Dye-Sensitized Photooxidation of the Escherichia coli Ribosome

Abstract. *Chemical modification of ribosomes from* Escherichia coli *by oxidation in the presence of selected photosensitizing dyes causes a rapid loss of their amino acid–incorporating ability. By comparing the efficiency of dyes that sensitize the photooxidation of either guanine in ribonucleic acid or amino acid residues in proteins, inactivation of one or more functional ribosomal proteins is inferred.*

Little is known about the manner in which the ribosomal protein and nucleic acid constituents relate to the various functions which the ribosome is required to perform. Much of the difficulty in finding this relation arises from the variety of these constituents (approximately 30 to 40 proteins and at least three nucleic acid species) (*1*). Methods which distinguish between "surface" structures and more protected or buried regions could aid in studying the structure-function relations, since it is at least reasonable that the exposed portions are likely to be involved more directly in binding and synthetic functions, whereas the interior is more apt to play a purely structural role.

Dye-sensitized photooxidation methods have several virtues which could contribute to studying specific components involved in ribosomal function. Typically, in dye-sensitized photooxidation, a substance such as a protein or nucleic acid is exposed simultaneously to a dye and light in the presence of oxygen, causing a chemical alteration of susceptible amino acids or nucleotides (*2*). Photosensitive moieties include the side chains of histidine, tryptophan, methionine, tyrosine, and cysteine, and the base guanine. Because the dye does not normally have access to internal regions of protein structures, only the surface residues are readily photooxidized (*3, 4*). Other advantages of the technique are (i) the backbone of the polymer (protein or nucleic acid) is not ruptured and the residues affected suffer only minor modifications (*5*); (ii) harsh reaction conditions are not required (*6*); (iii) only a few residues are affected (*7*); and (iv) some degree of specificity may be derived from the use of different dyes (*8, 9*).

We have found dye-sensitized photooxidation to be extremely effective for the inactivation of the *Escherichia coli* ribosome. The ribosome very rapidly loses the ability to function in polyadenylate (poly A)–directed synthesis of polylysine in vitro upon photooxidation in the presence of Rose Bengal (Fig. 1).

Total inactivation caused no change in the sedimentation pattern obtained with the analytical ultracentrifuge. The presence of dye with no exposure to light, or exposure to light in the absence of dye produced no loss of ribosomal activity.

Because both protein and ribonucleic acid (RNA) can be altered by the process of dye-sensitized photooxidation, we used the known specificity of various sensitizers for the side chains of proteins as compared to guanine. Singer and Fraenkel-Conrat (*10*) found that thiopyronin was approximately 1000-fold more effective than methylene blue or proflavin for the photooxidation of guanine in RNA from tobacco mosaic virus. Bellin and Yankus (*8*) reported that, in general, Rose Bengal was more effective than methylene blue, which in turn was more effective than thiopyronin in the dye-sensitized photooxidation of the five photosensitive amino acids. In comparing the ability of various dyes to sensitize the photochemical degradation of deoxyribonucleic acids (DNA), Bellin and Grossman (*9*) determined that methylene blue and thiopyronin were the most effective, whereas Rose Bengal was about tenfold less effective.

A comparison of these four dyes as sensitizers for the photooxidation of *E. coli* ribosomes (Fig. 2) revealed that the relative efficiency of the dyes is roughly similar to the rates found for the photooxidation of histidine, cysteine, methionine, and tyrosine (*8*). The fact that both methylene blue and thiopyronin are less efficient than Rose Bengal implies that a protein or proteins are the primary substrates being

