Tyrosine and Thyronine Analogs as Inhibitors of the Dye-Sensitized Photoinactivation of Lysozyme (34086)

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Enzymes can be inactivated on illumination in the presence of sensitizing dyes and molecular oxygen, a phenomenon termed "photodynamic inactivation" (1). We showed earlier that a number of thyronine and tyrosine analogs protect trypsin against dye-sensitized photoinactivation (2). The present paper describes similar studies of the protection of lysozyme against photodynamic inactivation. The protective compounds used were: Potassium iodide, L-tyrosine (TYR), 3-monoiodo-L-tyrosine (MIT), 3, 5-diiodo-L-tyrosine (DIT), DL-thyronine (THYR), 3,-4-diiodo-L-thyronine (T_2), 3, 3′, 5-triiodo-L-thyronine (T_3), and L-thyroxine (T_4).

Materials and Methods. The quantum yields for the photodynamic inactivation were measured over the range of 0–700 μM protective agent concentration. Reaction mixtures were 40 μM in lysozyme and 0.125 M in sodium phosphate buffer of pH 8. The sensitizing dyes used were riboflavin-5′-phosphate (FMN, at 150 μM), eosin Y (25 μM), or methylene blue (12.5 μM). The reaction mixture was illuminated at 15° while being stirred. The light source used was either a General Electric A-H6 high pressure mercury arc lamp (for FMN systems at 4370Å) or a 500-W slide projector (for eosin Y systems at 5170Å and methylene blue systems at 6750Å). Reasonably monochromatic light at the wavelengths indicated was obtained by using Baird-Atomic multilayer interference filters. The light energy absorbed by the reaction mixture was measured with a thermopile-millimicrovoltmeter combination calibrated with standard lamps from the National Bureau of Standards. Lysozyme (Calbiochem No. 4402, A grade, from egg white) activity was measured after 0, 5, 10, and 15 min of illumination using *Micrococcus lysodeikticus* as substrate. Under the reaction conditions used, enzyme inactivation was first order with respect to time of illumination. The quantum yield of inactivation was defined as (dS/dt) / (dQ/dt) where (dS/dt) is the rate of loss of enzyme activity and (dQ/dt) is the rate of absorption of photons by the reaction system, both as measured at the beginning of illumination. We have described these experimental methods in detail elsewhere (3–4).

Results and Discussion. The effects of the protective agents listed above on the dye-sensitized photoinactivation of lysozyme are shown in Figs. 1–3 for experiments with FMN, eosin Y, and methylene blue, respectively. As may be seen in Fig. 1, all eight compounds showed very similar protective effects as a function of concentration using FMN as sensitizer even though they differ markedly in chemical properties. These results are not easy to interpret, since one would expect that some of these compounds might protect by different mechanisms. For example, potassium iodide quenches the long-lived excited states of FMN presumably involved in photodynamic reactions (5–8), whereas tyrosine and thyronine (and possibly their congeners) might be expected to act by competing with the enzyme for the oxidizing entity involved in photodynamic action (9–11). Further, these studies are complicated by the fact that the iodinated congeners of tyrosine and thyronine are deiodinated upon illumination in the presence of FMN (12–15). If deiodination occurred during the experiments, the concentrations and types of

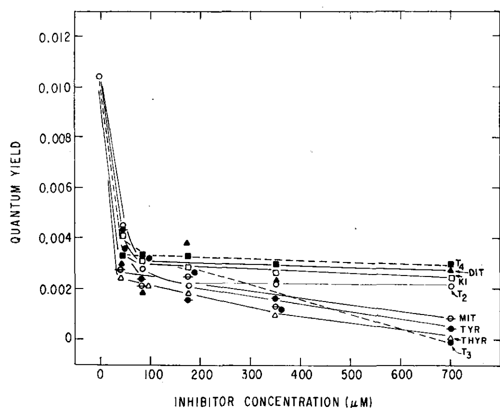

FIG. 1. Effects of potassium iodide, and tyrosine and thyronine analogs on the photodynamic inactivation of lysozyme in the presence of 150 μM FMN. The reaction mixture was 40 μM in lysozyme and 0.125 M in sodium phosphate buffer at pH 8 and 15°. The following inhibitors were used: Potassium iodide (KI), L-tyrosine (TYR), 3-monoiodo-L-tyrosine (MIT), 3,5-diiodo-L-tyrosine (DI T), DL-thyronine (THYR), 3,5-diiodo-L-thyronine (T_2), 3,3′, 5-triiodo-L-thyronine (T_3), and L-thyroxine (T_4).

protective compounds present would thus change with time of illumination. The family of curves shown in Fig. 1 is very similar to that obtained for the FMN-sensitized photoinactivation of trypsin (2). This suggests that these compounds protect by similar mechanisms in both systems, probably by acting on the excited states of the dye rather than on the enzymes.

In contrast to the results with FMN, the different compounds tested vary widely in their protective efficiencies with eosin Y and with methylene blue as sensitizers (Figs. 2, 3). Again, these results are similar to those obtained with trypsin (2), suggesting that the protective agents act on the dyes, or with the oxidizing entity produced in photodynamic action, rather than on the enzyme as such. Eosin Y, but not methylene blue, sensitizes the photochemical deiodination of T_4 (16). With methylene blue, the efficiency of protection by the various compounds is simi-

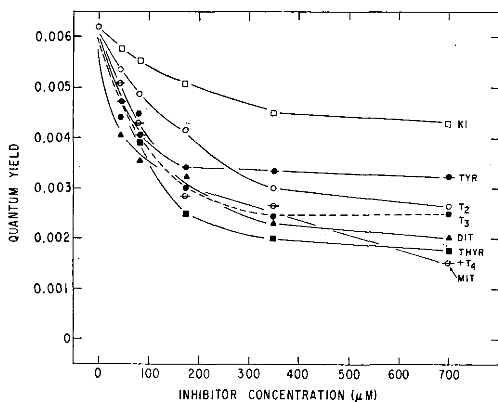

FIG. 2. Effects of potassium iodide, and tyrosine and thyronine analogs on the photodynamic inactivation of lysozyme in the presence of 25 μM eosin Y. The reaction mixture and protective agents used were the same as given for Fig. 1.

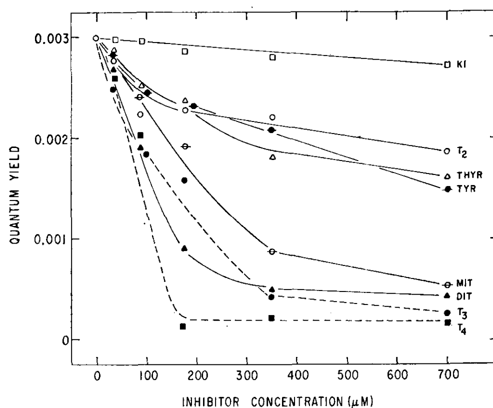

FIG. 3. Effects of potassium iodide, and tyrosine and thyronine analogs on the photodynamic inactivation of lysozyme in the presence of 12.5 μM methylene blue. The reaction mixture and protective agents used were the same as given for Fig. 1.

lar to their known metabolic activities (17), with the exception that T_4 is more potent than T_3. Thus these photodynamic systems seem potentially useful for the study of the pharmacological effects of thyroid congeners at the energy transfer level.

Summary. The efficiency of iodinated congeners of thyronine and tyrosine in protecting lysozyme against dye-sensitized photoinactivation was determined. With methylene blue as sensitizer, the relative protective efficiency is similar, in general, to the known metabolic efficiencies of these compounds.

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