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Structural Gene for Ornithine Decarboxylase in *Neurospora crassa*

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To define the structural gene for ornithine decarboxylase (ODC) in *Neurospora crassa*, we sought mutants with kinetically altered enzyme. Four mutants, PE4, PE7, PE69, and PE85, were isolated. They were able to grow slowly at 25°C on minimal medium but required putrescine or spermidine supplementation for growth at 35°C. The mutants did not complement with one another or with ODC-less *spe-1* mutants isolated in earlier studies. In all of the mutants isolated to date, the mutations map at the *spe-1* locus on linkage group V. Strains carrying mutations PE4, PE7, and PE85 displayed a small amount of residual ODC activity in extracts. None of them had a temperature-sensitive enzyme. The enzyme of the PE85 mutant had a 25-fold higher K_m for ornithine (5 mM) than did the enzyme of wild type or the PE4 mutant (ca. 0.2 mM). The enzyme of this mutant was more stable to heat than was the wild-type enzyme. These characteristics were normal in the mutant carrying allele PE4. The mutant carrying PE85 was able to grow well at 25°C and weakly at 35°C with ornithine supplementation. This mutant and three ODC-less mutants isolated previously displayed a polypeptide corresponding to ODC in Western immunoblots with antibody raised to purified wild-type ODC. We conclude that *spe-1* is the structural gene for the ODC.

The polyamines (putrescine, spermidine, and spermine) and a key enzyme of their synthesis, ornithine decarboxylase (ODC), have been investigated in a large number of eucaryotes (25). The polyamine pathway appears to become active in cells undergoing rapid growth, differentiation, and conversion to the neoplastic state (2, 4, 15, 28). Despite the relationship of the pathway to the growth process, the roles of polyamines and the elaborate regulation of ODC in most organisms are only recently becoming understood at the molecular level (4, 14, 20). Mutants affecting ODC have been recovered in a number of microbial and higher eucaryotic systems (5, 6, 10, 18, 20–23, 23a, 24, 27), but until recently clear evidence that any of these mutants affected the structural gene was not available.

ODC in *Neurospora crassa* is regulated over a large range of activity (20), as it is in most organisms. Part of the regulatory mechanism is enzyme inactivation, accompanied by a slower loss of the enzyme protein (R. H. Davis, G. N. Krasner, J. J. DiGangi, and J. L. Ristow, Proc. Natl. Acad. Sci. U.S.A., in press). To determine the mechanisms of the regulation of ODC, we examined mutations affecting the enzyme. We have previously obtained one mutant lacking ODC (20). It was not known whether this mutant and two others isolated previously (18) were structural gene mutants. We therefore attempted here to define the structural gene for ODC by isolating mutants with kinetically altered enzyme and by defining immunologically the cross-reacting material in ODC-less mutants.

MATERIALS AND METHODS

Strains, growth, and genetic techniques. The strains of *N. crassa* used here are listed in Table 1. The basic medium used was Vogel's medium N (26). Stocks were maintained in agar medium supplemented appropriately for the nutritional requirements imposed by auxotrophic mutations; polyamine-requiring strains were grown on 1 mM spermidine · 3HCl. Techniques and media for crosses and complementation tests have been described previously (7). Growth of strains for the determination of growth rate, enzyme activity, and polyamine pools was carried out in 1-liter cultures as described previously (7). Stationary growth in liquid media for dry-weight determinations with different supplements was done in 10 ml of media in 50-ml Erlenmeyer flasks at 25 and 35°C.

Mutant selection. Conidia of strain IC-40 (*aga inl*), grown previously in 1 mM arginine, inositol (50 μg/ml), and 0.05 mM spermidine, were used. In this strain, arginine creates a polyamine requirement through ornithine starvation (8), and the small amount of spermidine was enough to support growth while limiting endogenous pools of polyamines. Conidia were irradiated with UV irradiation to 50% survival. They were then suspended in 100 ml of minimal medium (in a 500-ml Erlenmeyer flask) with 2% sucrose and *myo*-inositol (0.025 μg/ml) and put in a shaking water bath. The small amount of inositol was designed to support limited growth until the small endogenous polyamine pools (and the inositol in the medium) were exhausted. As shaking continued, auxotrophs unable to grow further survived, whereas prototrophs suffered inositol-less death. Periodic filtrations through cheesecloth were necessary in the earlier stages to remove clumps of mycelia. After 120 to 130 h (at 25°C) or 50 to 60 h (at 35°C), the conidia were concentrated by centrifugation and plated on media containing inositol (50 μg) and 1 mM spermidine. Colonies were transferred to individual tubes and later spot tested for their ability to grow on minimal medium and medium supplemented with putrescine or spermidine.

