Effect of the Proline Analogue Baikiain on Proline Metabolism in *Salmonella typhimurium*

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A proline analogue, 4,5-dehydro-L-pipecolic acid (baikiain) induces the formation in *Salmonella typhimurium* of the two enzymes catalyzing the degradation of proline, proline oxidase and Δ^1-pyrroline-5-carboxylic acid (P5C) dehydrogenase. The level of induction by 20 mM baikiain is about 10% of the maximum level induced by proline. Since the analogue is a substrate of proline oxidase the first enzyme of the proline catabolic pathway, the oxidation derivative rather than baikiain itself might be the actual effector. Baikiain is also an inducer of proline oxidase in *Escherichia coli* K-12 and *E. coli* W. An additional effect of this analogue on proline degradation in *S. typhimurium* is inhibition of P5C dehydrogenase. At a concentration of 5×10^{-4} M, baikiain inhibits completely the growth of strains constitutive for proline oxidase. This inhibition, which can be overcome by proline, occurs in the presence or absence of P5C dehydrogenase activity. Three spontaneously occurring mutants resistant to baikiain were isolated from constitutive strains. All are pleiotropic-negative for the proline-degrading enzymes. The sites of these mutations are linked to the *put* region. Although the mechanism of toxicity has not been determined, baikiain provides a simple and direct selection for obtaining mutants unable to degrade proline. In addition, it allows selection for strains with an inducible rather than constitutive phenotype.

The induction by L-proline and repression by catabolites of the enzymes required for the degradation of L-proline in *Salmonella typhimurium* strain 15–59 have been described in earlier papers from this laboratory (5, 9). Catabolism of proline to glutamate in *S. typhimurium* is catalyzed by two enzymes (5): proline → Δ^1-pyrroline-5-carboxylate (P5C) (proline oxidase); P5C ⇌ glutamic γ-semialdehyde (nonenzymatic); glutamic γ-semialdehyde → glutamate (P5C dehydrogenase).

Metabolite analogues have been exploited as powerful tools in revealing biochemical relationships and in identifying and studying gene functions in microbial systems (14). The present work describes a proline analogue, 4,5-dehydro-L-pipecolic acid (baikiain), which induces the proline degradative enzymes. Besides inducing, this analogue has several additional effects on the enzymes of proline metabolism.

MATERIALS AND METHODS

Chemicals. L-Proline, nicotinamide adenine dinu-

cleotide phosphate (NADP), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. *o*-Aminobenzaldehyde (*o*-AB) was obtained from K & K Laboratories. Baikiain was purchased from Calbiochem. Not a trace of proline, as tested with an amino acid analyzer, was found in the baikiain. The other proline analogues were all from Aldrich Chemical Co. Δ^1-Pyrroline-5-carboxylic acid was synthesized by the method of Strecker (13).

Bacterial strains. The strains of *S. typhimurium* used in this study are listed in Table 1. The parent strain is *S. typhimurium* strain 15–59. The ability to utilize proline as sole source of nitrogen is designated phenotypically as Put or genotypically as *put*. *Escherichia coli* K-12 was obtained from W. S. Reznikoff and *E. coli* W from J. Adler.

Cultivation of bacteria. Cells were cultivated in a modified minimal medium (8), consisting of medium E of Vogel and Bonner plus trace elements and lacking citrate. This medium, supplemented with 0.2% sodium succinate and 0.2% ammonium sulfate, is designated as SN medium. Medium selective for proline utilization, designated as SP medium, was supplemented with 0.2% sodium succinate and 0.2% L-proline.

Enzyme assays. The assays for proline oxidase and P5C dehydrogenase have been described previously (5). The latter was modified to include 20

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TABLE 1. *Strains of Salmonella typhimurium 15-59*[a]

Strain	Pertinent genotype	Reference or derivation
AR	Wild type	B. Magasanik
AR27	*putA26*	(5)
AR28	*putB27*	(5)
AR63	*putC1*	(5)
AR65	*putC3*	(5)
AR74	*putC3,P61*	(5)
AR111	*putC3,B86*	(5)
AR182	*putC3,P108*	Spontaneous mutation of AR65
AR183	*putC1,P109*	Spontaneous mutation of AR63
AR185	*putC3,P111*	Spontaneous mutation of AR65

