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Carbon starvation mediated changes in carbohydrate metabolism in *Neurospora crassa*

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Abstract. Carbon starvation conditions were found to increase the activities of gluconeogenic enzymes such as malic enzyme, cytosolic malate dehydrogenase and isocitrate lyase along with proteases and inhibition in glucose catabolic enzymes such as G6P dehydrogenase and FDP aldolase in *Neurospora crassa.*

Keywords. *Neurospora crassa;* carbohydrate metabolism; starvation.

Introduction

A number of studies on starvation are concerned with situations in which traces of nutrients are available transiently (Novitsky and Morita, 1977; Jones and Rhodes-Roberts, 1981). Responses to such conditions of starvation include the development of specific systems for chemotaxis, to obtain scarcely available nutrients (Geesey and Morita, 1979), miniaturization of cells (Casida, 1977) and upkeep of readily available machinery for macromolecular synthesis (Koch, 1971). Starvation mediated changes have also been implicated as a decrease in the rate of protein synthesis (Calzone *et al.,* 1983) and accumulation of reserve materials (Matin *et al.,* 1979). The present study deals with the effect of starvation on changes in carbohydrate metabolism in *Neurospora crassa.*

Materials and methods

The carotenogenic strain of *N. crassa* (wild type) was obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, and was maintained on Saboraud's agar slants. The composition of the synthetic medium employed was the same as that described earlier (Acharya and Chhatpar, 1981). The culture was grown in 50 ml liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (180 rpm) at 30°C for 96 h. The mycelia were harvested by filtration and stored at — 5°C before use. For starvation experiments, *N. crassa* was grown for 72 h, the mats were removed aseptically, washed with sterile distilled water and transferred to

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either complete medium (normal condition) or to a medium lacking carbon sources (carbon starvation condition) and incubated for 24 h. Resupplementation was done by transferring the mats again to normal medium under aseptic conditions. The incubation was continued for 24h. Filter-sterilized cycloheximide was added at 0·5 μg/ml.

For enzyme assays, a cell-free extract was prepared in 0·05 M Tris-HCl buffer, pH 7·2 by grinding the frozen mycelia mixed with glass powder in a chilled mortar. The extract was centrifuged at 15,000 *g* for 30 min at 4°C. The supernatant solution was used for cytosolic enzyme assays.

The assay methods used for FDP aldolase (fructose-1,6-diphosphate D-glyceraldehyde 3-phosphate-lyase, EC 4.1.2.13), malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) glucose-6-P dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49), isocitrate lyase (threo-D-isocitrate glyoxylate lyase, EC 4.1.3.1) and malic enzyme (L-malate: NADP oxidoreductase (decarboxylating) EC 1.1.1.40) were the same as described by Jagannathan *et al.* (1956), Ochoa (1955a) Kornberg and Horecker (1955), Dixon and Kornberg (1959) and Ochoa (1955b), respectively. Protease activity was assayed by the method of Ong and Gaucher (1973). The substrate used for neutral protease was 0·5% casein in 0·1 M Tris-HCl buffer (pH 7·2) and for alkaline protease, 0·5 % casein in 0·1 M Tris-HCl buffer (pH 8·6). Protein and keto acids were determined according to the methods of Lowry *et al.* (1951) and Friedemann (1957), respectively. Lipids were extracted by the method of Folch *et al.*(1957) and estimated according to the method of Bragdon (1951). Polyacrylamide gel electrophoresis was carried out according to the procedure of Davis (1964). Activity staining of glucose-6-P dehydrogenase and malate dehydrogenase was carried out by the method of Corman *et al.* (1967).

Results and discussion

N. crassa when grown in a normal medium for 72 h in shake culture and transferred to starvation conditions for 24 h, showed a loss of F D P aldolase and G6 P dehydrogenase activities (table 1). Activity staining on polyacrylamide gels for G6P dehydrogenase showed 3 prominent bands under normal conditions while 3 very faint bands with identical electrophoretic mobilities were detectable under conditions of carbon

Table 1. Effect of carbon starvation on the activities of FDP aldolase, G6P dehydrogenase, malic enzyme and the levels of keto acids and lipids in *N. crassa*.

