EFFECT OF CHLORAMPHENICOL ON SOME OF THE CYTOSOLIC ENZYMES FROM *ASPERGILLUS NIGER*

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ABSTRACT

Chloramphenicol was found to inhibit the activities of cytosolic FDP aldolase, isocitrate dehydrogenase, malate dehydrogenase and isocitrate lyase from *Aspergillus niger*. The significance of these studies has been discussed.

INTRODUCTION

CHLORAMPHENICOL is a broad spectrum prokaryotic protein synthesis inhibitor which acts on the 50 S subunit of ribosome and prevents the peptide bond formation[1]. However, mammalian cells are usually found resistant to chloramphenicol[2]. In higher plants it has little effect on protein synthesis unless used at a high concentrations[3]. Studies are lacking regarding the effect of chloramphenicol on eukaryotic cytosolic systems. It is being used to understand the mechanism of biogenesis of mitochondria or chloroplasts since it inhibits specifically the protein synthesis in mitochondria[4], or chloroplasts[5] but not eukaryotic cytosolic systems. The present study focusses attention that eukaryotic cytosolic system is also affected drastically either directly or indirectly at higher concentrations of the antibiotic and hence suggests that the data should be interpreted carefully when chloramphenicol is used to study the roles and mechanisms of biogenesis of mitochondria or chloroplasts.

MATERIALS AND METHODS

Aspergillus niger (wild type, citric acid producing) used in the present studies was obtained from the culture collection department, Hindustan Antibiotics, Poona, India. The culture was grown and maintained on saboraud's agar slants. The composition of the synthetic medium employed was the same as that described earlier[6] except sucrose concentration which was added 7.5% in our studies. The culture was grown in 50 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (180 rpm) at 30° C for 7 days. The mycelia were harvested by filtration and stored at −5° C before use. For growth measurement, the mycelia were dried at 50° C to a constant weight. The growth was expressed as dry mat weight per flask.

For enzyme assays, a cell-free extract was prepared in 0.05 M Tris-HCl buffer of pH 7.5 containing 0.4 M sucrose by grinding the frozen mycelia with a pestle in a chilled mortar with glass powder so as to get a 30% (wet/vol) extract. The extract was centrifuged at 5000 g for 10 min. The supernatant was recentrifuged at 15,000 g for 30 min. and the 2nd step supernatant was used for cytosolic enzyme assays. The 15,000 g pellet obtained was sonicated 4 times for 30 sec, with intervals of 30 sec, in the cold, in the same buffer. The sonicated material was then centrifuged at 15,000 g for 30 min and the resulting supernatant was used for the assay of mitochondrial malate dehydrogenase activity.

The assay methods used for FDP aldolase (fructose-1, 6 diphosphate D glyceraldehyde-3phosphate lyase, EC 4.1.2.13), isocitrate dehydrogenase (three-D-isocitrate: NADP oxidoreductase (decarboxylating), EC 1.1.1.42), malate dehydrogenase (L-malate: NAD oxidoredutase, EC 1.1.1.37), and isocitrate lyase (three-D-isocitrate glyoxylate lyase, EC 4.1.3.1) were the same as described by other workers[7-9]. Invertase (B fructo furanoside Fructohydrolase, EC 3.2.1.26) activity was measured by estimating reducing sugar released by Bernfeld method[10] and a unit is defined as the amount of enzyme which causes the liberation of one μmole of reducing sugar at 37° C per hour. Protein was estimated according to the method of Warburg and Christian[11]. Acidity was measured by titrating clear culture filtrate with 0.1 N NaOH using phenolpthalein as an indicator and is expressed as ml of 0.1 N NaOH needed to neutralize total acids present in 50 ml of culture filtrate.

RESULTS AND DISCUSSION

Chloramphenicol when added in 4 mg/ml did not affect the growth of *Aspergillus niger*. The acidity which is chiefly due to citric acid production in the growth medium was found to be decreased when *Asp. niger* was grown in the presence of chloramphenicol (table 1). The possibilities of low acidity, *i.e.* low citric acid production may be due to (a) inhibition of mito-

TABLE 1

Effect of chloramphenicol on growth, acidity and invertase activity from Aspergillus niger

Conditions of the growth	Growth (dry mat wt/ (g) flask)	Acidity	Invertase activity				
			Intracellular (Units/ mat)	Units/mg protein)	Extracellular	Total (Extracellular + intracellular)	Ratio (Extracellular to Intracellular)
Control	0.79	64	100	13.94	408.6	508.6	4.09
With Chloramphenicol (2 mg/ml)	0.94	45	38	6.63	548.0	596.0	14.42
With Chloramphenicol (4 mg/ml)	0.74	40	45	5.6	381.7	426.7	8.48

chondriogenesis resulting in decreased synthesis of citric acid or (b) if glycolytic enzyme(s) is inhibited either directly or indirectly by chloramphenicol then the availability of the substrate like pyruvate may be limiting due to less production in the cytosol and hence less transport into the mitochondria to be converted into acetyl CoA for citrate synthase reaction or (c) the inhibition of invertase if any by chloramphenicol, would result in less degradation of sucrose (7.5% sucrose is used as a carbon source) and hence less supply of reducing sugars.