Enzyme extraction and determination. One-liter cultures were harvested on Whatman no. 1 filter paper, and the moist mycelium was ground with an equal weight of acid-washed sand in a buffer solution (0.05 M potassium phosphate [pH 7.1], 1 mM EDTA). After centrifugation at 18,000 × *g*, the supernatant was removed and desalted on a Sephadex G-25 column equilibrated with the extraction buffer. Extracts were stored at −70°C. Enzyme determinations were made at

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TABLE 1. Strains used in this study[a]

Strain	Loci and mating type	Alleles (in order)
IC-1	Wild-type 74A	
IC-3	*aga A*	UM-906
IC-36	*spe-1 a*	462JM
IC-37	*spe-1 A*	462JM
IC-38	*spe-1 a*	PE4
IC-39	*his-1 a*	Y155M302
IC-40	*aga, inl a*	UM-906, 89601
IC-41	*spe-1, aga A*	521KW, UM-906
IC-9	*spe-1, aga A*	TP138, UM-906
IC-42	*spe-1, inl A*	PE4, 89601
IC-43	*spe-1, aga, inl A*	PE4, UM906, 89601
IC-44	*spe-1, aga, inl A*	PE7, UM906, 89601
IC-45	*spe-1, aga, inl a*	PE69, UM906, 89601
IC-46	*spe-1, aga, inl a*	PE85, UM906, 89601
IC-47	*am, his-1, cot-1, rec3-3 A*	am^{118}, K83, C102(t) *rec3-3*

[a] All but one strain were obtained from the collection of R.H.D. or from the Fungal Genetics Stock Center, Humboldt State University Foundation, Arcata, Calif. Strain IC-47 was the gift of John A. Kinsey.

37°C in 0.3-ml reaction mixtures by a method described previously (20) which depends upon the capture of $^{14}CO_2$ liberated from [1-^{14}C]ornithine. Extracts with low activity were assayed with ornithine of 5- to 10-fold the normal specific radioactivity. Protein was measured by the method of Lowry et al. (14) with ovalbumin as a standard. Specific activities were expressed as nanomoles of product per hour per milligram of protein.

Polyamine pools. Cultures used for enzyme determinations were also used for the determination of polyamine pools. Volumes (10 to 20 ml) of culture were harvested and washed on 2.5-cm membrane filters (5 μm, pore size), and the mycelium was immediately put in 1 mM cold 0.4 M $HClO_4$ with 2 mM EDTA. An internal standard (10 or 100 nmol of 1,7-diaminoheptane) was added. After standing for at least 20 min, the cells were centrifuged, and most of the supernatant was recovered. A 100-μl portion of this supernatant was dansylated by adding an equal volume of a dansyl chloride solution (5 mg/ml of acetone), followed by solid sodium carbonate to saturate the solution and mixing. After standing overnight, the solutions were mixed and extracted with 0.2 ml of ethyl acetate. The ethyl acetate extract was filtered through 13-mm membrane filters (pore size, 0.45 μm; Alpha-450; Gelman Sciences, Inc.). The polyamines were determined with a Gilson high-pressure liquid chromatography apparatus by using an Altex Ultrasil ODS column (0.4 by 4.5 cm, 10-μm particle size). Elution was started with 50% acetonitrile-water, which was increased linearly over 7.5 min to 95% acetonitrile and then decreased to 50% over 3 min. The effluent was monitored with a fluorescence detector, and the data were integrated with the Gilson Datamaster. Samples were determined against calibration runs of the normal polyamines and the internal standard, dansylated at the same time as the unknowns.

