ENZYMES IN *CANDIDA ALBICANS*[1]

I. Pathways of Glucose Dissimilation

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Among the several yeastlike fungi of the genus *Candida*, only *Candida albicans* is potentially pathogenic to laboratory animals and to humans. The study of the metabolism of this organism has attained importance in view of the observation that treatment with broad spectrum antibiotics would destroy even the normal nonpathogenic bacterial flora of the body and bring in its train the adverse effects of this altered ecological relationship of the microorganisms in the host, chief among which is the overgrowth of *C. albicans*.

A few studies have been carried out, mainly with whole cells, to investigate the detailed metabolism of this fungus. Van Niel and Cohen (1942) showed that *C. albicans* fermented sucrose more slowly than glucose in anoxia. Thjotta and Thorheim (1955) dealt with the fermentation of various carbohydrates such as lactose, maltose, glucose, sucrose, and galactose by various strains of different species of *Candida*. Ramachandran and Walker (1957) showed the growth of *Candida* species in a simple medium containing glucose as carbon source. A detailed study of the respiratory metabolism of normal and divisionless strains of *C. albicans* was conducted by Ward and Nickerson (1958). Respiration studies with *Candida stellatoidea* with various substrates, which included sugars, amino acids, fatty acids, and intermediates of the citric acid cycle, were conducted by Bradley (1958). In view of the fact that diabetic patients, in whose systems the carbohydrate metabolism has been altered, are more prone than normal persons to infection by *C. albicans*, it was decided to investigate the pathways of glucose dissimilation in this organism. This paper presents the results of these investigations employing both whole cells and cell-free extracts of *C. albicans*.

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Abbreviations employed: G-6-P, glucose 6-phosphate (B. D. H.); F-6-P, fructose 6-phosphate; F-1,6-P, fructose 1,6-diphosphate; R-5-P, ribose 5-phosphate; ATP, adenosine triphosphate (Nutritional Biochemical Corporation); 6-PG, 6 phosphogluconate (Schwartz); TPN, triphosphopyridine nucleotide (Sigma Chemical Company); DPN, diphosphopyridine nucleotide (Pabst Laboratories); G-3-P, glyceraldehyde 3-phosphate.

MATERIALS AND METHODS

Test organism. C. albicans strain Z248, obtained from the London School of Hygiene and Tropical Medicine, has been used. The pathogenicity of this organism was established by infecting mice and noting the mortality and typical lesions in the kidneys. Stock culture was maintained on slants of Sabouraud's glucose agar at room temperature.

Medium employed. The medium used for growing *C. albicans* was essentially that of Bradley (1958) with the following composition: glucose, 20 g; yeast extract, 2.0 g; potassium nitrate, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.50 g; calcium carbonate, 0.25 g; deionized water to 1.0 liter. The medium was adjusted to pH 6.5 before sterilization.

Preparation of cells for manometric studies. The organism was grown in the above medium by incubating at 37 C for 20 hr. Cells were harvested by centrifugation and washed three times with ice cold 0.03 M phosphate buffer, pH 7. Finally the washed cells were suspended in the same buffer and used in Warburg experiments.

Preparation of cell-free extracts. The harvested cells were pressed between filter paper folds and mixed with twice their weight of Alumina 301 (325) and ground in a precooled mortar for 10 to 15 min. The enzymes were extracted with 0.03 M phosphate buffer, pH 7.0, and centrifuged at 13,780 $\times$ *g* for 30 min in a Servall centrifuge. The cell-free supernatant, dialyzed against 0.003 M phosphate buffer, pH 7, at 2 to 5 C for 10 hr, was used in the present study.

TABLE 1

*Utilization of intermediate of glycolytic and hexose-monophosphate shunt pathways by Candida albicans**

Substrate†	Oxygen Uptake‡ at Time, in Min:		
	60	120	180
Glucose	18	32	40
Glucose 6-phosphate	2	3	3
Fructose 6-phosphate	4	6	6
Fructose 1,6-diphosphate	1	2	2
Ribose 5-phosphate	13	20	22
6-Phosphogluconate	15	24	26

* Each flask contained 6.7 mg of whole cells (dry weight).

† Each flask contained 5 μmoles of the substrate.

‡ In μl per mg whole cells, dry weight, corrected for endogenous activity, at 30 C.

Protein determination. The protein content of the cell-free extract was determined according to the Biuret method (Gornell, Burdawill, and David, 1949).

