JOURNAL OF BACTERIOLOGY, Nov. 1982, p. 932–934
0021-9193/82/110932-03$02.00/0

Vol. 152, No. 2

UDP Glucuronate Decarboxylase and Synthesis of Capsular Polysaccharide in *Cryptococcus neoformans*

ERIC S. JACOBSON* AND WILLIAM R. PAYNE

Research and Medical Services (151), McGuire Veterans Administration Medical Center, Richmond, Virginia 23249, and Division of Infectious Diseases, Department of Medicine, Medical College of Virginia-Virginia Commonwealth University, Richmond, Virginia 23298

Received 17 May 1982/Accepted 19 July 1982

UDP glucuronate decarboxylase activity was comparable in encapsulated and non-encapsulated strains of *Cryptococcus neoformans*, required NAD (K_a = 0.2 mM), and was inhibited by NADH (K_i = 0.1 mM) and UDP xylose.

Cryptococcus neoformans is the etiological agent of a life-threatening meningitis. The best-understood virulence factor of this organism is its extracellular polysaccharide capsule, which inhibits phagocytosis (5, 6, 11, 12). The structure of an acidic extracellular polysaccharide of a nonpathogenic cryptococcus, *C. laurentii*, is known; this consists of a mannose-containing backbone with xylosyl and glucuronyl residues as side groups (1). Two enzymes presumably involved in the biosynthesis of the glucuronyl and xylosyl moieties have been studied (2, 3); these were UDP glucose dehydrogenase, which catalyzes the conversion of UDP glucose to UDP glucuronic acid, and UDP glucuronic acid decarboxylase (formerly termed carboxy-lyase), which catalyzes the irreversible conversion of UDP glucuronic acid to UDP xylose.

A sexual cycle has been discovered in *C. neoformans* (13), and we and others have demonstrated that auxotrophic (7, 16) and acapsular (9) mutants can be readily obtained and studied genetically. We have recently demonstrated that these acapsular mutants have decreased virulence for mice (7a). The major component of the capsule in the pathogen consists of an α(1→3)-linked mannan with side groups of xylose and glucuronic acid (4, 7). This component is missing in our acapsular mutants (9). Thus, the acapsular mutants represent potential tools for studying the biosynthesis of the major capsular polysaccharide. In the present study we describe the properties of the UDP glucuronic acid decarboxylase (EC 4.1.1.35) of *C. neoformans* in two wild-type, encapsulated strains and in various capsule mutants.

The wild-type, encapsulated strains used were B-3501 and B-3502 from the National Institutes of Health, Bethesda, Md., and ATCC 7472 from the American Type Culture Collection, Rockville, Md. All were of the D serotype. Capsule mutants were obtained by treatment of one or another of the wild-type strains with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or, in one case, with UV light (9). Stocks were maintained on solid brain heart infusion medium (BBL Microbiology Systems, Cockeysville, Md.). UDP [*U*-^{14}C] glucuronic acid was obtained from New England Nuclear Corp., Boston, Mass. Other reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

Extracts of the wild-type, encapsulated strains B-3501 and B-3502 contained maximum specific activities in the range of 6 to 12 nmol/min per mg of protein (Table 1). The enzyme was present in highest activity during the early-to-middle logarithmic phase of growth (optical density < 0.6). The enzyme remained in the supernatant fraction of disrupted cells after centrifugation at 105,000 × *g* for 1 h. There was a narrow pH optimum at pH 7.5. The decarboxylation reaction was linear with respect to time and enzyme concentration throughout the range of time and protein concentrations used. The maximum extent of reaction in kinetic studies was 10%. Activity depended upon exogenous NAD, with a K_a of 0.16 mM NAD. The substrate saturation curve for UDP glucuronic acid was essentially hyperbolic in form, with a K_m of 1.0 ± 0.3 mM UDP glucuronic acid. UDP glucuronic acid decarboxylase was inhibited competitively by the product, UDP xylose, with a K_i of 2.0 ± 0.5 mM UDP xylose (data not shown). We studied the interaction of UDP xylose and NAD and found that UDP xylose did not affect the binding of NAD. The enzyme was inhibited competitively by NADH (K_i = 0.1 mM NADH) and by UDP glucose (K_i = 5.0 ± 1.0 mM UDP glucose). UDP glucuronic acid decarboxylase was inactivated when incubated at 37°C in the absence of substrates or substrate analogs. However, it was stabilized by UDP glucose (Fig. 1). We screened 30 independently derived capsule mutants for genetic defects in the UDP

TABLE 1. Properties of UDP glucuronic acid decarboxylase from various strains of *C. neoformans*

Strain[a]	Sp act[b] (nmol/mg of protein per min)	% Inhibition by:	
		4 mM UDP xylose	1 mM NADH
B-3501	11.9	44	73
B-3502	6.0	63	58
602	8.6	54	84
Cap64	14.3	70	76
Cap55	6.9	43	78
Cap59	6.4	50	73
Cap66	9.3	76	85
Cap67	3.6	52	76
Cap70	12.9	72	84
Cap52	5.1	70	70
Cap53	4.1	43	79

[a] B-3501 and B-3502 are normally encapsulated, wild-type background strains; all others are capsule mutants.