Conditions of growth	FDP aldolase U/mg protein	G6P dehydrogenase U/mg protein	malic enzyme U/mg protein	Keto acids mg/mg protein	Lipids g/100 g dry wt.
Normal	119	136	255	0·06	3·01
Carbon starvation	ND	ND	962	0·02	1·7

ND, Not detectable; U, units. Units for FDP aldolase and G6P dehydrogenase are described as the amount of enzyme which brings about a change of 0·001 absorbance at 240 and 340 nm/min respectively at 30°C.

starvation (figure 1). In order to examine whether there was any possibility of an increase in the levels of these enzymes, if starved cultures are resupplemented with carbon source, starved cultures were transferred to normal medium. Under these conditions, a significant increase in the activity of FDP aldolase was observed suggesting the possibility of *de novo* synthesis of this enzyme in response to starvation-refed conditions (table 2). Starvation has been found to reduce the activities of a number of enzymes in rat liver (Szepesi *et al.,* 1975). Szepesi and Berdanier (1971) have shown a decrease in the activities of G6P dehydrogenase and malic enzyme by starvation in rats. When starved animals were refed with a diet rich in carbohydrates, activities of liver G6P dehydrogenase and malic enzyme rose significantly.

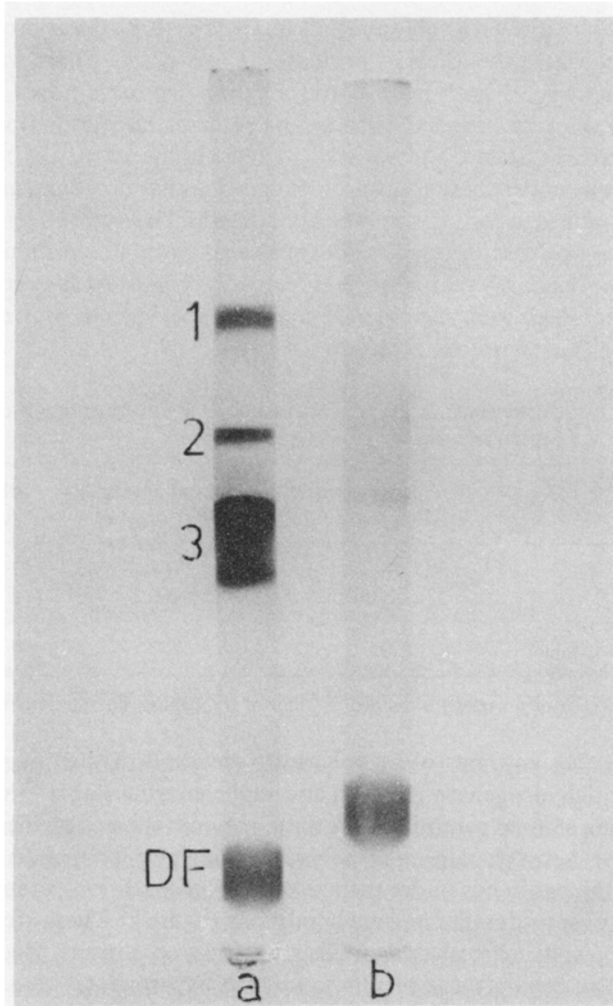

Figure 1. Activity staining for G6P dehydrogenase after Polyacrylamide gel electrophoresis. (a), Normal condition; (b), carbon starvation condition. DF indicates Dye Front.

Table 2. Effect of carbon starvation and refeeding on the activities of isocitrate lyase, malate dehydrogenase and FDP aldolase in *N. crassa.*

Conditions of growth	Isocitrate lyase U/mg protein	Malate dehydrogenase U/mg protein	FDP aldolase U/mg protein
Normal	104	20	119
Carbon starvation	407	283	ND
Refeeding of carbon starved cells	98	164	262

ND, Not detectable. Units for isocitrate lyase and malate dehydrogenase are described as the amount of enzyme which brings about a change of 0·001 absorbance at 324 and 340 nm respectively per min at 30°C.

Since proteases are known to play a significant role in turnover of proteins, attempts were made to determine the levels of proteases in starvation. There was a significant increase in the activity of both neutral and alkaline proteases (table 3). In order to xamine whether there was *de novo* synthesis of proteases, starvation was carried out in the presence of cycloheximide. There was no increase in the activity of neutral protease or alkaline protease under these conditions suggesting that proteases might have been synthesized in response to carbon starvation conditions. Drucker (1972) has also shown the production of protease in response to carbon starvation conditions. Hanson and Marzluf (1975) have shown that when *Neurospora* is grown in starvation medium in which an endogenous protein serves as the major or sole carbon source, it synthesizes and secretes a protease into the medium.