Determination of enzyme activities. The photometric method of Wajzer (1949) was used for the detection of hexokinase. Slein, Cori, and Cori's (1950) spectrophotometric determination of the reaction product of the hexokinase reaction, hexose 6-phosphate, was also used for testing hexokinase activity of the cell-free extract. The resorcinol method of Roe (1934) as used by Slein (1955) was employed to detect the presence of phosphoglucose isomerase. Aldolase activity was measured by the colorimetric method of Sibley and Lehninger (1949). The presence of phosphohexokinase was demonstrated by the method of Kuo-Huang Ling, Birne, and Lardy (1955). The method of DeMoss (1955) was used for testing the glucose 6-phosphate and 6-phosphogluconic dehydrogenase activity of the cell-free extract. Pentose phosphate isomerase occurrence was demonstrated according to the method of Dische and Borenfreund (1951) as modified by Axelrod and Jang (1954). The formation of sedoheptulose, the product of transketolase activity, was shown by the method of Dische, Shettles, and Osnos (1949) as modified by Axelrod et al. (1953). Lactic, glycerol, and α-glycerophosphate dehydrogenases were measured by determining the reduction of either

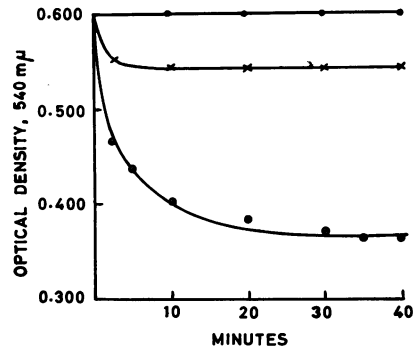

Figure 1. Hexokinase activity. Additions: glucose (0.025 M), 0.2 ml; ATP (0.02 M), 0.7 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.2 ml; phenol red (0.1 per cent), 0.4 ml; cell-free extract (0.32 mg protein), 0.2 ml; H_2O to 3.0 ml. ●——●, Enzyme omitted; ×——×, glucose omitted; ⊙——⊙, complete system.

di- or triphosphopyridine nucleotide in the presence of the appropriate substrate. Increase in optical density due to the reduction of DPN or TPN was measured at 340 mμ with Beckman model DU spectrophotometer using 3-ml quartz cuvettes; light path, 1 cm.

Measurement of oxidation. Conventional Warburg (Umbreit et al., 1957) technique was used to measure the utilization of various substrates. Final volume in each flask was 3.0 ml; gas phase, air; temperature, 30 C. The central well contained 0.2 ml of 20 per cent potassium hydroxide.

RESULTS

Studies with whole cells: glucose, 6-PG, and R-5-P were very well utilized by the organism, and G-6-P, F-6-P, and F-1,6-P were not utilized to any significant extent (table 1).

Studies for the hexokinase activity of the cell-free extract showed a marked activity as tested by both the methods (figures 1 and 2).

The cell-free extract showed phosphoglucose isomerase activity. Under the test conditions 0.71 μmole of F-6-P:min:mg of protein was formed.

The presence of phosphofructokinase was shown by the reduction of TPN in presence of F-6-P (figure 3, curve *A*). The activity was observed only with TPN and not with DPN, and since in this reaction TPN oxidizes glyceraldehyde 3-phosphate formed during incubation, the G-3-P dehydrogenase is TPN-specific. From figure 3, curves *B* and *C*, it appears that there

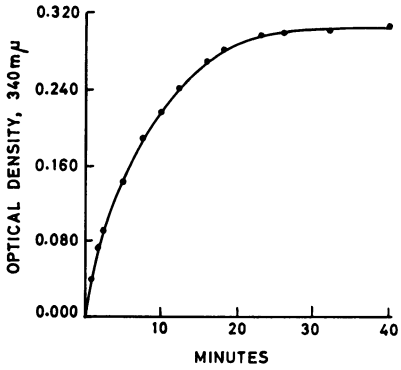

Figure 2. Hexokinase activity. Additions: glucose (0.5 M), 0.2 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.15 ml; ATP (0.02 M), 0.5 ml; tris(hydroxymethyl)-aminomethane buffer (0.05 M), pH 7.9, 0.8 ml; TPN (1.0 mg per ml), 0.2 ml; cell-free extract (0.08 mg protein) 0.5 ml. Volume adjusted to 3.0 ml with water.

