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FREE AMINO ACIDS IN *PHYCOMYCES BLAKESLEEANUS* DURING DEVELOPMENT

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Abstract—In *Phycomyces blakesleeanus* the total level of free amino acids and their relative distributions change strikingly during the development of sporangiophores. Very high levels of serine, glutamate and histidine are found. The variety of amino acids becomes more restricted in sporangiophores grown on nitrogen-rich media; levels of arginine increase dramatically. The distribution differs in mycelia. No unusual amino acids or oligopeptides were found.

The sporangiophore of *Phycomyces blakesleeanus* has long been studied as a model sensory transduction system [1]. However, with the exception of a preliminary report on the involvement of cyclic nucleotides (2), little molecular biology has been attempted to correlate molecular events with this process. The sporangiophore may also be useful for the study of development since its maturation occurs in well defined stages. Developmental mutants are also available. In order to study these processes at the molecular level, it is important to know the amino acid pools. Furthermore, the possible direct involvement of amino acids in these processes cannot be discounted. Therefore, we have examined the free amino acid pool during development in three commonly used growth media.

Development proceeds in well defined stages; however, no cell fission takes place. Five hr after activation of vegetative spores of *P. blakesleeanus* germinal hyphae develop. The hyphae branch and develop very rapidly, forming no cross-walls or septa. After 60 hr, sporangiophore growth is initiated. The maturation of the sporangiophore is divided into five stages [1]. *Stage I*: The sporangiophore is a simple pointed tube approximately 70 μm in diameter at the base and usually less than one cm tall. The sporangiophore is growing 1–2 mm/hr. *Stage II*: The tip swells and a yellow spherical sporangium forms, eventually reaching about 800 μm in diameter. The sporangiophore is not elongating. *Stage III*: Neither enlargement of the sporangium nor growth occurs. *Stage IVa*: Growth resumes and the sporangium darkens. The elongation is accompanied by a counterclockwise twist (as seen from above). *Stage IVb*: The mature sporangiophore grows at a constant rate, about 3 mm/hr, and the twist has become clockwise. The sporangiophore is several cm tall with a stalk approximately 100 μm in diameter. The sporangium contains approximately 5×10^4 spores. The stalk is nonseptate but multi-nucleate.

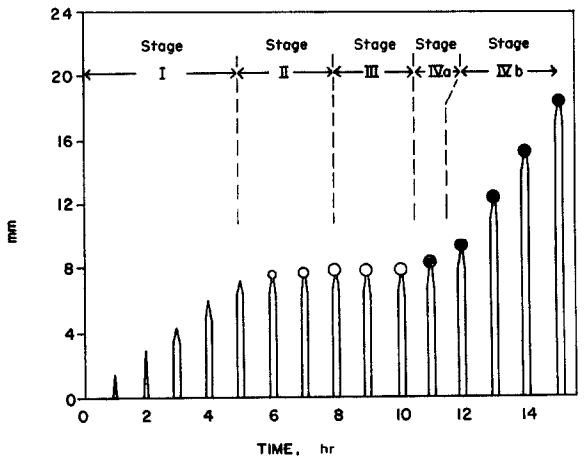

Fig. 1. The development of the *Phycomyces* sporangiophore.

RESULTS

Table 1 indicates the changes in free amino acids as a function of developmental stage for *Phycomyces* growing in PDA + Y. The total amount of amino acids decreases during maturation. The most significant findings however, are the very high concentrations of serine and glutamic acid which constitute about half the available pool. The latter is perhaps to be expected because of the central role of glutamate in transamination. However, the relative amount of glutamate peaks sharply in stages II and III. The relative rise in glutamate coupled with the loss of the basic amino acids in stage II and III results in a very acidic amino acid pool. The relative level of histidine in the mature stage IVb is higher than that reported in any other microorganism. Very little of the aromatic amino acids phenylalanine and tyrosine and virtually no tryptophan are found in any of the samples.

