A PHOTO-INDUCED REACTION OF CHLORAMPHENICOL WITH E. coli RIBOSOMES: COVALENT BINDING OF THE ANTIBIOTIC AND INACTIVATION OF PEPTIDYL TRANSFERASE

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Summary: On irradiation of 50S ribosomal subunits with C^{14}-chloramphenicol, radioactivity becomes covalently attached to the ribosomes and irreversible loss of peptidyl transferase activity occurs. The label is distributed among most of the ribosomal proteins. Inactivation, but not covalent binding, was prevented when the irradiated mixture contained also erythromycin.

Introduction

Affinity labeling has found wide application as a method for identifying active sites of proteins. An important variant of affinity labeling that is in principle capable of a higher degree of specificity is photo-affinity labeling (1). In this method a reagent is used that under ordinary conditions combines reversibly with the active site of a protein but is converted on irradiation into a reactive intermediate capable of binding covalently to amino acid residues in the active site.

We have formerly studied by affinity labeling the peptidyl transferase site of 50S ribosomal subunits (2), using as a label a synthetic analog of chloramphenicol, an antibiotic that normally binds reversibly to ribosomes at or close to the peptidyl transferase site (3, 4). We report here a photochemical reaction of unmodified chloramphenicol with 50S ribosomal subunits of E. coli. When the ribosomes are irradiated with C^{14}-chloramphenicol labeled in the N-dichloroacetyl moiety, radioactivity becomes covalently attached to the ribosomes and irreversible inactivation of peptidyl transferase occurs. The nature of the photochemical reaction is not understood as yet, and it is not clear whether the inactivation is directly related to the covalent binding.

Experimental

Ribosomes from E. coli MRE-600 were prepared as described (5). Assay of peptidyl transferase activity and determination of covalent binding of chloramphenicol (4.91 C/mol, New England Nuclear) to ribosomes by trichloroacetic acid precipitation, were performed essentially as previously reported (2). Peptidyl transferase assay mixtures contained 80 µg of 50S subunits and acid insoluble radioactivity was determined with samples containing 160 µg of ribosomes.

Results

Isolated 50S ribosomal subunits were irradiated with C^{14}-chloramphenicol under the conditions and for the periods specified in Figure 1. Following irradiation, the ribosomes were dialyzed and tested for trichloroacetic acid insoluble radioactivity (Fig. 1a) and peptidyl transferase activity (Fig. 1b).

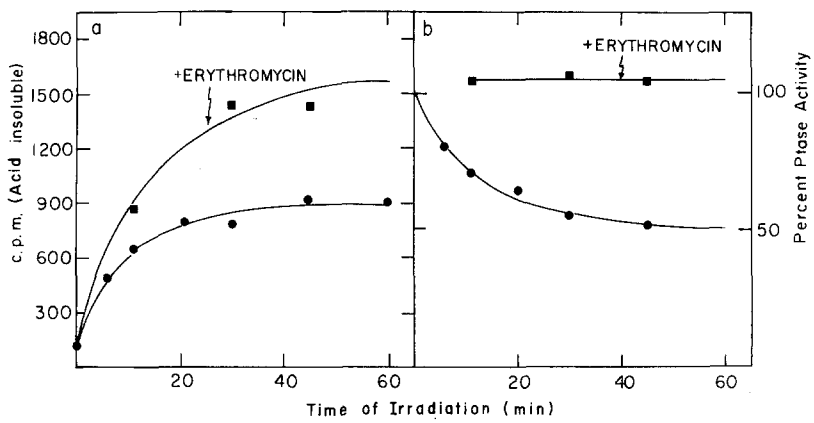

Figure 1: Effect of irradition of 50S ribosomal subunits with C^{14}-chloramphenicol

50S subunits were suspended to a concentration of 18.8 mg/ml in an aqueous solution containing 1 mM Mg acetate, 0.1 M NH_4Cl, 0.02 M Tris-HCl (pH 7.4), 10% methanol, 1.6 mM C^{14}-chloramphenicol and when indicated also 0.018 M erythromycin (Sigma). The samples were placed in a water bath at room temperature and irradiated for the indicated periods at a distance of 8 cm from a mercury high presure lamp (450 W, Hanovia). Light of wavelength below 300 nm was eliminated by the use of Pyrex glassware and a Pyrex jacket around the lamp. Following irradiation the mixtures were dialyzed against 0.01 M Mg acetate, 0.1 M NH_4Cl and 0.02 M Tris-HCl (pH 7.4) and tested for acid insoluble radioactivity - (a), and peptidyl transferase activity - (b). 100% activity was obtained with ribosomes incubated with chloramphenicol at room temperature for 1 hour and then dialyzed as above. The activity measured (12.3 pmol of fMet-puromycin formed in 8 minutes) was lower by 10% from the activity of untreated ribosomes.

