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Externally Suppressible Frameshift Mutant of *Salmonella typhimurium*

JOSEPH YOURNO, DONALD BARR,[1] AND SHEGEJI TANEMURA
Biology Department, Brookhaven National Laboratory, Upton, New York 11973

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Prototrophic revertants of ICR-191A-induced frameshift mutant *hisD3018* have been induced spontaneously by ICR-191A and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) treatment. In each case two genetically distinct prototroph classes were differentiated by transducibility into *his* deletion recipients: (i) transducible, generally fast-growing revertants within the *hisD* gene producing from 10 to 100% of normal amounts of histidinol dehydrogenase and (ii) nontransducible slow-growing prototrophs with very low levels of enzyme activity of which at least some arose by external suppression. These nontransducible revertants, whether arising spontaneously or in the presence of ICR-191A or NG, contain histidinol dehydrogenase which is electrophoretically similar to the wild-type enzyme.

The *hisD* gene of the histidine operon of *Salmonella typhimurium* encodes the enzyme histidinol dehydrogenase, which catalyzes the final step of histidine biosynthesis in this bacterium (1). Frameshift mutant *hisD3018* was isolated by Oeschger and Hartman from the LT-2 strain of *S. typhimurium* after induction by the acridine half-mustard derivative ICR-191A (2) and penicillin selection. The hisD3018 mutation shows the strongly polar characteristics associated with frameshifts (4) and is not phenotypically curable by streptomycin nor revertable by 2-aminopurine. But this mutant is unusual in two respects. (i) It is one of a newly isolated set of ICR-induced frameshifts in the *his* operon from which prototrophic revertants can be induced not only spontaneously and by ICR treatment, but also by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) and diethyl sulfate (DES), agents generally observed to cause base substitutions only (8). (ii) It produces, in addition to a class of revertants transducing *hisD* deletion mutants to prototrophy, another class incapable of such transduction, possibly arising by nontransducible chromosomal aberrations through *hisD* or by external suppression (N. Oeschger, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1967).

We have produced a series of prototrophic revertants from *hisD3018*, spontaneously arising and induced with ICR-191A and NG and have thereby also found external suppressors of this mutant. To characterize the revertants genetically and to increase the levels of revertant histidinol dehydrogenase preparatory to protein analysis, revertants producing the enzyme constitutively are normally prepared by transduction into *his-2236*, a deletion covering almost all of *hisD*, including the *3018* site and carrying the operator constitutive *hisO1242* mutation. On high glucose-agar prototrophic transductants carrying the *hisO1242* mutation form readily distinguishable wrinkled colonies (6) and account for 2 to 5% of the transductants.

It was expected that all revertants would be easily transducible into the *his* deletion recipient, since frameshift mutants were considered to revert only by true back mutation or internal suppression within the mutant gene. Surprisingly, spontaneous, NG-induced and ICR-191A-induced prototrophs formed two genetically distinct classes based on transducibility into *his-2236*: (i) transducible revertants (internal suppressors) within the *hisD* gene for which operator constitutive strains were readily constructed and (ii) nontransducible revertants, of which at least some arose by external suppression of the *hisD3018* mutation.

Riyasaty and Atkins have recently described a suppressible frameshift mutant in the anthranilate synthetase gene of *S. typhimurium* with grossly similar but inverted reversion characteristics, *trpA91* (5). In that system most, if not all, ICR-191A-induced prototrophs were revertants within the *trpA* gene, whereas all NG-induced prototrophs apparently resulted from external sup-

[1] Present address: School of Medicine, University of California, San Francisco, Calif. 94117.

pression of *trpA91*. As with *hisD3018*, spontaneously arising prototrophs were of both kinds. The authors concluded that at least some of the *trpA91* suppressors were umber (UGA) suppressors. They proposed a suppressor mechanism, suggested by B. N. Ames, based on reinitiation of protein synthesis in *trpA91* in a phase compensatory to the frameshift and concomitant suppression of a critical UGA codon generated by the out-of-phase reinitiation.