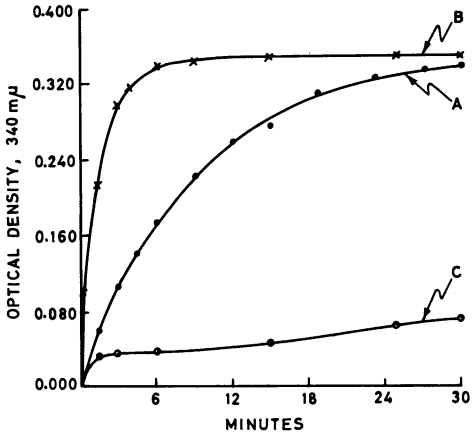

Figure 3. 6-Phosphofructokinase activity. Additions: fructose 6-phosphate (0.025 M), 0.2 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.1 ml; cysteine HCl (0.2 M), 0.2 ml; sodium arsenate (0.4 M), 0.2 ml; ATP (0.02 M), 0.3 ml; tris(hydroxymethyl)aminomethane buffer (0.05 M) pH 7.9, 0.9 ml; cell-free extract (0.08 mg protein), 0.5 ml; TPN (1.0 mg per ml), 0.2 ml. Volume adjusted to 3.0 ml with water. Lactic dehydrogenase activity with TPN (*B*) and DPN (*C*) as coenzymes. Additions: sodium lactate (0.5 M), 0.15 ml; phosphate buffer (0.2 M) pH 7, 1.0 ml; cell-free extract (0.27 mg protein), 0.2 ml; TPN or DPN (1.0 mg per ml), 0.2 ml. Volume adjusted to 3.0 ml with water.

are two distinct enzymes which function in dehydrogenation of lactate. The one which requires TPN is highly active compared to the other which needs DPN.

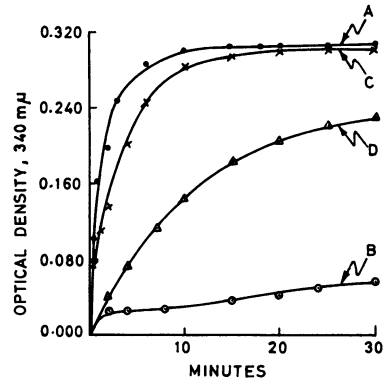

Figure 4. Glycerol (*A*, *B*, *C*) and α-glycerophosphate (*D*) dehydrogenases activity. Additions: glycerol or α-glycerophosphate (0.5 M), 0.5 ml; phosphate buffer 0.2 M) pH 7, 1.0 ml; cell-free extract (0.27 mg protein) 0.2 ml; TPN or DPN (1.0 mg per ml) 0.2 ml. Volume adjusted to 3.0 ml with water. *A*. TPN; *B*. DPN; *C*. TPN with added ATP (0.02 M), 0.3 ml and $MgSO_4 \cdot 7H_2O$, (0.1 M), 0.15 ml; *D*. TPN.

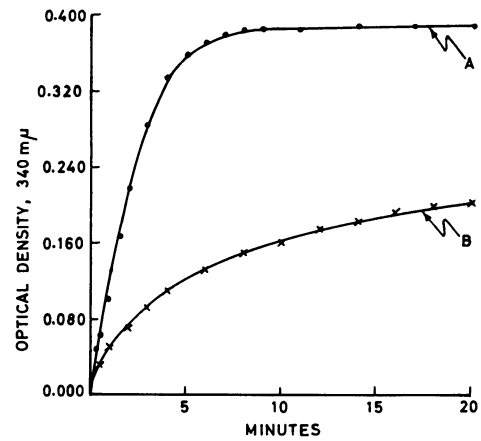

Figure 5. Glucose 6-phosphate (*A*) and 6-phosphogluconic (*B*) dehydrogenase activity. Additions: glucose 6-phosphate or 6-phosphogluconate (0.025 M), 0.4 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.2 ml; tris(hydroxymethyl)aminomethane buffer (0.05 M) pH 7.9, 0.9 ml; cell-free extract (0.08 mg protein), 0.5 ml; TPN (1.0 mg per ml), 0.2 ml. Volume adjusted to 3.0 ml with water.

The cell-free extract had an aldolase activity of 16 units when tested by the colorimetric method of Sibley and Lehninger (1949). One unit is defined as that which produces an absorption of 0.100:min:mg of protein under the assay conditions.

Glycerol and α-glycerophosphate dehydroge-

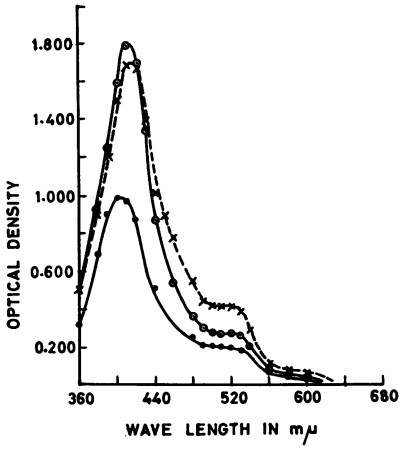

Figure 6. Absorption curves for the products of ribose 5-phosphate metabolism by the cell-free extract of *Candida albicans*. Additions: ribose 5-phosphate (0.025 M), 0.3 ml; tris(hydroxymethyl)-aminomethane buffer (0.1 M) pH 7, 0.2 ml; thiamine HCl (0.15 M) pH 7.5, 0.05 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.05 ml; cell-free extract (0.408 mg protein), 0.3 ml; water to 1.0 ml. Incubation at 37 C for: ●——●, 0 min; ×——×, 30 min; ⊙——⊙, 180 min.