[a] Symbols used are: *putA*, loss of proline oxidase activity; *putB*, loss of P5C dehydrogenase activity; *putC*, constitutive synthesis of the proline-degrading enzymes; *putP*, pleiotropic loss of the proline-degrading enzymes.

mM $MgCl_2$. The P5C reductase assay is an adaptation of that described by Yura and Vogel (15). The decrease in absorbance at 340 nm resulting from the oxidation of reduced NADP was followed by using a Gilford 2400 recording spectrophotometer. Assays were run at 37 C. The complete system contained 0.05 ml of 1 M potassium phosphate buffer at pH 7.0, 0.15 ml of 2 mM NADPH, 0.06 ml of 0.0195 M P5C, extract, and water to 0.5 ml. After 4 min of preincubation the reaction was initiated by the addition of extract. Activity was determined by correcting for the rate observed when the reaction mixture contained no extract. Specific activity is expressed as micromoles of NADP formed per minute per milligram of protein. Protein concentration was estimated by the method of Lowry et al. (7) with lysozyme as the standard.

Genetic analysis. The preparation of phage lysates, transduction procedures, and the spot test for constitutivity have been described (5).

RESULTS

Induction of the enzymes of proline degradation. Cells of *S. typhimurium* grown in the presence of proline have a high level of the two proline-degrading enzymes, proline oxidase and P5C dehydrogenase (5). In an attempt to find a gratuitous inducer, many homologues and substituted derivatives of proline were screened for the ability to induce proline oxidase: 4,5-dehydro-L-pipecolic acid, N-acetyl DL-proline, 4-hydroxy L-proline, thioproline, L-pyro-glutamic acid, L-prolyl-β-naphthylamide hydrobromide, pyrroline-2-carboxylic acid, 5-phenyl-2-pyrrolepropionic acid, L-2-pyrrolidone, DL-pipecolic acid, L-2-azetidine-carboxylic acid, tropacocaine hydrochloride, atropine, diethyl 2,4-dimethylpyrrole-3,5-dicarboxylic acid, scopolamine hydrobromide trihydrate, and homatropine hydrobromide. Of the compounds tested, only 4,5-dehydro-L-pipecolic acid (baikiain) induces

proline oxidase. It gives only 13% of the level obtained when proline is used as inducer. Figure 1 shows the specific activity of proline oxidase induced by various concentrations of proline or baikiain. The maximum level induced by proline is achieved at a concentration of 5 mM. When baikiain is the inducer, even at the high concentration of 100 mM, this maximum level is not reached.

To see whether this low inducible level could be raised by growth for many generations in the presence of baikiain, cells were grown overnight in SN medium plus 20 mM baikiain. They were then diluted into the same medium and allowed to grow for several generations before being assayed. The same specific activity was obtained, whether the inoculum was cells grown in the presence or absence of baikiain.

Cells grown in the presence of baikiain were assayed for activity of the other proline-degrading enzyme, P5C dehydrogenase. Table 2 shows that baikiain does induce this enzyme, and, like its induction of proline oxidase, the level is low compared to the level induced by proline. Baikiain at a concentration of 20 mM results in about 10% of the maximum proline-
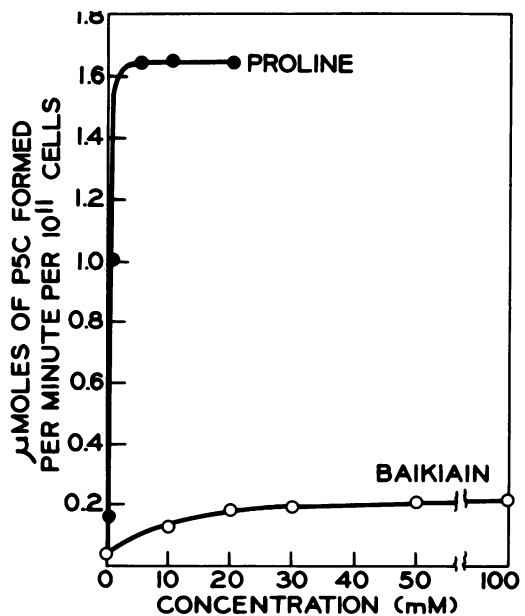

FIG. 1. *Induction of proline oxidase as a function of proline or baikiain concentration. The cultures assayed were grown for at least five doublings on SN medium containing various concentrations of inducer. Assay conditions are those described in Materials and Methods.*

induced level; proline oxidase is induced approximately to the same extent.