The basic reinitiation mechanism proposed by Ames for the *trpA91* system may also apply to *hisD3018*. Our results suggest, however, that in this case the *hisD3018* suppressors are missense rather than nonsense suppressors. In the absence of a reinitiation mechanism, these could be explained as true frameshift suppressors. In either case the response of *hisD3018* to external suppressors and its revertability by NG and DES do not appear to be necessarily related phenomena. This paper describes some basic genetic and enzymological characteristics of the *hisD3018* revertants. The accompanying paper is concerned with the characterization of the *hisD3018* mutation as a +1 frameshift as deduced from amino acid replacements in revertant histidinol dehydrogenase (9). In a forthcoming communication (*in preparation*), we shall present detailed genetic evidence for the existence of highly specific external suppressors of *hisD3018*.

MATERIALS AND METHODS

Isolation of prototrophs from hisD3018. For isolation of R1 to R36, 12 parallel clones of *hisD3018* were established by single-colony isolation and growth in nutrient broth. From each clone 2×10^8 cells per plate were spread on plates of minimal E medium (7) agar containing 0.2% nutrient broth (doubly enriched E or 2EM agar). After 48 hr of incubation at 37 C, spontaneous revertants were independently isolated from the 12 parallel clones in one experiment. In a later experiment, 24 additional 2EM spread plates, 2 from each clone, were prepared as described. On the center of the plates was placed a drop of freshly prepared ICR-191A solution at 1 mg/ml in sterile water, or a few tiny crystals of NG. After 72 hr of incubation at 37 C, a single revertant was isolated from the center of the treated area in the ICR-191A-containing plate and at the periphery of the zone of killing in the NG-containing plate. Thirty-six additional spontaneous revertants, R37-R72, were later independently isolated from 2EM-spread plates after 72 hr of incubation at 37 C. The revertants were purified by streaking twice on 2EM agar, and nutrient broth cultures were established for each.

Growth characteristics of ICR-191A and NG-induced revertants. Single spread plates of *hisD3018* on 2EM agar were treated as described above with ICR-191A and NG. From the zone of mutagen-induced reversion, 100 colonies were picked contiguously to obtain a random sample after 72 hr of incubation at 37 C. The revertants were then purified by streaking on 2EM agar and scored daily for growth characteristics of streaks on E and E plus histidine (15 μg of L-form per ml) agar during a 96-hr incubation period at 37 C.

Transductions. All transductions were performed on 2EM agar containing 2% glucose. Recipients were spread at 2×10^8 cells per plate with 1×10^9 particles of the nonlysogenizing L4 strain of phage P22. After 72 hr of incubation at 37 C, transductant colonies were scored. When desired, transductants were purified and cultures established as described above.

Preparation of crude extracts for histidinol dehydrogenase assay. To measure histidinol dehydrogenase specific activity of *hisD3018* revertants, carrying the *hisO1242* mutation, 200 ml of 2EM cultures of each revertant and a *hisO1242* control were grown at 37 C overnight on a rotary shaker. The centrifuged, washed cells in 0.02 M tris(hydroxymethyl)aminomethane (Tris)-succinate buffer (pH 6.5) were sonically disrupted, and enzyme activity and protein concentration was determined as previously described (10). To measure enzyme specific activity in *hisD3018*sup strains carrying the O^+ gene, the cells were grown in either of two ways selecting against wild-type secondary revertants: (i) in 200 ml of E medium containing 15 μg of L-histidine per ml at 37 C overnight as described above or (ii) in 200 ml of nutrient broth at 37 C overnight as described above, followed by centrifugation and saline washing under sterile conditions and resuspension in 200 ml of E medium with 4 hr of incubation at 37 C on a rotary shaker to derepress the histidine enzymes.