Purification of ODC and detection of cross-reacting material. An ODC preparation was purified to homogeneity (approximately 600-fold over the starting material) from strain IC-3 *aga*, which had been grown on arginine and thus already had highly augmented ODC activity. The purification steps were ammonium sulfate fractionation, gel filtration on Bio-Gel P-200, DE-52 chromatography, and high-pressure liquid chromatography with a Bio-Gel TSK DEAE 5PW column (7.5 by 75 mm). The pure enzyme reveals two bands (M_r, 52 and 44 kilodaltons [kDa]) on denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis after silver staining. The 44-kDa form is a minor and variable proportion of the whole; it is labeled by incubation of the native preparation with the ODC suicide inhibitor, difluoromethylornithine, and is probably a proteolytic product of the main 52-kDa form. The details of this work (J. J. DiGangi and R. H. Davis, unpublished data), together with a characterization of the enzyme, will be published elsewhere. The enzyme of strain IC-46, carrying *spe-1* allele PE85, was partially purified by ammonium sulfate fractionation and gel filtration on a Sephacryl S-200 column, resulting in an 18-fold increase over the starting specific activity.

The pure wild-type enzyme was used to raise an antiserum in New Zealand White rabbits. Western immunoblots (3) of sodium dodecyl sulfate-polyacrylamide gels of crude mycelial extracts, visualized by ^{125}I autoradiography, revealed about eight polypeptides. The most prominent one corresponded to the M_r of ODC (52 kDa) and was regulated appropriately by the growth of cells in minimal medium, medium supplemented with 1 mM spermidine, and medium supplemented with arginine (see below). These treatments led to corresponding changes in ODC specific activity; no other bands on the immunoblots responded to these nutritional manipulations. The ^{125}I-labeled ODC bands were cut from the blots and counted in a gamma counter for quantification.

Chemicals. All biochemicals were purchased from Sigma Chemical Co. [1-^{14}C]ornithine was purchased from International Chemical and Nuclear Co. ^{125}I-protein A was the gift of Daniel Knauer. [^{14}C]difluoromethylornithine was purchased from Amersham Corp.

RESULTS

Mutant selection and genetic characterization. Four mutants were selected from strain IC-40, carrying the *aga* and *inl* mutations, as described above. Two, carrying mutations PE4 and PE7, were found after enrichment at 25°C; two others, carrying mutations PE69 and PE85, were found after enrichment at 35°C. All four strains had exponential doubling times two to four times greater than normal at 25°C (see below), and none grew significantly at 35°C. The four mutants did not complement one another, nor did they complement a strain carrying a preexisting *spe-1* allele, 462JM. Controls established that the lack of complementation with the latter was not due to heterokaryon incompatibility.

The strain in which the new alleles originated carried the *inl* mutation linked to the previously isolated *spe-1* mutations. In an outcross of each of the new mutants to wild type, all four strains displayed linkage of the polyamine requirement to the inositol requirement, and the recombination percentages (4 to 7%) were in the same range established previously for the *spe-1-inl* interval (18, 20). As in the case of previous *spe-1* mutants, the Spe$^-$ progeny of the new mutants germinated poorly.

Using the PE4 allele, a more definite map position was sought for *spe-1*. A cross of strain IC-42 to strain IC-39, involving the PE4, *his-1*, and *inl* mutations, yielded the gene order *spe-his-inl*, with distances of 1.2 map units for the first interval and 10.0 map units for the second. A second cross of strain IC-38 (carrying *spe-1* allele PE4) to strain IC-47, carrying the am^{118} and *his-1* mutations, was performed. The ascospores were plated on medium containing histidine only, thereby selecting for am^+ $spe\text{-}1^+$ progeny. Only two isolates were obtained in a progeny of 660, and both were His$^-$. This demonstrates that *spe-1* is between *am* and *his-1*.

Because it is known that the *his-1* mutation is centromere distal to the *am* mutation (i.e., toward the *inl* locus), the overall order is *am-spe-1-his-1-inl*. There are too few progeny to calculate a reliable map distance, which varies in any case among *N. crassa* crosses involving mixed backgrounds. However, the order has been confirmed by the more extensive data of John R. Kinsey in the same cross (personal communication).