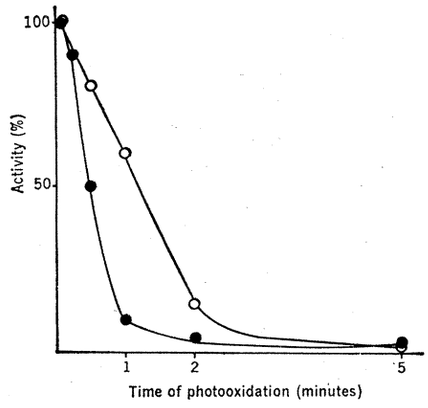

Fig. 1. Photooxidation of *E. coli* Q13 ribosomes in 0.45 ml buffer [0.08*M* NH_4Cl; 0.011*M* $Mg(OCH_2CH_3)_2$; 0.1*M* tris(hydroxymethyl) aminomethane-hydrochloride (tris-HCl); *p*H adjusted to 7.8] and 0.2 μmole of Rose Bengal at 0°C. The photooxidation was carried out in 12-ml conical centrifuge tubes placed in an ice bath positioned 10 cm from the lens of a 500-watt slide projector in the dark. The projector was operated for the times shown on the graph, after which the tubes were placed in light-free aluminum envelopes equipped with removable caps. The remainder of the incubation ingredients was added, and peptide synthesis was initiated by addition of poly A. Each tube contained the following ingredients: 0.5 μmole adenosine triphosphate; 0.015 μmole each of cytidine triphosphate, uridine triphosphate, and guanosine triphosphate; 2.5 μmole phosphoenol pyruvate; 1.5 enzyme units (10 μg) of pyruvate kinase; 0.000415 μmole C^{14}-labeled lysine (301 μc/μmole); 100 μl of *p*H 5 enzyme solution, prepared according to Julian (*12*); 0.6 mg of *E. coli* tRNA; 625 μg (●——●) or 1250 μg (○——○) of ribosomes; 6 μmole of mercaptoethanol; dye; and 50 μg of poly A. The final volume of the mixture was 1 ml. Following a 30-minute incubation at 37°C, each reaction was terminated by the addition of 0.04 mg of polylysine, 6 ml of tungstate solution (0.3*N* trichloroacetic acid (TCA); 0.01*N* sodium tungstate), and 0.2 ml of 10 percent TCA, in the order listed. Plating and counting of the radioactive synthetic peptides was done according to Julian (*12*).

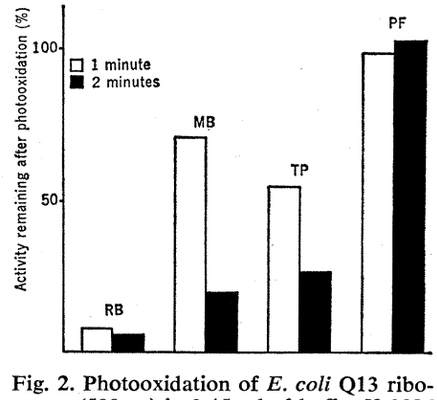

Fig. 2. Photooxidation of *E. coli* Q13 ribosomes (500 μg) in 0.45 ml of buffer [0.08*M* NH_4Cl; 0.011*M* $Mg(OCH_2CH_3)_2$; 0.1*M* tris-HCl; *p*H adjusted to 7.8] and 0.2 μmole of dye at 0°C. Abbreviations are: *RB*, Rose Bengal; *MB*, methylene blue; *TP*, thiopyronin; and *PF*, proflavin. Biologically active ribosomes of *E. coli* strain Q13 were prepared from frozen commercial cells and purified by chromatography on diethylaminoethyl-cellulose. Photooxidation, incorporation, plating, and counting procedures are given under Fig. 1.

altered by the photooxidation, and it is therefore unlikely that guanine residues of the RNA are involved in the inactivation process.

An extensive investigation of the effect of sulfhydryl reagents on ribosomal activity (*11*) showed that the effect of sulfhydryl reagents was primarily that of imposing a conformational change on the ribosomes. Some of the less bulky reagents were found actually to activate, while the largest of the reagents was the most potent inhibitor. The most nearly complete inhibition obtained was 70 percent after 12 hours incubation at 5°C. On the basis of our results (no conformational change; short photooxidation time) we exclude the possibility that dye-sensitized photooxidation of cysteine residues is primarily responsible for the inactivation process.

We feel that these initial photochemical studies provide evidence for the critical involvement of a ribosomal surface protein (or proteins) in the protein-assembly function of the ribosome. Minor modification of the photolabile side chains of the ribosomal protein or proteins *in situ* is sufficient to completely inactivate one or more essential steps of protein synthesis; the extreme rapidity of the inactivation process at very low dye concentrations (concentrations as low as one dye molecule per ribosome were found to be effective) implies an exposed catalytic site.