Polyacrylamide gel electrophoresis of revertant enzymes. Polyacrylamide gels were prepared, and samples were run at *p*H 9.5 as previously described (10). For enzyme staining samples of 300 to 1,500 μg of crude extract protein or 100 to 200 μg of partially purified enzyme of strains carrying the *hisO*$^+$ gene and grown under repressing or derepressing conditions as described above were examined with a crystalline *hisO1242* control. After electrophoresis the gels were stained for protein or for histidinol dehydrogenase as described (3) except that for the enzyme stain the gels were immersed completely in the dye-substrate mixture and allowed to react for up to 3 hr at room temperature. The reacted gels were then stored in 7% acetic acid.

Purification of revertant histidinol dehydrogenase. Large-scale growth of cells and purification of enzyme from *hisD3018* revertant strains carrying the *hisO1242* mutation were performed as previously described (10).

Purification of histidinol dehydrogenase from hisD3018R20. It was necessary to maintain selection against wild-type secondary revertants in carboy cultures of *hisD3018*R20, a suppressed mutant carrying the O^+ gene, grown under physiologically derepressing conditions. Therefore, nutrient broth cultures were established from a single colony immediately prior to the large scale growth in 2EM medium, and each carboy received a 400-ml inoculum

of a saturated nutrient broth culture. The following growth and purification steps were performed as previously described (10). Samples of 1 ml of each harvested 2EM carboy culture were taken and combined for nutritional and genetic tests to ascertain that wild-type cells were absent.

RESULTS AND DISCUSSION

Isolation and genetic characterization of prototrophic revertants from hisD3018. The spontaneous reversion frequency of *hisD3018* to prototrophy is 2×10^{-9} to 5×10^{-9} after 72 hr of incubation of spread cultures on 2EM agar. This reversion frequency is greatly enhanced under the same conditions by ICR-191A or NG. Typically about 200 prototrophs per plate are produced in the exposed area by ICR-191A and about the same number by NG at the periphery of the zone of killing. We have not determined the values exactly but this represents roughly a reversion frequency of the order of 50-fold above background in both cases.

Originally 12 spontaneous (R1–R12) revertants were independently isolated from 2EM-agar plates after 48 hr of incubation at 37 C. To obtain recombinants carrying the operator constitutive *hisO1242* gene for protein analysis and to confirm that the frameshift reversions occurred within the *hisD* gene, P22 L4 phage was prepared from R1 to R12 and transduced into deletion *his-2236* as recipient. The expected results were obtained with these spontaneously arising revertants. Prototrophic tranductants were obtained at a frequency similar to that for the wild-type LT-2 strain donor control (Table 1). The desired prototrophs carrying the *hisO-1242* allele constituted from 2 to 5% of the transductants and were easily identifiable by their characteristic wrinkled colony morphology on the agar plates containing 2% glucose. More careful examination of the original reversion plates, however, revealed the presence of late-appearing ($\geq$72 hr of incubation) small-colony prototrophs. Therefore, during the isolation of 12 ICR-191A-induced (R13–R24) and 12 NG-induced (R25–R36) revertants, the reversion plates were incubated for 72 hr at 37 C prior to isolation, and care was taken to isolate a sizeable number of the small colony prototrophs arising in the presence of both types of mutagen. Donor phage from large colony revertants of both origins again transduced as expected into *his-2236*, and operator constitutive strains were readily constructed. Donor phage from small colony isolates, however, was unexpectedly not transducible into *his-2236*, while transducing normally the *leu-428* outside marker control (Table 1).

To determine if prototrophs not transducible into deletion *his-2236* arise spontaneously, 36 additional spontaneous revertants (R36–R72) were isolated from independent *hisD3018* cell lines after incubation of the reversion plates for 72 hr at 37 C. Several small colony types were isolated. Donor phage was prepared from each revertant and spot tested (D. Sheppard, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1963) for transducibility into *his-2236* and *leu-428* recipients. All large colony- and three small colony-type revertants proved capable of