Baikiain also induces proline oxidase in *E. coli*. Like the induction in *S. typhimurium*, this level is low compared to the level induced by proline. The specific activity of proline oxidase in two strains, *E. coli* W and *E. coli* K-12, is shown in Table 3.

Studies to determine the mechanism of induction. Baikiain might induce the proline-degrading enzymes directly, by mimicking the natural inducer, or it might induce indirectly, e.g., as a result of derepressing the proline biosynthetic enzymes. In *E. coli* K-12, strains excreting proline are partially constitutive for the degradative enzymes (4). This latter mechanism of induction has been demonstrated in the histidine degradative system (11). Induction of these enzymes by a histidine analogue, α-methylhistidine, is a result of derepression of the biosynthetic enzymes. Cells grown in the presence of this analogue have high levels of the anabolic enzymes, which result in histidine excretion and consequent induction of the catabolic enzymes. To see whether baikiain induces similarly, by derepressing the proline

TABLE 2. *Induction of the proline-degrading enzymes by baikiain*

Additions to growth medium[a]		Specific activity	
Proline	Baikiain	Proline oxidase	P5C[b] dehydrogenase
+	−	1.65	22
−	−	0.04	1
−	+	0.18	2

[a] Proline or baikiain was added to SN medium at a final concentration of 20 mM.

[b] P5C, Δ^1-pyrroline-5-carboxylate.

TABLE 3. *Induction by baikiain of proline oxidase in Escherichia coli*

Strain	Additions to growth medium[a]		Specific activity of proline oxidase
	Proline	Baikiain	
E. coli W	−	−	0.03
	+	−	1.76
	−	+	0.12
E. coli K-12	−	−	0.04
	+	−	1.85
	−	+	0.16

[a] Cells were cultivated in SN medium plus 16 mM proline or 20 mM baikiain where indicated.

biosynthetic enzymes, we attempted to look at the biosynthetic reactions.

Glutamic acid kinase presumably catalyzes the first step of proline biosynthesis in *E. coli* W (1). Attempts to duplicate the glutamic acid kinase assay with *S. typhimurium* were unsuccessful. The activity of P5C reductase, catalyzing the final step in proline biosynthesis, was determined under various conditions. Before determining whether baikiain derepresses the synthesis of this enzyme, assays were performed to determine whether proline has any effect on its synthesis. In *E. coli* W the synthesis of this enzyme is not repressed by 0.01% proline (2). In repeated assays, the specific activity of cells grown in the presence of 0.2% proline was lower than that of cells grown without proline. Levels ranged between 53 and 80% of the level in cells grown without end product. Table 4 shows the average values from four such assays. Both proline, at a concentration of 10 mM, and baikiain, at a concentration of 40 mM, were tested as inhibitors of P5C reductase. Neither had an effect.

Cells grown in the presence of 20 mM baikiain have a lower specific activity of P5C reductase than cells grown without baikiain (Table 4). Thus, baikiain does not derepress the synthesis of this biosynthetic enzyme but, rather, represses it. Although the effect of baikiain on the synthesis of P5C reductase was determined, it is not possible to extrapolate this information to the enzyme(s) mediating the conversion of glutamate to P5C. The locus determining P5C reductase, *proC*, cannot belong to the same operon as the loci involved in the conversion of glutamate to P5C (*proA* and *proB*) because in *S. typhimurium* the attachment site for the phage P22 is located between *proB-proA* and *proC* (10).