ODC, polyamine pools, and growth. Strains IC-43, -44, and -46, carrying alleles PE4, PE7, and PE85, respectively, when grown on polyamine-free medium at 25°C, had 10 to 20% of normal ODC activity in the standard reaction mixture with 2 mM L-ornithine (Table 2). Extracts of these mutants, after growth on spermidine-supplemented medium, lacked detectable activity, while the activity of the parental strain was reduced only to 25% of normal (Table 2). This indicates that the mutants, grown in unsupplemented medium, might actually be at the limit of their capacity to express ODC activity in response to polyamine starvation. The parental strain, IC-40, carries the *aga* mutation, which leads to ornithine deprivation (and thus to polyamine deprivation) when it is grown on arginine (8). The capacity of the parental strain to augment ODC activity in response to polyamine starvation was thus seen when it was grown on arginine (Table 2). The augmentation was almost 90-fold. (Growth continues because lysine is decarboxylated by high levels of ODC and provides polyamine analogs which substitute for the normal polyamines [20].) In the case of the mutants, which also carry the *aga* mutation, the addition of arginine led to no further augmentation of activity (a slight loss was usually seen) and an even more severe growth impediment (Table 2). The poor growth was clearly the result of ornithine deprivation in combination with the low ODC activity of the mutants; indeed, arginine led to lower polyamine pools in all mutants (Table 3). Strain IC-45 (carrying the *spe-1* allele PE69) had too little ODC activity to measure dependably.

TABLE 2. Ornithine decarboxylase activity of mutants

Genotype (strain)	Supplement[a]	Growth temp (°C)	Doubling time (h)	ODC sp act (U/mg of protein)[b]
aga, inl (IC-40)	Inos	25	3.0	18.5
	Inos + Spd		3.3	4.3
	Inos + Arg		5.5	1,658.0
	Inos	35	2.8	16.5
	Inos + Spd		2.4	4.3
	Inos + Arg		4.25	1,656.0
PE4 *aga inl* (IC-43)	Inos	25	6.8	2.3
	Inos + Spd		4.0	<0.1
	Inos + Arg		12.0	1.5
	Inos	35	NG[c]	
PE7 *aga inl* (IC-44)	Inos	25	9.8	1.8
	Inos + Spd		4.3	<0.1
	Inos + Arg		15.0	<0.1
	Inos	35	NG	
PE69 *aga inl* (IC-45)	Inos	25	11.0	<0.7
	Inos + Spd		3.0	<0.3
	Inos + Arg		17.0	<0.2
	Inos	35	NG	
PE85 *aga inl* (IC-46)	Inos	25	8.5	3.9
	Inos + Spd		3.5	<0.2
	Inos + Arg		14.0	2.2
	Inos	35	NG	

[a] Inos, Inositol; Spd, spermidine; Arg, arginine.
[b] Specific activities are expressed as nanomoles of product per hour per milligram of protein.
[c] NG, No growth.

In crude extracts of all ODC-positive mutants and the parental strain, the ratios of enzyme reaction rates at 35 and 25°C of the mutants and parental strain were similar (1.6 to 2.5). The temperature sensitivity of the mutants, therefore, did not reflect temperature sensitivity of the enzyme, at least not in vitro. It is possible that at the higher temperature the temperature-sensitive phenotype is an exacerbation of the requirement imposed by the ODC deficiency or that the amount of the enzyme is less.

The polyamine pools of the mutants, grown in medium lacking polyamines, were similar but very low in comparison to that of the parental strain (Table 3). The wild-type strain has a substantial amount of bound (sequestered) polyamines, the loss of which would probably not lead to a growth requirement (19). The ODC deficiency is great enough in the case of the mutants, however, to limit the metabolically important free-polyamine pool and thus to limit growth rates. Ornithine did not stimulate growth of the mutants carrying alleles PE4, PE7, or PE69, but it markedly stimulated the growth of the mutant with the PE85 allele, even at 35°C (Table 3).