TABLE 1. *Transduction of hisD3018 revertants into his-2236 and leu-428*

Donor			Recipient (no. of transductants per plate[a])	
Phage	Colony size[b]	Origin	*his-2236*	*leu-428*
R1	L[c]	Spontaneous	1,410	54
R2	L		1,020	110
R3	L		890	37
R4	L		1,150	89
R5	L		1,680	94
R6	L		>2,000	450
R7	L		600	44
R8	L		1,000	65
R9	L		860	19
R10	L		>2,000	690
R11	L		1,080	43
R12	L		1,130	46
R13	L	ICR-191A	>2,000	210
R16	L		1,800	100
R17	L		220	15
R18	M		120	59
R20	S		0	180
R21	S		0	160
R22	M		1,580	56
R23	S		0	71
R24	S		0	210
R25	L	NG	1,020	210
R26	L		>2,000	250
R28	M		>2,000	1,130
R29	L		830	44
R30	VS		0	210
R32	VS		0	160
R33	VS		0	120
R35	VS		0	82
LT-2			420	45
3018			0	91
No phage			0	0

[a] Average for two plates, scored after 72 hr of incubation at 37 C on 2EM agar; numbers above 100 given to nearest 10.

[b] Scored after 20 hr of incubation of streaks on 1/2EM agar.

[c] L, large; M, medium; S, small; VS, very small.

transducing *his-2236* to histidine-independence and therefore contain internal suppressors of *hisD3018* conferring full or partial prototrophy. The remaining three small-colony revertants were not capable of transducing *his-2236* to prototrophy (Table 2). It is therefore concluded that nontransducible prototrophs can arise spontaneously from *hisD3018*.

Extensive genetic tests have shown that the prototrophs of each origin not transducible into *his-2236* contain the original *hisD3018* mutation and that these stains can recombine normally with nonoverlapping *his* mutations. Some can transduce *hisD3018* to small colony prototrophy but have no effect on several other *hisD* mutants whether nonsense, missense, frameshift, or deletion type (*manuscript in preparation*). This is evidence against nontransducible chromosomal aberrations and for the presence of specific external suppressors in these strains. We shall refer to these revertants henceforth as *hisD3018*sup genotypes.

Nutritional characteristics of hisD3018 revertants. As can be seen in Tables 1 and 2, revertants within the *hisD* gene are generally fast-growing on growth medium lacking histidine. Of a total of 45 such spontaneous strains carrying the *hisO*$^+$ gene, only 3 grew at a rate noticeably slower than normal on streak plates. All ICR-191A- and NG-induced internal suppressors isolated, a total of 12, grew at normal or near normal rates. All *hisD3018*sup isolates, however, grew very slowly in the absence of histidine, as mentioned above, and were greatly stimulated in growth by histidine. Those *hisD3018*sup strains which arose in the presence of NG grew considerably more slowly than spontaneous or ICR-191A-induced *hisD3018*sup strains (Table 1), and may therefore reflect a different mutational basis.

TABLE 2. *Transduction spot test to distinguish external from internal suppressors among 36 additional spontaneous revertants (R37-R72) from hisD3018*

No. of revertants as donors	Colony size[a]	Recipient[c]	
		his-2236	*leu-428*
33	27L[b], 3M, 3S	+	+
3	3S	−	+
hisD3018 R1 Control		+	+
hisD3018 Control		−	+

[a] Scored after 20 hr of incubation of streaks on 1/2EM agar at 37 C.

[b] L, large; M, medium; S, small.

[c] Symbols; +, indicates at least 15 transductant colonies on the spot; −, indicates no transductant colonies on the spot.