Small amounts of citrulline and β-alanine are evident, but no ornithine, hydroxyproline, oxidized glutathione or γ-aminobutyrate. No ninhydrin positive substances appear in the amino acid analysis other than those in

Table 1. Levels of free amino acids in cytoplasm

Medium	PDA + Y						Commercial instant potatoes	
Developmental Stage	I		II-III		IVb		IVb	
Amino acid	Levels [nmol/ml cytoplasm] and % total free amino acid							
Trp	20	(0·0)	0	(0·0)	0	(0·0)	0	(0·0)
Lys	3900	(4·9)	390	(1·1)	520	(4·0)	480	(4·6)
His	2400	(3·0)	760	(2·0)	1080	(8·3)	1350	(13·9)
Arg	4750	(5·9)	650	(1·7)	460	(3·5)	220	(2·3)
Asp	4310	(5·4)	1880	(5·0)	1530	(11·7)	760	(7·8)
Thr	0	(0·0)	Trace	(Trace)	120	(0·9)	390	(4·0)
Ser	22100	(27·6)	8770	(23·6)	3620	(27·8)	3600	(37·1)
Gln	7600	(9·5)	3540	(9·5)	90	(0·7)	ND	(ND)
Glu	12900	(16·1)	11800	(31·7)	2380	(18·3)	1090	(11·2)
Pro	1920	(2·4)	550	(1·5)	130	(1·0)	Trace	(Trace)
Cit	290	(0·4)	180	(0·5)	130	(1·0)	ND	(ND)
Gly	4020	(5·0)	1800	(4·8)	890	(6·8)	610	(6·3)
Ala	8220	(10·3)	3260	(8·8)	590	(4·5)	560	(5·8)
Val	1540	(1·9)	600	(1·6)	290	(2·2)	160	(1·7)
1/2 Cys	2210	(2·8)	1090	(2·9)	430	(3·3)	100	(1·1)
Met	390	(0·5)	270	(0·7)	30	(0·2)	20	(0·2)
Ile	1020	(1·3)	460	(1·2)	230	(1·8)	90	(0·9)
Leu	1540	(1·9)	720	(1·9)	240	(1·8)	110	(1·1)
Tyr	220	(0·3)	150	(0·4)	120	(0·9)	100	(1·0)
Phe	510	(0·6)	310	(0·8)	130	(1·0)	100	(1·0)
β-Ala	230	(0·3)	50	(0·1)	30	(0·2)	ND	(ND)
Total	80090	(100·1)	37230	(99·8)	13040	(99·9)	9740	(100·0)
NH_3	16000		15400		3380		ND	
Dry weight of extracted material	51 mg/ml		54 mg/ml		22 mg/ml		28 mg/ml	

* ND = Not determined.

the tables and trace amounts of materials which sometimes appear where taurine, urea, glucosamine and 3,4-dihydroxyphenylalanine (L-DOPA) chromatograph.

Free amino acids in stage IVb grown on commercial instant potatoes supplemented with yeast extract were in substantially the same order of abundance except that histidine was increased relative to glutamate and aspartate.