It is evident from Fig. 1a that very little radioactivity becomes acid insoluble in non-irradiated mixtures. Radioactivity becomes increasingly acid insoluble when the mixtures are irradiated. The acid-insolubility is most probably due to the covalent binding of chloramphenicol, or part thereof, to the ribosomes since radioactivity is not rendered acid insoluble when the irradiation mixture does not contain ribosomes. Furthermore, the radioactivity remains bound throughout the treatments employed to dissociate ribosomes into their protein and RNA components. The maximal binding observed in the experiment shown corresponded to approximately one molecule of chloramphenicol

bound per ribosome. However, higher levels of binding were observed by increasing the concentration of chloramphenicol in the irradiated mixtures.

It is evident from Fig. 1b that during the irradiation with chloramphenicol the ribosomes also lose their activity in catalyzing peptidyl transfer. In the experiments shown activity dropped to 50% of the original level. When irradiated for 60 minutes under similar conditions but in the absence of the antibiotic the ribosomes lose at most 10% activity. When on the other hand the antibiotic was irradiated alone and then added to non-irradiated ribosomes, no irreversible binding and loss of activity were noted.

To test whether the photo-induced inactivation depends on the specific reversible binding of chloramphenicol to the ribosome, irradiation was carried out in the presence of erythromycin. This antibiotic is known to inhibit the reversible binding of chloramphenicol and also to displace chloramphenicol after it has been bound to the ribosome (6). By itself, erythromycin had no effect on activity of ribosomes irradiated in its presence. As is shown in Fig. 1b, the addition of erythromycin to mixtures containing chloramphenicol, fully protects the ribosomes against photo-inactivation. However, as shown in Fig. 1a, the extent of covalent binding is much increased under these conditions.

Analysis of C^{14}-chloramphenicol labeled ribosomes showed the label to be predominantly associated with the protein fraction with at most 17% bound to the RNA. Most of the proteins were labeled and no specific labeling pattern was obvious. The distribution of label was not significantly altered in ribosomes irradiated in the presence of erythromycin.

Discussion

The irradiation of 50 S ribosomal subunits with chloramphenicol results in two effects; the covalent binding of chloramphenicol, or part of it, mostly to the ribosomal proteins, and the inactivation of peptidyl transferase. The relationship between these effects as well as the nature of the photo-chemical reactions involved are unknown at present.

The observed covalent binding is most probably not restricted to the specific chloramphenicol binding sites(s). This is indicated by the wide distribution of label among the ribosomal proteins and by the enhancement of the labeling reaction when the specific reversible binding of chloramphenicol was abolished by erythromycin. Nevertheless, the inactivation of peptidyl transferase activity indicates that an irreversible reaction, perhaps different from the observed covalent binding, must have taken place at functionally significant sites. The protection against inactivation provided by erythromycin possibly indicates that chloramphenicol must first be specifically bound to the ribosome for inactivation to occur. However, the protection could also result

from the binding of erythromycin itself to the ribosome which might render the functional sites inaccessibe to chemical attack. An alternative possibility, that erythromycin functions as a scavenger of the reactive species derived from chloramphenicol is unlikely in view of the overall increase in covalent binding observed is the presence of erythromycin.

As indicated, the covalent binding appears to be largely non-specific and the possibility arises that inactivation is due to a reaction other than covalent binding. A possible reaction could be a photo-oxidation mediated by chloramphenicol of some essential aminoacid residues, analogous to photo-oxidation of enzymes induced by bound dyes or prosthetic groups (cf. 7, 8).

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References

1. Knowles, J.R., Accounts of Chemical Research 5, 155 (1972).
2. Sonenberg, N., Wilchek, M. and Zamir, A., Proc. Nat. Acad. Sci. USA 70, 1423 (1973).
3. Pestka, S., Proc. Nat. Acad. Sci. USA 64, 709 (1969).
4. Celma, M.L., Monro, R.E. and Vasquez, D., FEBS Letters 13, 247 (1971).
5. Miskin, R. and Zamir, A., J. Mol. Biol. in press.
6. Tanaka, K., Tamaki, M., Takata, R. and Osawa, S., Biochem. Biophys. Res. Comm. 46, 1979 (1972).
7. Scoffone, E., Galiazzo, G. and Jori, G., Biochem. Biophys. Res. Comm. 38, 16 (1970).
8. Mauk, M.R., Girotti, A.W., Biochemistry 13, 1757 (1974).