Chloroplast Ribosomes: Stereospecificity of Inhibition by Chloramphenicol

Abstract. *Chloroplast ribosomes from tobacco leaves show the same stereospecificity of inhibition by chloramphenicol as bacterial ribosomes do. Cytoplasmic ribosomes from the same leaves are unaffected by chloramphenicol. These results remove doubts raised by nonstereospecific effects of chloramphenicol on higher plant cells and support the concept that chloroplasts have evolved from prokaryotes.*

Chloroplasts from higher plants contain ribosomes which resemble bacterial ribosomes in their sedimentation behavior (*1*), in the sizes of their RNA components (*2*), and in the inhibition of their amino acid incorporating ability by chloramphenicol (*3*, *4*). These similarities support the suggestion (*5*), in which interest has recently revived (*6*), that chloroplasts have evolved from symbiotic prokaryotes. In contrast, cytoplasmic ribosomes from both green and nongreen tissues of higher plants are larger than chloroplast ribosomes (*1*), and those present in several nongreen tissues are insensitive to chloramphenicol both in vitro (*7–9*) and in vivo (*10*). It has not been established that the cytoplasmic and chloroplast ribosomes isolated from the same higher plant cells differ in their response to chloramphenicol, although this has been shown for *Euglena* (*11*).

There are four stereoisomers of chloramphenicol (D- and L-*threo*, and D- and L-*erythro*); the naturally occurring antibiotic is the D-*threo* isomer. The inhibition by chloramphenicol of amino acid incorporation by bacterial ribosomes is highly specific for the D-*threo* isomer (*12*), but a similar stereospecificity has not been reported for chloroplast ribosomes. It is important to establish this point because other processes carried out by higher plants, such as ion uptake (*13*) and oxidative phosphorylation (*14*), are inhibited by several isomers of chloramphenicol including the D-*threo* isomer; in bacteria these processes are not inhibited by chloramphenicol (*15*). If a similar lack of stereospecificity occurs in the inhibition of protein synthesis by chloroplast ribosomes, serious doubt would be cast on the interpretation of the similarity between bacterial and chloroplast ribosomes. Experiments were therefore carried out to determine (i) whether cytoplasmic and chloroplast ribosomes isolated from tissues of the same higher plant differ in their sensitivity to chloramphenicol, and (ii) whether chloroplast ribosomes resemble bacterial ribosomes in the stereospecificity of inhibition by chloramphenicol. The effect of cycloheximide was also investigated because this antibiotic reportedly inhibits protein synthesis by cytoplasmic ribosomes from higher plants (*9*) but not that by bacterial ribosomes (*16*).

Surfaces of expanding leaves from young plants of *Nicotiana tabacum* L. were sterilized with hypochlorite, washed in sterile water, and deribbed. The laminae were sliced in Honda medium (*17*) by a hand-chopping procedure (*4*) to minimize breakage of the chloroplasts. The chloroplasts were sedimented at 1500*g* for 10 minutes and resuspended in Honda medium. The supernatant was centrifuged at 15,000*g* for 15 minutes to remove mitochondria, and dialyzed for 3 hours to lower the endogenous pool of amino acids before use as a source of cytoplasmic ribosomes. Incorporation of L-leucine-C^{14} into protein was measured as described (*9*). All glassware and solutions were sterilized before use.

Intact chloroplasts incorporate 50 to 100 pmole of leucine per milligram of chlorophyll into protein when incubated with guanosine triphosphate (GTP) and adenosine triphosphate (ATP) (Table 1). The incorporation is sensitive to small amounts of ribonuclease, and the labeled protein is rendered almost totally soluble by treatment with the detergent Triton X-100. These criteria indicate that the incorporation is due to chloroplasts and not to bacteria or nuclei (*18*). Direct sampling of assay tubes (*9*) revealed less than 100 bacteria per tube. The inhibition by chloramphenicol is stereospecific; the D-*threo* isomer inhibits 80 percent at 300 μg/ml but the L-*threo* and D-*erythro* isomers are inactive. Cycloheximide does not inhibit incorporation by chloroplasts, even at much higher concentrations than those which inhibit protein synthesis in vivo, but inhibits slightly incorporation by cytoplasmic ribosomes. Ribosomes from beetroot are inhibited markedly by cycloheximide (*9*) but those from castor-bean embryos are not (*8*), so there appear to be differences between species in this respect. The same results are obtained with chloroplasts treated with Triton X-100 which renders the chlorophyll completely soluble (*4*); thus the negative effects are not due to failure of the compounds to penetrate the chloroplast membranes. Incorporation by cytoplasmic ribosomes is unaffected by all the isomers of chloramphenicol.