In another attempt to gain some insight into the mechanism of induction by baikiain, the initial kinetics of induction were measured. We wished to contrast the length of the lag

TABLE 4. *Effect of proline or baikiain on the synthesis of the proline biosynthetic enzyme Δ^1-pyrroline-5-carboxylate (P5C) reductase*

Additions to growth medium[a]		Specific activity of P5C reductase
Proline	Baikiain	
−	−	0.56
+	−	0.40
−	+	0.41

[a] Where indicated, 16 mM proline or 20 mM baikiain was added to SN medium.

periods upon induction by proline and induction by baikiain. When baikiain is added to an exponentially growing culture at a final concentration of 50 mM, proline oxidase activity remains at the uninduced level for 5 min and then begins to rise. With 16 mM baikiain as inducer, the length of the lag period before enzyme appearance is the same. The initial rate of enzyme synthesis was likewise the same with the two inducer concentrations (Fig. 2). With proline as the inducer, proline oxidase activity starts to rise after 3 min (Fig. 2). If baikiain itself is not the actual effector, the conversion to the effector must be rapid.

Inhibition of P5C dehydrogenase. In addition to being tested as an inducer, baikiain was also tested as an inhibitor of proline oxidase and P5C dehydrogenase. P5C dehydrogenase was assayed by first adding the analogue at a concentration of 20 mM and then adding the substrate, P5C, at a concentration of 3.9 mM. Baikiain inhibits this enzyme. Enzyme activity as a function of substrate concentration in the presence and absence of baikiain was measured. The data, plotted by the method of Lineweaver and Burk (6), suggest that baikiain is a competitive inhibitor of P5C dehydrogenase (Fig. 3). This is not surprising because baikiain is a structural analogue of P5C as well as of proline.

Baikiain as a substrate of proline oxidase. A culture of proline-induced cells was used to test baikiain as an inhibitor of proline oxidase. Baikiain was added at a final concentration of 0.1 M with three concentrations of substrate used: 0.45 M, 4.5×10^{-2} M, and 4.5×10^{-3} M. With the two higher concentrations of proline, no inhibition by baikiain was observed. With 4.5×10^{-3} M proline, a pink color was observed rather than the usual yellow color resulting from the complex of o-AB and P5C, the product of proline oxidation.

When uninduced cells were assayed in a similar manner, no pink color was seen. This
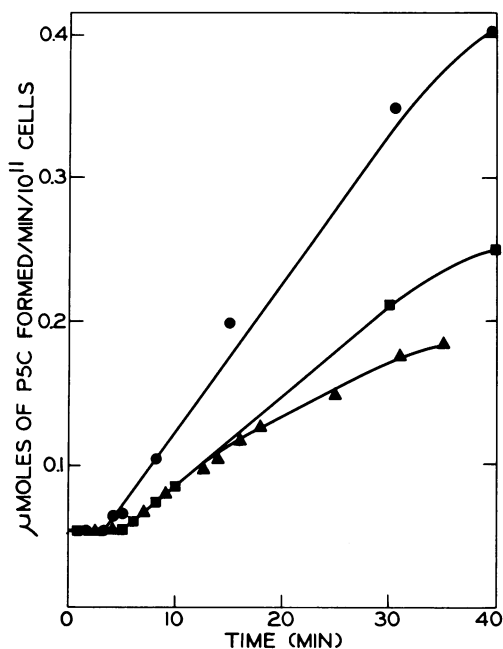

FIG. 2. *Formation of proline oxidase after addition of baikiain or proline to the growth medium. Cultures were growing exponentially with 0.2% sodium succinate and 0.2% ammonium sulfate. Baikiain or proline was added at zero time. At the indicated times, 1.5-ml samples were removed and added to tubes containing sodium azide (final concentration, 5.0 mM) at 0 C. After all samples were collected they were assayed for proline oxidase activity. Symbols:* ●, *10 mM proline;* ■, *50 mM baikiain;* ▲, *16 mM baikiain.*
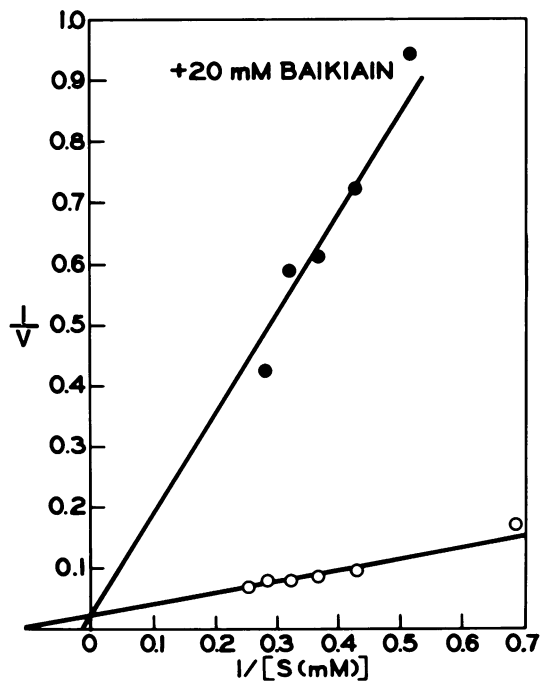