The levels of extracellular invertase were not decreased significantly. The secretion of enzyme might have been influenced since the ratio of extracellular to intracellular invertase was increased in the presence of chloramphenicol where intracellular enzyme was decreased while total enzyme (extracellular + intracellular) was not affected significantly (table 1). These results indicate that invertase may not play a significant role in changing the acidity in the medium in these conditions.

To see the inhibition of mitochondrial enzyme, one enzyme malate dehydrogenase was seen. The total activity of malate dehydrogenase per total mitochondria of complete mat was inhibited (growth is not affected) in the presence of chloramphenicol (table 2). It is therefore assumed that mitochondriogenesis had been affected due to chloramphenicol which might have resulted in decreased production of citric acid and hence lower acidity in the medium. Surprisingly, the activity of cytosolic FDP aldolase was also affected considerably which may be another reason for the low citric acid production (table 2). Here chloramphenicol in the concentrations of 1–2 mg/ml was used.

TABLE 2

Effect of chloramphenicol on FDP aldolase and mitochondrial malate dehydrogenase activities from Aspergillus niger

Condition of the growth	FDP aldolase activity	Mitochondrial MDH
	(Units/mg protein)	(Total Units/ Mat)
Control	854.7	1219.8
With chloramphenicol (1 mg/ml)	243.0	656.8
With chloramphenicol (2 mg/ml)	166.9	455.2

Attempts were made to see whether other cytosolic enzymes were also affected. Cytosolic isocitrate dehydrogenase, malate dehydrogenase and isocitrate lyase were also affected significantly (table 3). However, it is not clear why the cytosolic enzymes should get affected in the presence of chloramphenicol or what is the role of mitochondria in the synthesis of cytosolic enzyme directly or indirectly. The objective of the present investigation was to see whether cytosolic enzymes do get affected with mitochondrial protein synthesis inhibitor. Chloramphenicol has been reported to inhibit the activities of α-amylase in the seedlings of barley[12] and phosphatase and amylase in pea[13]. The inhibition of induction of nitrate reductase in bean[14] and other plants[15] has also been reported. The activity of nitrate reductase in cucumber cotyle-

dons, however is not affected by chloramphenicol[16] while in rice[17,18] and maize seedlings[19] it is induced by the antibiotic. Dixit *et al.*[20] reported the inhibition of induction of nitrate reductase by chloramphenicol in maize leaves. Such conflicting results exist in the literature. The present study therefore suggests the need for investigating and understanding the effect of chloramphenicol directly or indirectly on eukaryotic protein synthesis inhibition.

TABLE 3

Effect of chloramphenicol on cytosolic isocitrate dehydrogenase malate dehydrogenase and isocitrate lyase activities from Aspergillus niger

Conditions of the growth	Isocitrate dehydrogenase*	Malate dehydrogenase*	Isocitrate lyase*
Control	13.9	556.2	34.8
With chloramphenicol (2 mg/ml)	6.6	328.4	18.4
With chloramphenicol (4 mg/ml)	5.6	206.7	18.5

*(units/mg protein)

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REGULATION OF NITROGENASE EXPRESSION IN *RHIZOBIUM*

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INTRODUCTION

THE catalytic reduction of atmospheric dinitrogen to ammonia by nitrogenase and its subsidiary enzyme systems (nitrogen fixation), is of immense agricultural importance. Research on biological nitrogen fixation was pioneered by intensive studies of legume-*Rhizobium* symbioses, since legumes contribute more to the nitrogen economy of the world than any other system. Reductionist efforts to characterise these symbioses have been made with excised root cultures[1,2], detached leaves and pods[3], nodules[4], nodule protoplasts[5]; and isolated bacteroids[6]. Although potentially valuable, the application of these isolated experimental systems is limited because of the enormous complexities involved in the symbiosis. Development of an effective symbiosis comprises a multi-step cascade of events, namely root colonisation, root hair adhesion, infection, nodule initiation and development, bacteroid development and ultimately nitrogen fixation[7]. This requires a tightly co-ordinated reciprocal communication between plant and bacterium[7,8] as shown in figure 1. The range of non-fixing *Rhizobium* mutants isolated[9]