Table 3. Effect of carbon starvation in the presence and absence of cycloheximide on the activities of G6P dehydrogenase and neutral and alkaline proteases in *N. crassa.*

Conditions of growth	G6P dehydrogenase (U/mg protein)	Neutral protease (U/mg protein)	Alkaline protease (U/mg protein)
Normal	55	0·1	0·2
Carbon starvation	ND	0·3	0·5
Carbon starvation in the presence of cycloheximide	25	ND	0·2

ND, Not detectable. Unit of proteases is defined as μmol of tryptophan liberated per hour at 37°C.

In *N. crassa,* other enzymes of carbohydrate metabolism such as isocitrate lyase, cytosolic malate dehydrogenase (table 2) and malic enzyme (table 1) were studied in normal and carbon starved conditions. All these enzymes showed an increase in activity during starvation. Activity staining of Polyacrylamide gels for malate dehydrogenase showed two prominent bands under carbon starvation conditions as compared to two very faint bands observed under normal conditions (figure 2). These are gluconeogenic enzymes. These results suggest that under starvation conditions, gluconeogenesis is favoured. To substantiate these results, refeeding experiments were carried out to measure isocitrate lyase, malate dehydrogenase and FDP aldolase levels. Isocitrate lyase and malate dehydrogenase showed a considerable decline in activity when starved cultures were resupplemented with normal medium. FDP aldolase on the contrary

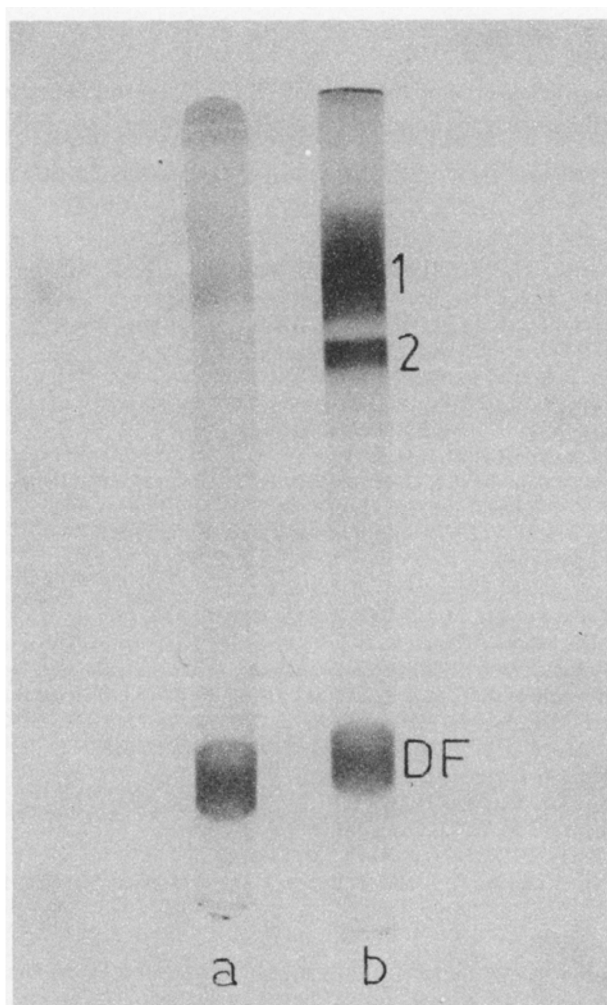

Figure 2. Activity staining for malate dehydrogenase after polyacrylamide gel electrophoresis, (a), Normal condition; (b), carbon starvation conditions. DF indicates Dye Front.

showed an increase in the activity suggesting the need for this enzyme under these circumstances.

Lipids and keto acids were also found to show substantial changes under carbon starvation conditions (table 1). The decrease in the levels of G6P dehydrogenase, an enzyme important for the generation of reducing power may suppress lipogenesis. The lipid content was found to be 42 % less under carbon starvation conditions.

Carbon starvation requires energy conservation and for replenishment small metabolic products are rechanneled in an organised way for better survival conditions. The decrease in catabolic enzymes such as FDP aldolase and G6P dehydrogenase and an increase in anabolic enzymes such as malate dehydrogenase, malic enzyme and isocitrate lyase fulfill these requirements.

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