Table 2. Levels of free amino acids in cytoplasm when grown on GA medium

Developmental stage	Mycelium		II-III		IVb	
Amino acid	Levels [nmol/ml cytoplasm] and % of total free amino acid					
Trp	0	(0·0)	Trace	(Trace)	70	(0·1)
Lys	1900	(3·1)	2160	(2·4)	2890	(3·3)
His	510	(0·8)	2990	(3·4)	2560	(3·0)
Arg	25400	(41·8)	18800	(21·1)	23400	(27·1)
Asp	3120	(5·1)	2620	(2·9)	3190	(3·7)
Thr	0	(0·0)	0	(0·0)	0	(0·0)
Ser	7380	(12·10)	28300	(31·8)	32300	(37·4)
Asn	ND	(ND)	4350	(4·9)	ND	(ND)
Gln	0	(0·0)	7260	(8·2)	0	(0·0)
Glu	8100	(13·3)	7260	(8·2)	6090	(7·1)
Pro	190	(0·3)	2480	(2·8)	1150	(1·3)
Cit	80	(0·1)	180	(0·2)	210	(0·2)
Gly	2260	(3·7)	2510	(2·8)	1940	(2·2)
Ala	10300	(16·9)	6880	(7·7)	9580	(11·1)
Val	280	(0·5)	(1110)†	(1·3)†	(1030)†	(1·2)†
1/2 Cys	750	(1·2)				
Met	0	(0·0)	280	(0·3)	390	(0·5)
Ile	110	(0·2)	450	(0·5)	370	(0·4)
Leu	210	(0·3)	680	(0·8)	520	(0·6)
Tyr	40	(0·1)	210	(0·2)	230	(0·3)
Phe	50	(0·1)	380	(0·4)	290	(0·3)
β-Ala	140	(0·2)	60	(0·1)	60	(0·1)
Total	60820	(99·8)	88960	(100·0)	86270	(99·9)
NH_3	10700		57400		41430	
Dry weight of extracted material	48 mg/ml		66 mg/ml		37 mg/ml	

* ND = not determined. † Val and Cys were not resolved in these runs and the figure is the total for both.

When *Phycomyces* is grown in a nitrogen rich media where asparagine is the sole source of nitrogen, a somewhat different pattern emerges (Table 2). Most of the nitrogen in the free amino acids resides in arginine; the level of arginine is high enough to maintain a highly basic amino acid pool throughout development. (The amount of available carbon is not grossly different compared with the two other media.) The serine levels are again very high in the sporangiophore. Note however, that mycelial levels are not as high. Histidine and proline levels are also much lower in mycelia. The variety of amino acids is more restricted on a nitrogen rich source, in agreement with most microbial studies [3]. From 80–90% of the total level is composed of arginine, aspartic acid, serine, glutamic acid and alanine. The overall free amino acid pool is larger when *Phycomyces* is grown in this medium.

A problem encountered in automatic amino acid analysis is that asparagine is often not resolved from serine. Therefore a sample was split into two portions before analysis and one was hydrolyzed 24 hr in 6N HCl. The amide should revert to aspartate if present, reducing the serine peak and enhancing the aspartate. Asparagine was estimated from the increased aspartate peak. (Serine is somewhat degraded during hydrolysis and a slightly decreased amount was observed.)

DISCUSSION

The amino acid pool gives no clues to aid our understanding of either the sensory mechanism or developmental processes. No unusual amino acid, for example, appears during the most responsive stages I and IVb. Perhaps the large amount of serine and the presence of 3,4-hydroxyphenylalanine are most significant in these aspects. Since serine is the ultimate methyl donor (to tetrahydrofolic acid), its abundance in the sporangiophore may signify an important role for transmethylation in the stimulus-response system or in the maintenance of the normal cellular functions of the sporangiophore. Some of the sensory and developmental systems, especially the blue light response, are thought to be mediated by a flavoprotein. One possible candidate, if C_1 transfer is involved, would be the flavoprotein, N^5, N^{16}-methylene tetrahydrofolate reductase, the product of which would be available for the *de novo* genesis of methyl groups.

The presence of glutamine indicates a possible nitrogen storage utilization and is possibly related to the synthesis of glucosamine-6-phosphate, a precursor in the synthesis of chitin and chitosan, the major component of the cell wall of *Phycomyces*.

Our laboratory has recently discovered the polyamines putrescine and spermidine in the sporangiophore of *Phycomyces*. The size of the arginine pool is obviously important for the maintenance of polyamines. However, we have shown that the free arginine level is highly dependent on nutritional sources, so that care is necessary for comparative studies. *Dictyostelium discoideum* also shows remarkable changes in amino acid pools as a function of development [4, 5]. The one other study of physiological changes during sporangiophore development in *Phycomyces* indicates striking stage-specific alterations in the MW patterns of nonhistone chromosomal proteins during the maturation process [6].