Frequency of internal and external suppressors in revertants of various origins. Spontaneous *hisD3018*sup types arise at a significant but apparently rather low frequency (3 of 36) after 72 hr of incubation at 37 C on 2EM reversion plates. We did not examine this problem systematically, however, and the frequency may be considerably higher, particularly after times of incubation exceeding 72 hr. From single agar plates containing ICR-191A or NG 100 mutagen-induced colonies were isolated so as to obtain a random sample and purified by streaking. The isolates were tested for growth rate by streaking on minimal E medium in the presence and absence of histidine. NG induced predominantly large colony revertants which were not responsive to histidine. Only 3 of 100 isolates grew slowly and were noticeably stimulated by histidine (Table 3). Since we have never observed *hisD3018*sup strains to grow at a normal rate in the absence of histidine, we conclude that a maximum of 3% of these prototrophs were of such a genotype. This raises the question of whether or not such strains are actually spontaneously arising background revertants. In any event the frequency of NG-induced *hisD3018*sup strains isolated under these conditions must be low at best. ICR-191A, however, appears to induce a high frequency of *hisD3018*-sup strains. Of 100 isolates, 28 showed the nutritional characteristics associated with this genotype, although some of these may be poorly growing internal suppressors (Table 3). These characteristics are in direct contrast to those of the *trpA91* system (5) of *S. typhimurium* discussed above, for which NG appears to induce external suppressors exclusively, and ICR-191A internal suppressors exclusively.

Histidinol dehydrogenase of internally suppressed revertants. All internally suppressed

TABLE 3. *Growth classes of hisD3018 revertants induced by ICR-191A and NG*

Mutagen	Colony size	
	$E^LE + his^{L\,a}$	$E^SE + his^{L\,b}$
ICR-191A	72	28
NG	97[c]	3

[a] Large colonies on both E and E + histidine agar.

[b] Small colonies on E, large colonies on E + histidine agar.

[c] Includes 11 isolates giving medium colonies on both media.

hisD3018 revertants for which donor phage was preparable in high titer were examined for crude extract histidinol dehydrogenase after construction of operator constitutive strains by transduction into deletion *his-2236* as recipient. All ICR-191A- and NG-induced internally suppressed strains isolated produced enzyme indistinguishable in specific activity from that of the *hisO1242* control (Table 4). As with wild-type histidinol dehydrogenase, these enzymes were essentially stable to storage at 4 and 25 C for at least 24 hr. Most spontaneous isolates of this type (7 of 12) also produced normal enzyme by these criteria. Of the remaining five spontaneous isolates, at least two classes based on crude extract enzyme were evident: (i) a class producing stable enzyme at about 30% of normal specific activity, R7, R9, and R10, and (ii) one producing stable enzyme at about 10% of normal specific activity, R5 and R11. Whether enzymes in each class are identical structurally or merely fortuitously of similar activity has not been investigated, but the activity difference between R7 and R5 does reflect a structural difference (9). Crude extract enzyme specific activities of small overnight shake cultures are quite reproducible in separate experiments with a given strain, e.g., *hisO1242*, and also with parallel clones of the same strain, e.g., R7a–R7d. On the other hand, enzyme specific activities of cells grown in carboy cultures with vigorous aeration are always higher, but unreproducibly so. Wildtype values occasionally reach 0.5 in crude extract, a 2-fold increase, but the increase for R7 was 4-fold and for R5 four- and 10-fold in separate experiments. Enzymes from R7 and R5 have been recrystallized to constant specific activity, achieving values approximately 60 and 50%, respectively, of wild-type histidinol dehydrogenase. As judged by the electrophoretic homogeneity of these preparations (9), these specific activities are probably maximal or near maximal. Crystalline enzyme from the NG-induced revertant, R29, was close to the wild type in specific activity (Table 4).

TABLE 4. *Histidinol dehydrogenase specific activity of hisD3018 revertants containing hisO1242 mutation*

Revertant	Origin	Crude extract (specific activity)	Crystallized enzyme (specific activity)
R1	Spontaneous	0.20	
R2		0.19	
R3		0.24	
R4		0.22	
R5		0.017, 0.023[a]	5.27
R6		0.22	
R7		0.085, 0.080, 0.050, 0.055[a]	6.02
R8		0.24	
R9		0.080	
R10		0.060	
R11		0.030	
R12		0.21, 0.21[a]	
R13	ICR-191A	0.26	
R16		0.24	
R17		0.27	
R22		0.25	
R25	NG	0.26	
R26		0.22	
R28		0.23	
R29		0.22	9.24
hisO1242		0.19, 0.24, 0.22, 0.24, 0.25[b]	10.09

[a] Multiple values refer to results with parallel clones.

