DIFFERENTIAL EFFECTS OF CHLORAMPHENICOL ON THE INDUCTION OF NITRATE AND NITRITE REDUCTASE IN GREEN LEAF TISSUE

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This investigation shows that induction of nitrate reductase (NR)[1] and nitrite reductase (NiR)[1] is differentially repressed by chloramphenicol. These data support the conclusion that NR is synthesized by a cytoplasmic ribosomal system and NiR is synthesized by a chloroplastic ribosomal system.

Chloramphenicol is an effective inhibitor of protein synthesis in bacteria (Brock, 1961). With respect to its mode of action in inhibiting protein synthesis in E. coli, Wolfe and Hahn (1965) reported that chloramphenicol binds (1:1 ratio) to the 50 S ribosomal subunit. They suggest that this binding interferes "with some function of messenger RNA". Although Goffeau and Brachet (1965) and Spencer (1965) reported that low concentrations of chloramphenicol inhibited protein synthesis in chloroplasts, it is commonly held (Rabson and Novelli, 1960) that the antibiotic has little effect on protein synthesis in higher plants unless used at higher concentrations. Margulies (1962, 1964) has reported that synthesis of chloroplast proteins is inhibited by chloramphenicol in excised and intact plants. In contrast, protein synthesis in the cytoplasm of photosynthetic cells appears to be comparatively insensitive to chloramphenicol (Eisenstadt and Brawerman, 1964).

Some understanding of the differential inhibition of chloroplastic and cytoplasmic protein synthesis has been provided by recent investigations.

[1] The following abbreviations will be used: NR, nitrate reductase; NiR, nitrite reductase.

Clark-Walker and Linnane (1966) suggest that yeast mitochondrial ribosomes are "sensitive" to chloramphenicol while cytoplasmic ribosomes are "insensitive". They further suggest that protein synthesis in yeast mitochondria is mediated by ribosomes resembling the 70 S type from bacteria and chloroplasts. Anderson and Smillie (1966) showed that more ^{14}C-chloramphenicol was bound per unit of RNA by chloroplastic than by cytoplasmic ribosomes.

Chloramphenicol was previously reported (Beevers, et. al., 1965) to be relatively ineffective in inhibiting substrate induction of NR in radish cotyledons and corn seedlings unless used at levels exceeding 1 and 5 mg/ml, respectively.

The recent findings that (a) NiR in higher plants is also substrate inducible (Ingle, et. al., 1966) and (b) NiR is inside chloroplasts whereas NR is external to chloroplasts (Ritenour, et. al.,)[2] prompted the present investigation to ascertain whether the differential sensitivity of chloroplastic and cytoplasmic ribosomes to chloramphenicol would permit differential repression of the induction of the cytoplasmic enzyme NR and the chloroplastic enzyme NiR.

Materials and Methods

Growth of the corn seedlings, enzyme extraction, and induction was accomplished as previously described (Beevers, et. al., 1965). NR was assayed according to Hageman and Flesher (1960) and NiR was assayed according to Joy and Hageman (1966) using dithionite and benzyl viologen as electron donors.

Results and Discussion

Data for four separate experiments are presented in Table I.

The results (Table I) clearly demonstrate that the induction of NR and NiR respond quite differently to chloramphenicol. NR induction is actually

[2] Ritenour, G. E., Joy, K. W., Bunning, J., and Hageman, R. H. (Manuscript submitted to Plant Physiology)

Table I

Induction of Nitrate Reductase and Nitrite Reductase in Presence of Chloramphenicol

Expt.	Concentration of Chloramphenicol (mg/ml)	NR induction	NiR induction
I	0	0.61	0.66
	3.22	0.67	0.22
II	0	0.60	0.52
	3.22	0.65	0.05
III	0	0.58	0.54
	0.16	0.67	0.61
	0.32	0.79	0.57
	1.61	0.95	0.15
IV	0	0.60	0.50
	1.61	0.96	0.17
	3.22	0.77	0.01
	6.44	0.36	0.00

Corn seedlings were excised and preincubated 30 minutes in phosphate buffer (0.02 M, pH 4.0) and the concentration of chloramphenicol indicated above in experiments I and II before adding 0.1 M. nitrate for an additional 4 hours of incubation. The same techniques were used in experiments III and IV, except that the seedlings were preincubated 1 hour in chloramphenicol before adding the inducer. Nitrate (0.1 M.) was added in both experiments for an additional 4 hours and 0.001 M. $NaNO_2$ was also added in experiment IV.

NR induction is expressed as µmoles NO_2^- produced/mg protein/hr.

NiR induction is expressed as µmoles NO_2^- reduced/mg protein/hr.

The data for induction represent the net induction of the enzymes which occurred during the 4-hour induction period. Each figure represents the average of 3 replications.

stimulated by certain levels of chloramphenicol. In three experiments, an average stimulation of 74% was observed when 1.61 milligrams of chloramphenicol were added per milliliter of induction media. This is consistent with the recent report of Ramsey (1966) in which chloramphenicol (50 ug/ml) enhanced NR induction in Staphylococcus almost 4-fold in a 3-hour induction period, but inhibited ^{14}C-arginine incorporation by 88%.

In contrast, NiR induction was repressed by chloramphenicol (Table I).

Neither enzyme was inhibited by chloramphenicol in in vitro assays. Based upon these data and the report of Anderson and Smillie (1966), it is concluded that NR is synthesized by a cytoplasmic ribosomal system and NiR is synthesized by a chloroplastic ribosomal system. This conclusion is consistent with the observations of Ritenour, et. al.[2] which indicate that NR is located in the cytoplasm and NiR appears to be confined to the chloroplast.

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