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Preferential Synthesis of Ferritin and Albumin by Different Populations of Liver Polysomes

Abstract. *Free polysomes and a mixture of free and membrane-attached polysomes were isolated separately from rat liver, and each was added to a cell-free, protein-synthesizing system. The free polysomes showed a greater capacity than the mixed polysome population for incorporation of ^{14}C-leucine into ferritin, whereas the reverse was true for ^{14}C-leucine incorporation into albumin.*

The liver cell contains two populations of polysomes, one attached to the membranes of the endoplasmic reticulum and the other free in the cytoplasm. The membrane-bound and free polysomes are thought to be functionally distinct (*1*); the former seem to be active in the biosynthesis of proteins to be secreted by the cell and the latter in the biosynthesis of intracellular protein. Experimental evidence has recently been provided for the preferential synthesis of secreted proteins such as albumin (*2*, *3*) and other serum proteins (*3*) by the membrane-bound ribosomes of liver. However, there is yet no evidence available concerning the site of synthesis of retained proteins and of the function of free polysomes.

Ferritin is a retained liver protein that can be conveniently isolated by chemical and immunological means (*4*). With a cell-free protein-synthesizing system under conditions in which release of completed peptide chains is maximum, we examined the relative capacities of free liver polysomes and of the total liver polysomes (free and attached) to synthesize ferritin; we compared the capacity to synthesize ferritin with the relative activities of the two polysome populations to incorporate labeled amino acids into serum albumin. The results show that free polysomes are more active in the synthesis in vitro of ferritin, whereas the total polysome fraction is more effective for albumin synthesis.

With fasting adult male rats, free and total polysome populations were isolated from the homogenates of liver from which the mitochondria were removed (*5*). Each polysome population was incubated for 1 hour along with a mixture of ^{14}C-amino acids and activating and transferring enzymes in the form of the *p*H 5 fraction prepared from liver cell sap (*6*). The amount of the *p*H 5 enzyme fraction was, however, increased by five times, since it has been found (*7*) that this favors maximum release of completed peptide chains in a cell-free system. At the end of incubation, rat liver homogenate was added to provide unlabeled carrier ferritin, and the ferritin was isolated (*4*) from the incubation mixture and

Table 1. Relative incorporation of ^{14}C-leucine into ferritin and into serum albumin by free polysomes and total polysomes isolated from rat liver.

Exp. No.	Uptake into protein (% of total peptide incorporation)		Difference (%)
	Total	Free	
	*Ferritin**		
1	0.09	0.12	+33
2	.08	.14	+75
3	.10	.15	+50
4	.08	.15	+85
5	.06	.11	+80
Mean	.08	.13	+65
	Albumin†		
1	0.30	0.10	−67
2	.39	.21	−46
Mean	.35	.16	−56

* At the end of incubation, rat liver homogenate was added to provide ferritin as unlabeled carrier. The ferritin was then isolated by the procedure of Drysdale and Munro (*4*) with omission of the carboxymethyl-cellulose step, which reduces recovery of ferritin by about 20 percent. The final product was then precipitated by addition of antibody to ferritin, the precipitate was washed with cold 0.4*N* $HClO_4$, and the radioactivity was counted. The total incorporation of ^{14}C-leucine into peptides was also measured by precipitating a small amount of the same incubation mixture with 5 percent trichloroacetic acid (TCA), then heating the solution to 90°C to remove nucleic acids, washing with 0.4*N* $HClO_4$, and counting the radioactivity. † At the end of incubation, 100 μg of rat serum albumin was added to the incubation medium, and the protein was precipitated with an equal volume of 10 percent TCA. The precipitate was washed three times with 5 percent TCA and was then extracted with 1 ml absolute ethanol (*9*). After 30 minutes standing, the sample was centrifuged and the supernatant was then dialyzed for 12 hours against water and for 2.5 hours against 10 m*M* phosphate buffer, *p*H 7.5. To remove nonspecific protein that might coprecipitate, chicken albumin was added to the extract, followed by its antiserum (*10*). Rat albumin antiserum was then added to the supernatant; it was incubated for 2 hours at 38°C and for 18 hours at 4°C. The albumin-antiserum complex was then centrifuged, 5 percent TCA was added, and the solution was heated for 15 minutes at 90°C. The precipitate was washed with 0.4*N* $HClO_4$ on a Millipore filter and counted by scintillation.