BINDING OF CHLORAMPHENICOL BY RIBOSOMES FROM CHLOROPLASTS

Louise A. Anderson* and Robert M. Smillie

Plant Physiology Unit, C.S.I.R.O. Division of Food Preservation and School of Biological Sciences, University of Sydney, Australia.

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Although chloramphenicol is a potent inhibitor of protein synthesis in bacteria, it usually has little effect on protein synthesis in non-bacterial systems (Gale, 1963). Among higher organisms, green plants constitute an outstanding exception since chloramphenicol depresses protein synthesis in cells containing chloroplasts. The inhibitory action of chloramphenicol on these cells is largely confined to the synthesis of chloroplast protein, the synthesis of cytoplasmic protein being relatively unaffected. Thus concentrations of the antibiotic which inhibit the synthesis of chloroplast protein during greening of non-dividing cells of *Euglena gracilis*, or the growth rate of the cells grown autotrophically, have little effect on either the synthesis of certain cytoplasmic proteins during greening, or on the rate of growth of cells grown heterotrophically (Smillie *et al*, 1963). Similar differences have been found in cell-free systems. Though protein-synthesizing systems isolated from chloroplasts are sensitive to low concentrations of chloramphenicol (Goffeau and Brachet, 1965; Spencer, 1965), cytoplasmic systems from photosynthetic cells are comparatively insensitive (Eisenstadt and Brawerman, 1964).

Chloramphenicol is considered by Wolfe and Hahn (1965) to inhibit protein synthesis in bacteria by attaching to a site on the ribosome, thereby

*Present address : Microbiology Department, Dartmouth College, New Hampshire.

interfering with the function of messenger RNA. Should this be the case, the differential inhibitory effects shown by chloramphenicol on protein synthesis in cells containing chloroplasts may reflect differences between ribosomes in the chloroplasts and cytoplasm, respectively. Vazquez (1964) has shown that chloramphenicol is bound more strongly by bacterial ribosomes than by ribosomes isolated from higher organisms, and it is possible that, in contrast to cytoplasmic ribosomes, the chloroplast ribosomes contain sites that are either more accessible to chloramphenicol or can bind it more tightly.

Results obtained in the present study indicate that chloroplast ribosomes can in fact bind chloramphenicol more strongly than the neighbouring cytoplasmic ribosomes and suggest an explanation for the differential effects of chloramphenicol on protein synthesis in photosynthetic cells.

MATERIALS AND METHODS

Leaves of wheat (*Triticum vulgare* Mexico) or pea (*Pisum sativum* L. var. Yates Greenfeast) were harvested from ten-day-old plants. Algae were grown autotrophically (5% CO_2 in air as the carbon source) at 25°C and under 7,000 lux of white light; *Euglena gracilis*, strain Z, in the basal medium of Hutner *et al*. (1956) supplemented with vitamin B_{12} and *Anabaena cylindrica* Lemm. (Fogg's strain) in the medium described by Allen and Arnon (1955). Yeast (commercial Baker's yeast) and *Escherichia coli* were grown aerobically.

Extracts containing ribosomes were prepared in 0.02 M Tris, pH 7.4 containing 0.002 M $MgCl_2$. Leaves were extracted by grinding with a pestle and mortar, yeast by blending with glass beads, and the remaining microorganisms by passage through a French Pressure Cell. When isolating chloroplasts, sucrose to 15% was added to the extracting medium. Chloroplasts were collected by centrifuging at 1,000 x *g* for 20 min, and the supernatant fluid retained as the source of cytoplasmic ribosomes. The chloroplasts were washed once with the buffered 15% sucrose and were broken by suspension in 0.05% deoxycholate in buffer to release their ribosomes. Ribosomes were concentrated from the various extracts by centrifuging between 30,000 x *g* (20 min) and 144,000 x *g* (120 min). The binding of chloramphenicol by ribosomes was measured by Vazquez's (1964) procedure.

RESULTS AND DISCUSSION

In agreement with the results of Vazquez (1964), the binding of chloramphenicol by ribosomes isolated from cells, such as yeast, which contain a well-defined nucleus is considerably less than by ribosomes from bacteria, such as E. coli (Table 1). Chloramphenicol was also bound by ribosomes from the blue-green alga A. cylindrica, an organism which like the bacterial cell lacks a well-defined nucleus. As might be expected, less chloramphenicol was bound by the "total" ribosomes prepared from the other photosynthetic cells or tissues used, but the values were usually higher than those obtained from the corresponding etiolated tissues or yeast. More significantly, where cytoplasmic and chloroplast ribosomes from the same cells or tissue were compared, binding by preparations enriched for chloroplast ribosomes was invariably higher.

The possibility remained that the differences noted in Table 1 between ribosomes from chloroplasts and cytoplasmic ribosomes were due to increased non-specific binding of label by the preparations from chloroplasts. This is unlikely since erythromycin, which inhibits the specific binding of chloramphenicol by ribosomes from bacteria (Vazquez, 1963; Wolfe and Hahn, 1965), also inhibits the binding by ribosomes from chloroplasts. Thus erythromycin at 97 μg/ml reduced by 89% the binding of chloramphenicol by chloroplast ribosomes isolated from E. gracilis. Under similar conditions, the binding by cytoplasmic ribosomes from E. gracilis was reduced by 87% and that by ribosomes from E. coli by 84%.

Our results suggest that the synthesis of chloroplast protein either in vivo or in vitro is inhibited by chloramphenicol because chloroplasts contain a species of ribosome with a capacity to bind chloramphenicol. It is to be noted that the binding values shown for chloroplast ribosomes in Table 1 are underestimates since there was some cross contamination by cytoplasmic ribosomes. In contrast, since the binding of chloramphenicol to cytoplasmic ribosomes is lower, the synthesis of cytoplasmic protein is relatively insensitive to chloramphenicol.

Table 1. Binding of C^{14}-Chloramphenicol by Ribosomes from Various Sources.

Source	Fraction	Chloramphenicol Bound (mμg/mg RNA)
E. coli	total	88
Anabaena cylindrica	"	55
Yeast	"	6
Euglena gracilis	"	10
	chloroplast	13.2
	cytoplasm	6
Pea leaf : etiolated	total	4
green	"	11
	chloroplast	22
	cytoplasm	7
Wheat leaf : etiolated	total	9
green	"	17
	chloroplast	28
	cytoplasm	11

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