[b] Each strain was grown in Fries salts (8) containing glucose (2%, wt/vol) and thiamine (2 μg/ml) at 37°C with agitation, harvested by centrifugation when the optical density of the culture was between 0.2 and 0.7 at 700 nm, mixed with 10 times its wet weight of glass beads (diameter, 0.45 to 0.55 mm) and its weight of sodium phosphate buffer (0.2 M, pH 7.5), and homogenized in an MSK homogenizer (B. Braun Instruments, South San Francisco, Calif.) until phase-contrast microscopy indicated disruption of a majority of cells. During the later phase of our study, we routinely added 10 mM UDP glucose to the buffer to stabilize the enzyme. The glass beads were removed by filtration through cotton gauze, and cell walls and nuclei were removed by centrifugation at 27,000 × *g* for 20 min. All operations were performed at 0 to 4°C. Protein was measured by the method of Lowry et al. (14). The UDP glucuronic acid decarboxylase was assayed by trapping radioactive CO_2 cleaved from ^{14}C-labeled UDP glucuronic acid by the enzyme (10). The reaction was stopped by the addition of 0.2 ml of 2 N HCl. CO_2 was quantitatively trapped by a further 2-h incubation at 37°C and was then measured by liquid scintillation spectrophotometry. Enzyme activity was expressed as nanomoles of CO_2 per milligram of protein per minute. Putative UDP xylose (^{14}C labeled), the other product, cochromatographed with authentic UDP xylose on thin layers of polyethyleneimine-cellulose (J. T. Baker Chemical Co., Phillipsburg, N.J.) developed with 0.25 M LiCl. The radioactive spot corresponding to UDP xylose was eluted with 1 N HCl and hydrolyzed at 100°C for 60 min. The HCl was removed by evaporation, and the radioactivity was then found to cochromatograph with authentic xylose in phenol-water (15) and ethyl acetate-pyridine-water (10) systems.

glucuronic acid decarboxylase by measuring specific activity, inhibition by UDP xylose, and inhibition by NADH. Representative results are shown in Table 1.

Since UDP glucuronic acid decarboxylase converts one polysaccharide precursor irreversibly to another, a hypothetically defective enzyme might have either decreased activity or decreased susceptibility to inhibition. In the latter case one might suppose that the cellular UDP glucuronic acid pool would be depleted by uncontrolled decarboxylation, resulting in a failure to produce capsular polysaccharide. On the basis of this reasoning, we screened capsule mutants for both decreased specific activity and altered sensitivity to inhibitors. All mutants exhibited enzyme activity which is both qualitatively and quantitatively indistinguishable from that of the wild type. Since the K_i for UDP xylose is only twofold higher than the K_m, it is possible that product inhibition achieves control of the activity of the enzyme in vivo. On the other hand, it is also likely that intracellular NAD-to-NADH ratios regulate the activity of this enzyme, since the K_i for NADH was slightly lower than the K_a for NAD, and both values are

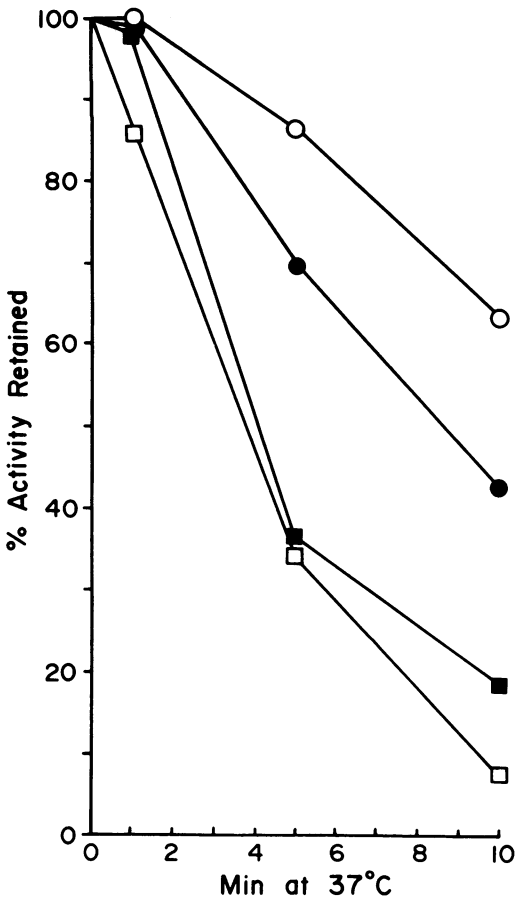

FIG. 1. Lability of enzyme and stabilization by UDP glucose. UDP glucose present during extraction of enzyme was diluted to 1 mM with buffer before the inactivation study. UDP glucose concentration during the inactivation step: □, 1 mM; ■, 2 mM; ●, 6 mM; ○, 11 mM.

in the physiological range for intracellular NAD and NADH concentrations.

This work was supported by the U.S. Veterans Administration and by Public Health Service grant AI-15752 from the National Institutes of Health.

We are grateful to John E. Bennett for serotyping strain 7472 for us and to Judith S. Bond for critically reading the manuscript.

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