[b] Multiple values refer to separate experiments.

Histidinol dehydrogenase of hisD3018sup strains carrying the hisO$^+$ gene. Cells from log phase E medium cultures contain low levels of what appears to be alcohol dehydrogenase, about 5% of R1, the wild-type control, histidinol dehydrogenase activity. This activity is apparent in crude extracts prepared from *hisD3018* when assayed for histidinol dehydrogenase and appears only after suspension of the cells in minimal E medium. The level of histidinol-oxidizing activity in crude extracts of suppressed mutants R20, R30, and R51 parallels that of the parent mutant under the aforementioned growth conditions, making accurate determination of the fraction attributable to the weak histidinol dehydrogenase activity impossible. When these strains are grown overnight under repressing conditions in E medium containing histidine, crude extracts from all show no detectable histidinol-oxidizing activity, whereas those of R1 are about 5-fold repressed. Crude extracts of these *hisO*$^+$ strains and controls grown in nutrient broth followed by E medium were subjected to acrylamide gel electrophoresis at *p*H 9.5 and stained for histidinol dehydrogenase activity; *hisD3018* showed two very closely spaced weakly active species, considered to be the alcohol dehydrogenases, migrating more slowly than the wild-type histidinol dehydrogenase. On the other hand, all the suppressed mutants tested showed in addition to the slow species an additional weak one which was electrophoretically indistinguishable from normal

histidinol dehydrogenase. R1 contained as expected a strongly staining electrophoretically normal enzyme and the weak slower species. Crude extracts of the same strains grown overnight on E medium containing histidine show the same gel species described above, but in greatly reduced amount as expected. Crystalline wild-type histidinol dehydrogenase controls did not show the slower weak species, even under conditions of large sample size or prolonged incubation (Fig. 1). To characterize further histidinol dehydrogenase of suppressed mutants 320 g (wet weight) of *hisD3018* R20 cells was grown under derepressing conditions as described. A sample of the harvested cells was plated at an appropriate dilution on 20 1/2EM plates, and colony size was scored after 24 hr incubation at 37 C. Among 11,000 colonies no large types were found, indicating that less than 0.01% of the cells were wild-type. Phage was prepared from the same sample of harvested cells and tested in quantitative transduction with *his-2236* and *leu-428* recipients. This phage and that prepared from wild-type controls transduced *leu-428* to prototrophy at high frequency, whereas only the wild-type phage was effective in transducing the *his* deletion mutant. No transductants were found with R20 phage under conditions that should have yielded 4,000 to 5,000 wild-type transductants. The enzyme from R20 was therefore purified by the standard procedure through the diethylaminoethyl-Sephadex A-50 step, followed by chromatography on Sephadex G-150 (2.5 by 200 cm) in 0.02 M Tris-succinate (*p*H 6.5). Because of the very low levels of enzyme in this preparation, quantitative measurements of activity in crude fractions is difficult. If the supernatant fraction at 55 C is taken as a starting point, a 44-fold purification was achieved with a 10% yield (Table 5). The partially purified enzyme was subjected to electrophoresis as described above and stained for protein and for histidinol dehydrogenase (Fig. 2). A relatively minor active band migrating as does the normal enzyme was found. To determine if a major gel band corresponds to an inactivated form of the enzyme, a 5-mg sample of the preparation was reduced and carboxymethylated, and a