My results show that chloroplast ribosomes resemble bacterial ribosomes in showing the same stereospecificity of in-

Table 1. Effect of chloramphenicol (CAM) isomers and cycloheximide on leucine incorporation into protein by chloroplast and cytoplasmic ribosomes. Chloroplasts were incubated in Honda medium containing a final concentration of 0.5 μc of L-leucine-C^{14}, 0.4 m*M* ATP, an ATP generating system, 0.4 m*M* GTP, 30 m*M* KCl, and 12 m*M* $MgSO_4$ in a final volume of 0.5 ml for 60 minutes at 25°C. Some chloroplast preparations were treated before assay with Honda medium containing 4 percent Triton X-100. The dialyzed cytoplasmic extract was incubated in the same medium as the chloroplasts except that the $MgSO_4$ was reduced to 8 m*M*. Protein was precipitated and washed, and the radioactivity was counted as described (*9*). The results are expressed as a percentage of the number of counts incorporated by the complete assay mixture. The chloroplasts incorporated 50 to 100 pmole of leucine per milligram of chlorophyll and the cytoplasmic ribosomes incorporated 5 to 10 pmole of leucine per milligram of protein.

Assay mixture	L-Leucine-C^{14} incorporation into protein (%)		
	Intact chloroplasts	Chloroplasts treated with Triton X-100	Cytoplasmic ribosomes
Complete	100.0	100.0	100.0
Zero time	1.5	0.5	11.0
− ATP and GTP	5.0	3.0	7.0
Complete, Triton X-100 insoluble	5.0		
+ Ribonuclease (1 μg)	20.0	2.5	18.0
+ D-*threo* CAM (150 μg)	23.0	23.0	95.0
+ L-*threo* CAM (150 μg)	90.0	98.0	93.0
+ D-*erythro* CAM (150 μg)	90.0	92.0	100.0
+ Cycloheximide (150 μg)	96.0	99.0	76.0
+ Cycloheximide (12 μg)	90.0	95.0	86.0

hibition by chloramphenicol and lack of sensitivity to cycloheximide, but differ from the cytoplasmic ribosomes isolated from the same tissue. Whether this resemblance extends to the location of the chloramphenicol-binding site, which is the 50*S* subunit in the case of bacterial ribosomes (*19*), remains to be investigated.

R. J. ELLIS*
Department of Botany, University of Aberdeen, Aberdeen, Scotland

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* Present address: Department of Biological Chemistry, University of Aberdeen.

2 October 1968

Solubility in Water of Normal C_9 and C_{10} Alkane Hydrocarbons

Abstract. *A new method for equilibrating water containing alkane hydrocarbons with a gas phase and analyzing the gas for hydrocarbon content by gas chromatography extends analytical sensitivity to better than 0.1 part per billion. The solubilities at 25°C of the normal C_9 (220 parts per billion) and C_{10} (52 parts per billion) alkanes decrease with increasing carbon number. A discontinuity occurs at the normal C_{11} alkane, probably because of a change from true solubility (molecular dispersion) to accommodation (aggregation).*

The solubilities in water of normal alkane hydrocarbons (*1*, *2*) decrease linearly from the normal C_4 alkane through the normal C_8 alkane, if the logarithm of the hydrocarbon solubility is plotted as a function of the molar volume (carbon number) of the hydrocarbon. It has been suggested that, if the relation is a continuous function, the solubilities in water of longer-chain normal alkanes could be predicted by extrapolation. However, recent measurements (*3*–*6*) indicate that the solubilities (or accommodation) of the normal alkanes from C_{12} to C_{36} are very much greater than would be predicted.