A more complete understanding of the amino acid pool in *Phycomyces* and other organisms is necessary before the changes in pools can be interpreted. Little is understood about the transport, especially intracellular, the turnover and most significantly the compartmentization of amino acids [3, 7]. The sporangiophore of *Phycomyces* has a large central vacuole throughout development; the mycelia also are vacuolated. The distribution of amino acids between vacuole and cytoplasm is important but would be difficult to examine in *Phycomyces*.

Our results are similar but do differ somewhat in detail from the semi-quantitative analysis of Close [8] who examined the free amino acids in the mycelia of a very closely related species *P. nitens* (+) (Agardh). He found moderate amounts of γ-aminobutyric acid and serine and a large amount of asparagine. However, our data (Table 2) also indicate a low serine level in mycelia. The possibility exists that γ-aminobutyric acid is present but has not been resolved from ammonia in our analysis. His mycelia were grown on GA for 16 days, far longer than ours, and changes in the media were noted. Quite possibly in Close's case when ammonia levels reached a toxic level, nitrogen storage was shifted to glutamine and asparagine.

We have grave doubts that any correlations in free amino acids between different taxonomic and physiological groups can be made. Free amino acid pools in fungi seem to be primarily dependent on nutritional sources, organelle, development and possibly age. However, in the case of *Phycomyces* extraneous variables may be eliminated and changes in amino acid pools may be of value in studying the development of normal and mutant fungi.

EXPERIMENTAL

Phycomyces blakesleeanus (—) (Burgeff) (NRRL1555) was grown on either (1) a complete medium of potato dextrose agar (Difco) supplemented with 0·2% yeast extract (PDA + Y), pH 5·6 or (2) commercial instant potato supplemented with 0·2% yeast extract or (3) on the minimal medium, GA, which is 30 g glucose, 2 g L-asparagine·H_2O, 0·5 g $MgSO_4 \cdot 7H_2O$, 1·5 g KH_2PO_4, and 0·25 ppm thiamine per l. of H_2O final pH 4·2. Before harvesting, mycelial growth was maintained for 4 days with oscillation and vigorous aeration in 500 ml Erlenmeyers containing 100 ml liquid minimal medium. Sporangiophores were more conveniently grown on a solid support made by the addition of 4% agar to the minimal medium. Spores in each case were heat shocked at 48° for 5 min to induce germination. Low densities of 1000 spores per 100 ml culture liquid GA and 2 spores/cm² on solid PDA + Y or GA were used to prevent crowding. Stage I sporangiophores were harvested 2–4 hr after their initial appearance, (about 60 hr after seeding of spores), stage II and III, 8–9 hr and stage IVb about 18 hr. The last were 3·5 cm tall. The degree of synchrony ranged from 85–100%; however, asynchronous sporangiophores could be easily plucked and discarded. Stages II and III could not be visually distinguished and were collected together. Mycelium was harvested by filtration through a cold Buchner funnel and washed with cold H_2O; sporangiophores were carefully plucked and washed. In each case the samples were cut into 3 mm pieces and squeezed through 3 layers of cheesecloth. From 0·5 to 4 ml of cytoplasm obtained in this manner was homogenized in 15 ml cold H_2O into which 0·5 nmol of 200 mC/mmol uniformly labelled [^{14}C]-L-valine was added to assess recoveries. 2 ml of the homogenate was rehomogenized in 15 ml 95% EtOH. The suspension was centrifuged for 15 min at 27 000 *g* and the supernatant soln made up to 20 ml with 95% EtOH. A 15 ml aliquot was evaporated to dryness and taken up in 0·5 ml 10 mM HCl for analysis. An aliquot was counted for recovery, which ranged from 75–90%. Another portion was dried for 18 hr at 105° to obtain the total weight of solid. Amino acids were quantitated using an amino acid analyzer.

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