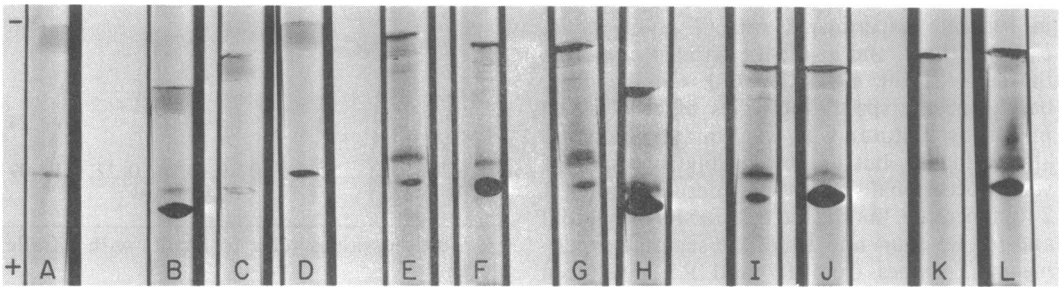

FIG. 1. *Polyacrylamide gel electrophoresis of crude extract histidinol dehydrogenase from hisD3018sup strains carrying the hisO⁺ gene. Cells were grown under conditions of derepression in nutrient broth and E medium, crude extracts prepared thereof, and gel samples run and stained for histidinol dehydrogenase for 3 hr unless otherwise noted. A, wild-type crystalline enzyme control (2 μg); B, R1 control (300 μg); C, R1 (300 μg), 20-min stain; D, wild type (1 μg) +R1 (300 μg) 20-min stain; E, R20 (1,500 μg); F, wild type (1 μg) +R20 (1,500 μg); G, R30 (1,500 μg); H, wild type (1 μg), +R30, (1,500 μg); I, R51, (1,500 μg); J, wild type, (1 μg), +R51, (1,500 μg); K, hisD3018 control (1,500 μg); L, wild type, 1 μg, +hisD3018 (1,500 μg). With the mixed samples it was necessary to overstain the wild-type enzyme to make the weaker species visible.*

TABLE 5. *Partial purification of histidinol dehydrogenase from hisD3018 R20, a suppressed mutant carrying the hisO⁺ gene*

Fraction	Total vol	Total enzyme	Total protein	Specific activity	Yield
	ml	*units*	*g*		%
Crude extract (55 C) supernatant fluid	1,020	—[a]	15.1	—	—
	760	45.1	5.5	0.008	100
Dialyzed ammonium sulfate ppt	135	20.3	2.6	0.008	44
DEAE-Sephadex eluate, concentrated	9	14.0	0.25[b]	0.056	31
Sephadex G150 eluate, concentrated	14	4.3	0.012[b]	0.36	10

[a] Indeterminate in crude extracts.
[b] Based on $E^{1\%}_{1\ cm} = 10$.

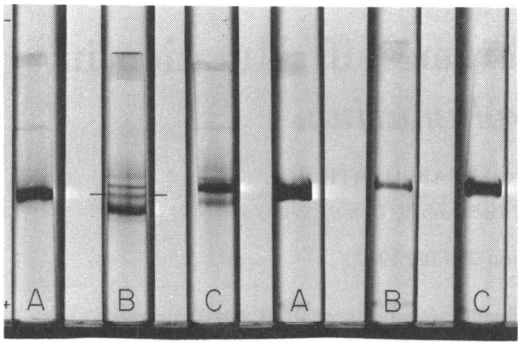

FIG. 2. *Polyacrylamide disc gel electrophoresis of partially purified hisD3018 R20 histidinol dehydrogenase. From left to right: (A) wild-type crystalline enzyme control, 50 μg; (B) hisD3018 R20, G-150 eluate 200 μg, (C) A + B, each 0.5 concentration. All samples stained for protein; (A, B, C) duplicate samples, all stained for histidinol dehydrogenase for 20 min. The arrow indicates the deduced position of histidinol dehydrogenase in the hisD3018 R20 preparation.*

peptide map of a tryptic digest was made. This map bore no resemblance to that from histidinol dehydrogenase, suggesting that the enzyme in this preparation comprises a relatively minor fraction of the total protein. A rough calculation, based on the assumption of 100% enzyme in the 55 C supernatant fraction and 10-fold derepression, yields a value of approximately 2% of wild-type levels of activity in the suppressed mutant. These observations suggest the presence of low levels of a reasonably active enzyme, possibly wild type, in the externally suppressed mutants. It follows that the efficiency of suppression is low, perhaps of necessity to prevent lethal effects. These questions can be answered more satisfactorily by purification of the enzyme from suppressed mutants to homogeneity.

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