nases activity of the cell-free extract was measured by noting the DPN or TPN reduction (figure 4). The reduction of TPN was very rapid compared to that of DPN when glycerol was the hydrogen donor. The reduction of TPN was comparatively slow when α-glycerophosphate was the substrate. Further, this α-glycerophosphate dehydrogenase is TPN-specific. Supplementing the glycerol with ATP and Mg^{++} has not improved the rate of reduction of TPN.

G-6-P and 6-PG dehydrogenases were demonstrated by the reduction of TPN (figure 5). DPN was ineffective. Calculated from the initial rates of reduction of TPN, the activity of G-6-P dehydrogenase is three times that of 6-PG dehydrogenase.

Pentose phosphate isomerase activity was detected by the formation of ribulose-5-phosphate from ribose 5-phosphate and estimating the keto sugar by the cysteine-carbazole reaction. The cell-free extract had an activity of 0.90 units; one unit is defined as that which produces an absorption of 0.100:min:mg of protein under the test conditions.

The method of testing transketolase activity is based on the increase in absorption corresponding to heptulose at 520 mμ. The absorption peaks observed at 415 and 520 mμ correspond to hexose phosphate and sedoheptulose respectively (figure 6), thus indicating the presence of ribose 5-phosphate isomerase, transketolase, and transaldolase. It is clear from the graph that prolonged incubation lowered the heptulose content which might be due to the conversion of the latter into hexose phosphate in presence of transaldolase.

DISCUSSION

The foregoing results show that *C. albicans* possesses the enzymes of both the glycolytic and hexose monophosphate shunt pathways, although the study is not exhaustive enough to prove unequivocally that these are the only two pathways of glucose dissimilation in the organism. The failure of the whole cells to oxidize the intermediates of the Embden-Meyerhof pathway is due to the nonpermeability of the cell wall, as was proved by the ready oxidation of these substrates by the cell-free extract of the organism.

It is interesting to note that most of the dehydrogenases in this organism are TPN-specific. They include G-6-P, 6-PG, G-3-P, and α-glycerophosphate dehydrogenases. Even in the case of certain enzymes like lactic dehydrogenase and glycerol dehydrogenase for which in other biological systems DPN is the preferred cofactor, the rate of the reaction with DPN is markedly lower than with TPN. This almost absolute requirement of the enzymes of the organism for TPN may be associated with the highly aerobic nature of the organism.

The equilibrium of both lactic dehydrogenase and α-glycerophosphate dehydrogenase has so far been reported to be very much toward the formation of the hydroxy compound so that a substance like cyanide, which binds the keto group, has to be used to pull the reaction in the opposite direction, or the reaction has to be carried at high pH values. In the case of *C. albicans*, however, both the hydroxy acids are oxidized in the presence of TPN even at neutral pH. This seems to be particularly true of lactic dehydrogenase. α-Glycerophosphate dehydrogenase is less active than glycerol dehydrogenase, presumably because of the presence of the active TPN-linked glycerol dehydrogenase. In the presence of TPN, rates of oxidation of glycerol with and without ATP are the same, either because the glycerol kinase is lacking in the organism or because of the stronger competition for

the TPN by glycerol dehydrogenase. It is also possible that the same enzyme is able to act on both glycerol and α-glycerophosphate and that the difference in the rates of metabolism of the substrates is due to the difference in the affinity of the enzyme for these substrates. Experiments are under way to isolate and purify the enzymes which catalyze these reactions and to study their kinetics. It is also proposed to obtain the actual equilibrium constants in these reactions by carrying out the reactions in the opposite direction using reduced TPN. The observed equilibrium for both the lactic and α-glycerophosphate dehydrogenases toward the formation of the oxidized substrates may also be due to the highly aerobic nature of *C. albicans*.

SUMMARY

Candida albicans possesses the enzymes of both Embden-Meyerhof and hexose monophosphate shunt pathways. Most of the enzymes were found to be highly specific for triphosphopyridine nucleotide. High activity of the glycerol and lactic dehydrogenases with equilibrium favorable to the formation of keto compounds has been observed.

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