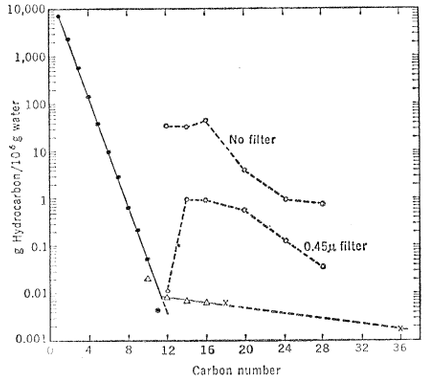

Fig. 1. Solubilities (or accommodations) of normal paraffin hydrocarbons in water at ≃ 25°C as a function of carbon number; ●, data from McAuliffe (*2*) and this work; ⊙, from Peake and Hodgson (*5*); △, from Franks (*3*); ×, from Baker (*6*).

The sensitivity of the present analytical method used to measure the solubilities of normal alkanes from C_1 to C_8 in water (*2*) is limited to about 0.1 ppm (by weight) because of the relatively small sample of water (50 μl) analyzed. The method reported here increases the sensitivity of the gas chromatographic method; the hydrocarbon content of the gas phase is measured after equilibration with 5 ml of hydrocarbon-saturated water. From the vapor pressures and solubilities of individual alkanes, the partitioning of the hydrocarbon between the water and gas phases can be calculated from the distribution coefficient

$$K = C_{HC(g)}/C_{HC(w)} = M_{(g)} \times N_{HC(g)}/M_{(w)} \times N_{HC(w)}$$

where C_{HC} and N_{HC} are the concentration and mole fraction of the hydrocarbon in the gas (g) and water (w) phases, and M is the number of moles.

Calculation of the distribution coefficients for alkanes from methane through normal octane indicates that from 96 percent (C_2) to over 99 percent (normal C_7 and C_8) of the hydrocarbon partitions into the gas phase when water saturated with that hydrocarbon is equilibrated with an equal volume of gas. The calculated distribution coefficients were experimentally confirmed for alkanes from methane through octane.

Normal alkanes from C_9 to C_{14} were equilibrated with distilled water at 25°C (*2*). Glass vials (30 ml) were filled with this hydrocarbon-saturated water; half of the water was then displaced with a glass rod. The displaced volume was replaced by air. Each vial was then quickly sealed with a metal screw cap. After the vials were shaken on a "wrist-action" paint shaker for 2 minutes, the gas phase was displaced through the 5-ml sample loop of a gas chromatograph by means of a sharpened stainless steel point 0.635 cm (0.25 inch) in diameter inserted through the cap. The stainless steel point has two openings; hydrocarbon-free water can be added through one port, and the gas phase can be displaced through the other. The puncturing device seals against the top of the metal screw cap and has suitable O-ring seals on the probe to prevent gas leakage (*2*).

The measured solubilities of the normal alkanes, expressed as parts per billion (by weight), are C_9, 220 ±21; C_{10}, 52 ± 4.3; and C_{11}, 4.4 ± 1.8. Although the analytical technique could measure less than 0.1 ppb, normal C_{12}, C_{13}, and C_{14} alkanes could not be detected in the gas phase. A predicted solubility for the normal C_{12} alkane, obtained by extrapolation, is 3.4 ppb. The value for the normal C_{11} alkane is also lower than predicted (14 ppb).

The low solubilities for normal alkanes beginning with C_{11} may well be due to the presence of hydrocarbon aggregates (*6*) or micelles arising from impurities in the water phase. During equilibration with the gas phase, the hydrocarbon aggregates or micelles compete with the gas phase for the individually dispersed hydrocarbon molecules in the water, which results in a decrease in the concentration in the gas phase. For the normal C_{11} alkane, the proportion of aggregates or micelles appears to be relatively small, resulting in a decrease in measured solubility from 14 to 4 ppb. However, for the