Enzyme characteristics. The small amount of ODC of strains IC-43 and IC-46 was tested for its sensitivity to high temperature. Preincubation over a range of temperatures showed that the enzyme of the mutant carrying PE4 resembled that of the parent, while that of the mutant carrying PE85 was less thermolabile in the 40°C range. A crude extract of the strain IC-46, carrying allele PE85, was tested for thermolability of ODC activity with time at 41°C. At the same time, an extract of the parental strain, IC-40, with augmented activity and a mixture of extracts of IC-40 and IC-46 were tested. (In the case of the mixture, the activity measured is >95% wild-type enzyme, while the proteins are mainly those of the mutant strain.) The results (Fig. 1) demonstrated that the activity of the PE85-carrying strain was more stable than that of the parental strain. This cannot be attributed to free factors in the extract of the PE85-carrying strain, because the lability of the parental enzyme persisted in the mixture (Fig. 1).

The growth of strain IC-46, carrying allele PE85, on ornithine suggested that its ODC had poor substrate affinity. The K_m of the mutant enzyme for ornithine was found to be 5.0 mM, some 25-fold higher than that of strain IC-40 (parental) or strain IC-43 (carrying allele PE4), which were both ca. 0.17 mM. The high K_m of the enzyme of strain IC-46 persisted upon partial purification of its enzyme (Fig. 2). A mixture of the partially purified enzyme from the PE85 mutant with that of the wild-type enzyme (purified through the DEAE chromatography step), in which the activity was mainly due to the parental enzyme, showed that the materials in the preparation of the mutant had no effect upon the parental enzyme activity (Fig. 2).

Western blots. Western blots of extracts of strains IC-40, grown in the presence of arginine, and IC-46, grown in minimal medium, displayed a prominent 52-kDa polypeptide corresponding to ODC (Fig. 3). The amounts of cross-reacting material in strain IC-46 (carrying allele PE-85) and IC-40, estimated by counting the ^{125}I associated with the 52-kDa

TABLE 3. Polyamine pools, growth rates, and ODC activity of mutants[a]

spe-1 Allele (strain)	Supplement	Growth temp (°C)	Doubling time (h)	ODC sp act (U/mg of protein)[b]	Polyamine pools		
					Put	Spd	Spm
Wild-type (IC-40)	Inos	25	3.0	24.2	0.9	18.3	0.2
	Inos + Orn		4.3	20.7	0.9	13.8	0
	Inos	35	2.8	16.5	ND	ND	ND
	Inos + Orn		3.5	23.6	0.7	21.5	0.5
PE4 (IC-43)	Inos	25	7.0	2.3	0	2.9	0.1
	Inos + Orn		10.0	ND	0	1.6	0.2
	Inos + Arg		12.0	1.5	0	0.3	0.1
PE7 (IC-44)	Inos	25	9.8	1.8	0	1.4	0
	Inos + Orn		13.0	ND	0	1.3	0.2
	Inos + Arg		15.0	<0.1	0	0	0
PE69 (IC-45)	Inos	25	11.0	<0.7	0	2.4	0.4
	Inos + Orn		12.5	ND	0	2.0	0.5
	Inos + Arg		17.0	<0.2	0	0.5	0
PE85 (IC-46)	Inos	25	9.5	3.3	0	3.5	0.3
	Inos + Orn		4.8	3.8	0	1.6	0.2
	Inos + Arg		14.0	2.2	0	0.4	0
PE85 (IC-46)	Inos	35	NG				
	Inos + Orn		5.8	2.0	0	3.8	0.5

[a] Orn, ornithine; Put, putrescine; Spm, spermine; ND, not determined. All other abbreviations are the same as in Table 2.
[b] Specific activities are expressed as nanomoles of product per hour per milligram of protein.

bands, were similar. Both strains had about 15-fold less of this material when mycelia were grown in the presence of spermidine. These results indicate that strain IC-46 has normal amounts of ODC protein and that it is normally regulated. Strains IC-9, -37, and -41, without detectable ODC activity, also had substantial amounts of ODC cross-reacting material when they were starved for polyamines. In these cases, however, the strains had to be grown with limiting putrescine (0.2 mM), and this was correlated with a lesser amount of cross-reacting protein than was seen in the arginine-grown strain IC-40. The amounts were 69, 54, and 31%, respectively, for the strains carrying alleles 462JM, 521KW, and TP138.

