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Genetic Map Position of the *pdxH* Gene in *Escherichia coli*

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The gene for pyridoxine phosphate oxidase, *pdxH*, is located 1.2 min beyond *aroD*, proximal to *trp*.

The locations on the *Escherichia coli* chromosome of three of the five known groups of pyridoxine genes are known (7). This report describes the location of a fourth gene, namely, that defined by *pdxH* mutants. *pdxH* mutants lack pyridoxine phosphate oxidase and consequently require pyridoxal rather than pyridoxine for growth in all media, including broth (2, 5).

Most of the pyridoxine mutants in this laboratory are in *Escherichia coli* strain B. To map the position of *pdxH*, we introduced *pdxH* into strain K-12 by the procedure we described earlier for mapping *pdxB* (4). Our results show that *pdxH* is located close to *aroD* and distal to *his*. Details and data are below.

The starting strain was WG1602, which bore the previously described (5) *pdxH181* allele as well as a spontaneous resistance to streptomycin and an additional mutation in an *ilv* gene. *E. coli* WG1602 was made lactose negative by the procedure described earlier (3). This strain was then converted to an Hfr strain by heat curing a thermosensitive F*lac* plasmid introduced from *E. coli* E'36 as described earlier (4) and selecting for Lac$^+$ Str^r types. Several individual single colonies were purified and used as donors of the *pdxH* gene into spectinomycin-resistant strains of *E. coli* AB1359 and *E. coli* AT724. The protocol was as described earlier (4). This time, however, we chose spectinomycin at 200 μg/ml to counterselect the donors instead of T6 phage infection. Selection was made in each cross for loss of the different amino acid requirements. Out of 500 amino acid-independent recombinants examined, 350 had gained both *pdxH181* and *ilv* genes from strain WG1602. This frequency was independent of which amino acid requirement was lost (Lys$^+$ was not used). Two strains, M and D, which retained useful genetic markers, were retained for further study (Table 1).

The restriction pattern of both strains M and D was found to be K-12 type by measuring the ability of P1*bt* and P1*kc* to plate on these strains. We found that P1*kc* had an efficiency of plating (EOP) of 0.4, and P1*bt* had an EOP of 0.06 on both strains M and D. For K-12 strains AB1359 and AT724, these EOPs were 0.5 and 0.01 for P1*kc* and P1*bt*, respectively.

We used the rapid mapping procedure of Low (6) to find that the *pdxH* gene was in the region near *his*. Time of entry experiments were performed as described earlier (4). The donor strain was KL96, and the recipient was strain M. We measured the numbers of His$^+$, AroD$^+$, and PdxH$^+$ recombinants at 2-min intervals for 76 min in five separate experiments. Data for

TABLE 1. *Description of strains*

Strain no.	Relevant genotype	Source or reference
AB1359	*proA2, his-4, aroD5, argE3*, F$^-$	A. L. Taylor
AT724	*his-1, lysA10, metB1, strA1*, F$^-$	A. L. Taylor
KL96	Hfr	B. Low
WG1602	*pdxH181, ilv-1, str^r*	Reference 5 and this work
M	*pdxH181, ilv-1, his-4, aroD5, spc^r, str^r*	This work
D	*pdxH181, ilv-1, metB1, spc^r, strA1*	This work
E'36	*lac^{del}*, F*lac^{TS}*	(4)

TABLE 2. *Entry time for genes His, AroD, and PdxH*[a]

Expt no.	Time of entry (min)		
	His	AroD	PdxH
1	14	25.2	28
2	9.5	21.8	25.8
3	16.5	29	31.5
4	13.3	27	29.3
5	13.0	24.3	26.8

[a] The data shown are times after mixing KL96 with strain M. The values shown are intercepts of the abscissa by the line describing the numbers of recombinants at different times.

the times of entry of these three genes are shown in Table 2.

The mean difference between the time of entry of *his* and *aroD* was found to be 12.2 ± 1.2 min, and the mean difference between the time of entry of *aroD* and *pdxH* was 2.8 ± 0.7 min. The published difference between *aroD* and *his* is 5.2 min. Normalizing our data to this time means that *pdxH* maps about 1.2 min after *aroD*.

We then transduced strain AB1359 with P1*kc* phage stocks that had been grown on strain D, and we plated for AroD$^+$ recombinants on pyridoxal-containing agar plates. Procedures used were as described earlier (3). Among 150 AroD$^+$ clones, we found that only 3 clones required pyridoxal for growth. When strain M served as the recipient and strain AT724 served as the donor, the selection was made for AroD$^+$, we found 5 PdxH$^+$ clones among 310 AroD$^+$ clones. When PdxH$^+$ transductants were selected and scored for AroD$^+$, we found 9 AroD$^+$ clones among 235 tested. These low linkages (2.0%, 1.6%, and 3.8%) suggest that the two markers are indeed separated by 1 min or more (7). Unfortunately, we were unable to obtain useful genetic markers between *pdxH* and *pyrF*, so we were unable to do 3-factor crosses. We conclude that the *pdxH* gene lies at 31 min on the 1972 version of the *E. coli* genetic map (7).

After this work had been submitted, a recalibrated linkage map for *E. coli* K-12 appeared (1). This new map contains the locations of both the *pdxH* gene and the *pdxJ* gene. When we recalculated the position of the *pdxH* gene, using our data and the new coordinates for *his* (44.15 min) and *aroD* (37.25 min), we found that the coordinate for *pdxH* is at 35.7 ± 0.4 min, which is in good agreement with its estimated position of 36 min on the new recalibrated map (1).

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