Utilization of Indole Compounds by *Cryptococcus neoformans* to Produce a Melanin-Like Pigment

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Several indoles served as substrates for the phenoloxidase of *Cryptococcus neoformans* and resulted in the production of a melanin-like pigment. In general, a higher percentage of *C. neoformans* var. *neoformans* (A and D serotypes) isolates could produce pigment from indoles than could those of var. *gattii* (B and C serotypes). Only compounds with a hydroxyl or an amino group on the phenyl ring produced pigment; methoxy, nitro, methyl, and fluorine substituents on the phenyl ring were inactive, as was a hydroxyl group at the 2 position on the indole ring. The phenoloxidase of *C. neoformans* thus appears to differ from that found in *Mycobacterium leprae*, which cannot use a hydroxyindole, desoxyfructo-5-hydroxytryptamine, as a substrate. In addition, *C. neoformans* differs from *M. leprae* in that desoxyfructo-5-hydroxytryptamine does not inhibit the uptake of dihydroxyphenylalanine into the cell.

The metabolism of dihydroxyphenylalanine (DOPA) and its derivatives appears to be important for both *Mycobacterium leprae* and *Cryptococcus neoformans*, human pathogens which are neurotropic. A small amount of DOPA appears to be essential for the survival and proliferation of *M. leprae* (12). Although DOPA is not required for the survival or growth of *C. neoformans*, the isolates that utilize DOPA to produce melanin appear to be more virulent for mice than those which do not (1, 4, 14). In both organisms, the phenoloxidases which oxidize DOPA have been reported to have similar characteristics. They are both membrane associated and utilize the same range of substrates to form a melanin-like pigment (5, 6, 9, 12). Nevertheless, the enzyme of *C. neoformans* is distinct from that of *M. leprae* in at least two respects (9). The phenoloxidase of *M. leprae* oxidizes DOPA to 5,6-dihydroxyquinone (11), whereas DOPA in *C. neoformans* follows a pathway that leads to dopachrome (5, 6, 9). The phenoloxidase of *C. neoformans*, unlike that of *M. leprae* (10), is completely inhibited by mimosine and cyanide (9).

Recent work indicates that the 5-hydroxytryptamine derivative, desoxyfructo-5-hydroxytryptamine (8), inhibits the uptake of L-DOPA and the multiplication of *M. leprae* in the mouse footpad (2, 7). Since both *M. leprae* and *C. neoformans* appear to utilize DOPA, we tested the effect of desoxyfructo-5-hydroxytryptamine on the uptake of DOPA by *C. neoformans*. We

have found that not only does this indolic compound fail to inhibit DOPA uptake, but it is also an effective substrate for melanin-like pigment production. Since a variety of other indoles can produce pigment formation as well, our data suggest that previous concepts regarding the substrate specificity of *C. neoformans* phenoloxidase (1, 9, 13) may need revision.

Twenty isolates each of *C. neoformans* var. *neoformans* (A and D serotypes) and of *C. neoformans* var. *gatti* (B and C serotypes) (3) were used for the study. The indole compounds tested were obtained from the Sigma Chemical Co. (St. Louis, Mo.), the Regis Chemical Co. (Chicago, Ill.), the Aldrich Chemical Co. (Milwaukee, Wis.), or through the courtesy of either A. Manian, Neurosciences Research Branch (National Institute of Mental Health, Rockville, Md.), or L. Mester (Centre Nationale de Recherche Scientifique, Gif-sur-Yvette, France). A loopful of cells from each isolate grown on malt extract agar for 48 h was streaked (a simple streak at the center of the plate) on indole media and incubated for 1 week at 30°C. The indole media were the same as the diphenol agar media (9), except that 0.5 mM indoles were substituted for the diphenols. The indole compounds (0.5 mM) were dissolved in 1 ml of ethyl alcohol (95%) before being added to the molten agar (45°C).

Desoxyfructo-5-hydroxytryptamine at a 0.5 mM concentration had no effect on the uptake of radiolabeled DOPA by *C. neoformans* (data not

TABLE 1. Indole compounds tested for the formation of melanin-like pigment by *Cryptococcus neoformans* at 30°C.

Compound	Chemical Structure	<i>C. Neoformans</i> ^a	
		Var. <i>Neoformans</i>	Var. <i>Gattii</i>
2-Hydroxyindole		0	0
4-Hydroxyindole		95	60
5-Hydroxyindole		90	85
5-Hydroxytryptophol		65	20
5-Hydroxyindolacetate		55	20
5-Nitroindole		0	0
5-Methoxyindole		0	0
4-Methoxyindole		0	0
Melatonin		0	0
6-Hydroxymelatonin		75	5
Tryptamine		0	0
4-Hydroxytryptamine		65	50
5-Hydroxytryptamine		100	100
1-Desoxyfructo-5-hydroxytryptamine		100	100
6-Hydroxytryptamine		100	100
4-Methoxytryptamine		0	0
5-Aminotryptamine		100	100
5-Methyltryptamine		0	0
6-Methoxytryptamine		0	0
Tryptophan		0	0
5-Hydroxytryptophan		100	100
5-Fluoro-tryptamine		0	0
6-Fluoro-tryptamine		0	0

^a Percentage of isolates which formed melanin-like pigment.

shown). However, tests with this compound, as well as with a number of indole compounds having at least one hydroxyl or amine group at any position in the phenyl ring, resulted in pigment formation (Table 1). Methoxy, nitro, methyl, or fluorine moieties at similar positions on the phenyl ring did not permit pigment formation, and a hydroxyl group on the indole ring at the 2 position also failed to permit any visible pigment formation. In general, a higher percentage of *C. neoformans* var. *neoformans* isolates used indole compounds to produce pigment than did those of var. *gattii*. For example, 20% or less of *C. neoformans* var. *gattii* isolates produced pigment with 5-hydroxyindole-3-acetate, 5-hydroxytryptophol, and 6-hydroxy-melatonin, whereas 55 to 75% of *C. neoformans* var. *neoformans* isolates did.

Previous work had suggested that cryptococcal phenoloxidase was limited in its substrate specificity to phenols with hydroxyl groups in the 3 and 4 positions, amino phenols, or diaminobenzenes (1, 9, 13). Presumably, the presence of two reactive moieties in these compounds permits oxidation to quinone-like structures, which can subsequently polymerize to doprachrome (5, 6, 9). With indolic compounds, the electron-withdrawing action of the indole nitrogen might be expected to render a single hydroxyl or amino group on the phenyl ring much more susceptible to oxidation, in effect mimicking the action of a second hydroxyl or amino group on a benzene ring. A hydroxyl in the 2 position would be expected to be more resistant to oxidation, since its close proximity to the indole nitrogen produces an oxidation-resistant structure like that of an amide.

The phenoloxidase of *C. neoformans* has not yet been purified, and our data show that the enzyme can act on a remarkably wide range of substrates. Although these phenomena suggest that there may be more than one enzyme, the genetic study by Kwon-Chung et al. (4) on the *Mel* isolate, and the study on the *Mel*⁺ revertant by Rhodes et al. (14), indicate that the phenoloxidase of *C. neoformans* is a single enzyme. The active site of the enzyme therefore appears to be relatively insensitive to the steric or electronic configuration of the molecule. Nevertheless, the reaction as a whole appears to proceed only if

the substrate molecule has the correct electrochemical potential to permit oxidation. Similar types of studies with *M. leprae* should permit determination of whether this phenomenon is restricted to *C. neoformans* or it represents a more general characteristic of this class of enzyme.

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