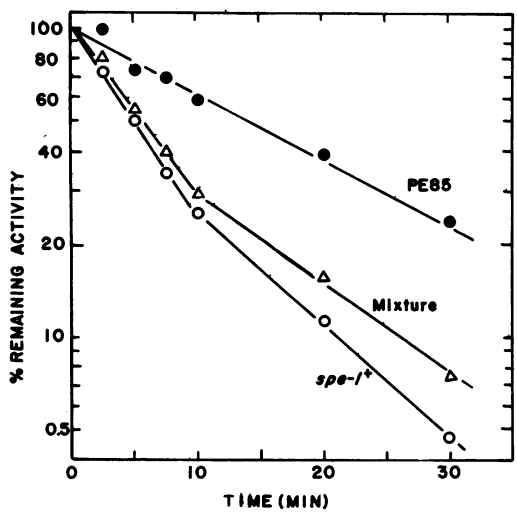

FIG. 1. Heat inactivation of ODC activity of the PE85 mutant at 42°C. Crude desalted extracts of strains IC-46 and IC-40 were used; the proportions of the mixture (*spe-1*$^+$/*spe-1*) were 1:4.3 with respect to protein and 134:1 with respect to initial activity in the standard reaction mixture.

DISCUSSION

Our results clearly indicate that the *spe-1* locus is the structural gene for ODC. This conclusion rests mainly upon differences between the enzyme of the strain carrying the *spe-1* allele PE85 and the wild-type enzyme in their substrate affinities and thermostabilities. These characteristics are normal in another mutant, carrying allele PE4. This shows that the changes imparted by the PE85 mutation are allele specific and not, for instance, the unmasking of a second form of the enzyme. The existence of ODC-less alleles at the *spe-1* locus reinforces this point. The mutant carrying the PE85 allele and the three previously isolated mutants completely lacking ODC activity all have a protein of the proper M_r which cross-reacts with antiserum to the wild-type enzyme. The protein of the PE85-carrying mutant (other mutants were not tested) is regulated similarly to that of the parental strain. These observations are all consistent with a role for the *spe-1* locus in the structure of ODC. The normal responses of mutants to spermidine starvation and excess and the allele-specific nature of the enzyme characteristics of the strain carrying the PE85 allele virtually exclude a regulatory or a modification function for the *spe-1* locus.

It has been suggested that a phosphorylated form of ODC performs an important accessory role in the RNA polymerase I reaction in the slime mold, *Physarum polycephalum* (1, 13). The fact that the growth of one mutant, carrying the TP138 allele, is impaired even on supplemented medium and that this mutant, when starved, has the least amount of cross-reacting material on Western blots justifies testing the possibility that ODC might have an indispensable role in *N. crassa*.

This genetic demonstration of the structural gene for ODC in *N. crassa* is among the first in eucaryotic organisms. Previously, however, extensive work on Chinese hamster ovary (CHO) cells yielded mutants with 1 to 3% of normal activity, though without a major change in the kinetic characteristics of the residual enzyme (24). A preliminary communication from the same laboratory has since indicated that other ODC-less mutants have been found that have ODC mRNA but lack ODC activity (23a). Unless these mutants reflect derangement of a translational control mechanism, they probably represent a structural gene for ODC in this organism. Even more recently, a variant form of ODC in a human lymphoblastoma cell line has been reported (22). In mouse lymphoma cells, amplification of the ODC gene brought about by selection for difluoromethylornithine resistance revealed chromosomal evidence for at least one ODC locus (16). Southern blotting of the DNA from such cells with ODC cDNA suggests that there is more than one locus containing a homologous sequence; but whether these represent loci encoding active ODC is not known (17). Other investigators have cloned ODC cDNAs from the mRNA of the kidneys of androgen-stimulated mice (12) and from mouse myeloma cells (11).

