	6
Tetrac (0.01 m*M*)	1.01	0.84	16
*p*CMB (3 m*M*)	1.58	1.26	20

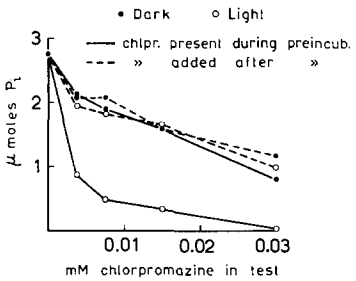

Fig. 3. Effect of chlorpromazine on ATPase when added before and after light exposure of the enzyme. Time of preincubation (dark or light), 15 min. The concentrations of chlorpromazine during preincubation were 10 times higher than those indicated in the figure.

$Na_2S_2O_4$, which was previously found to stimulate the liver-mitochondrial Mg^{++}-activated ATPase[2], had no effect on the light-inactivated enzyme. The dark control was stimulated to reach the same activity as was prevailing in the $Na_2S_2O_4$-stimulated, non-pretreated sample.

These results seem to provide further support to the idea that flavin is involved in mitochondrial ATPase[1,2].

While this paper was in preparation BEYER[7] reported that u.v. light inactivates the 2,4-dinitrophenol-induced ATPase reaction of intact rat-liver mitochondria, but not the Mg^{++}-activated ATPase reaction of mitochondria treated with 0.1 % deoxycholate.

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