The genetic information about ODC in another fungus, *Saccharomyces cerevisiae*, is rather complex. Whitney and Morris (27) isolated mutants of the *spe-1* locus which lacked ODC activity. More recently, Cohn et al. (6) isolated ODC-less mutants of yeast, with mutations which they designated *spe10*. These were thought to be regulatory mutations because several unusual kinds of revertants could be derived from them, but the mechanism of these reversions and of the *spe10* locus itself were not defined. Although neither study demonstrated that these mutants carried structural-gene mutations, it was later found that the Whitney and Morris *spe1A* mutation does not complement with the *spe10* mutation (25). Finally, the structural gene of *Saccharomyces cerevisiae* has been cloned, and shown to be a unique-sequence gene (9). The cloned DNA complements the *spe1A* mutant isolated by Whitney and Morris (27), as well as being expressed in an ODC-less *Escherichia coli* mutant (9). It is very likely, therefore, that the *spe1A* and *spe10* mutations represent the structural gene for the locus of yeast.

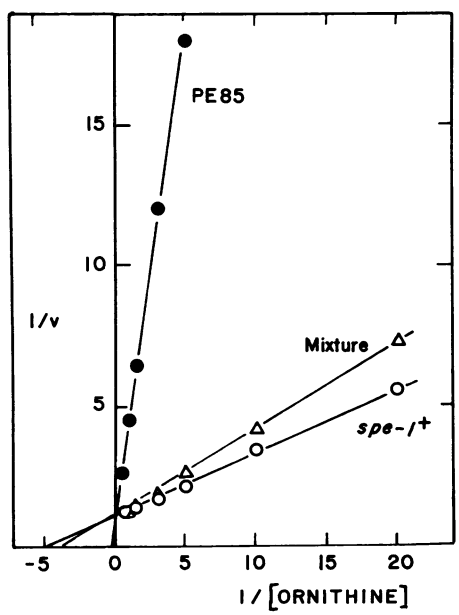

FIG. 2. Lineweaver-Burk plots of reaction velocity versus ornithine concentration for *spe-1* ODC and *spe-1*$^+$ ODC preparations. A crude extract of the IC-46 strain (carrying *spe-1* allele PE85) and a preparation from strain IC-3 (carrying the *spe-1*$^+$ allele and grown on arginine to induce highly augmented activity) were used. The mixture (*spe-1*$^+$/*spe-1*) was a 0.0013:1 ratio on a protein basis but a 73:1 ratio on the basis of activity because of the high specific activity of the preparation of wild-type enzyme. Symbols: ●, IC-46; ○, IC-3; △, mixture.

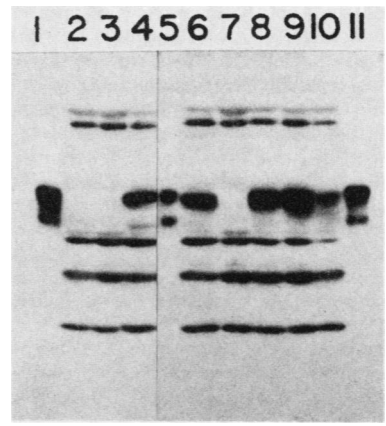

FIG. 3. Western blots of *spe-1* mutant extracts. Similar amounts of protein were loaded onto the gels. The position of wild-type ODC is shown by the arrow. Lanes 1, 5, and 11: purified wild-type ODC. Lanes 2, 3, and 4: *spe-1*$^+$ strain (IC-3) grown on spermidine-containing medium, minimal medium, or medium containing 1 mM arginine, respectively. Lanes 6 and 7: strain IC-46 (carrying *spe-1* allele PE85) grown in medium containing 1 mM spermidine or minimal medium, respectively. Lanes 8, 9, and 10: strains IC-37, IC-41, and IC-9, carrying alleles 462JM, 521KW, and TP138, respectively, displaying no ODC activity (grown on a suboptimal concentration [0.2 mM] of putrescine). The autoradiographic exposure shown here does not show the small amount of labeling in extracts of mycelia grown in minimal medium (lane 3) and in the presence of spermidine (lanes 2 and 7). Longer exposures and direct counting both show that labeled material is present at levels of one-eighth (minimal) to one-fifteenth (spermidine grown) that seen in polyamine-deprived cultures of IC-40.

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