FIG. 3. *Lineweaver-Burk plot of P5C dehydrogenase activity and inhibition by baikiain. The assay for enzymatic activity was performed on a crude extract of S. typhimurium grown on SN medium supplemented with 0.2% proline. The assay is that described in Materials and Methods but without* $MgCl_2$. *Velocity is expressed as nanomoles of reduced NADP formed per minute per milligram of protein.*

suggested that the pink color results from a complex consisting of o-AB and the oxidation product of baikiain, resulting from catalysis by proline oxidase. To test this hypothesis, strain AR27, deficient in proline oxidase activity, was assayed. In this experiment, the usual assay was performed but with 0.1 M baikiain as the substrate rather than proline. Cultures of strain AR27, both induced and uninduced, yielded no pink color; induced and uninduced wild-type cells resulted in a pink color with the former but not the latter. These results suggest that baikiain is a substrate of proline oxidase.

The ability of baikiain to serve as substrate suggested the possibility that the oxidized derivative, rather than baikiain itself, might be the actual inducer. If so, baikiain should be unable to induce P5C dehydrogenase in a strain deficient in proline oxidase activity. Strain AR27 was therefore assayed for induction by baikiain. In the wild-type strain, baikiain causes approximately a twofold increase over the basal level of P5C dehydrogenase. The basal level in strain AR27 is less than that found in the wild-type strain. Because of the limitations of this assay, the low basal level of strain AR27 and a twofold increase cannot be distinguished. Therefore, it is impossible at this time to determine whether or not baikiain induces P5C dehydrogenase in a proline oxidase-deficient strain.

Growth inhibition of constitutive strains. The finding that baikiain is a substrate for proline oxidase perhaps correlates with a previously made and unexplained observation: the growth of strains constitutive for proline oxidase is inhibited by baikiain. At a concentration of 5×10^{-4} M, baikiain completely inhibits growth on SN medium of the constitutive strain, AR65.

As Table 5 shows, it is the constitutive presence of proline oxidase that is required for baikiain to exert its inhibitory effect. Constitutive strains (AR65 and AR63) but not the wild-type strain are inhibited. The presence of P5C dehydrogenase activity is not necessary for inhibition since a double mutant, strain AR111, which is constitutive for proline oxidase and lacks P5C dehydrogenase activity is also inhibited by baikiain. Because baikiain is a substrate of proline oxidase, it is perhaps inhibitory by virtue of its conversion via proline oxidase to a toxic derivative. The lack of toxicity under conditions where baikiain induces the oxidase in the wild-type strain could be due to the low level of induction.

Inhibition is overcome by proline. On SN

TABLE 5. *Inhibition by baikiain of the growth of various strains altered in proline degradation[a]*

Strain	Phenotype	Growth
AR	Wild type	+
AR65	PutC	−
AR63	PutC	−
AR111	PutC,B	−
AR74	PutC,P	+
AR27	PutA	+
AR28	PutB	+

[a] A test tube containing 5 ml of SN medium supplemented with 5 mM baikiain was inoculated with 0.15 ml of an overnight culture grown on SN medium. "+" Indicates turbidity after 12 hr of incubation at 37 C. Strain AR65 is fully constitutive, and strain AR63 is partially constitutive (approximately 16% of the induced wild-type level).

medium containing 10^{-3} M baikiain, the presence of 8×10^{-4} M proline allowed growth.

Isolation of strains resistant to baikiain. SN medium supplemented with 5 mM baikiain will not support growth of the constitutive strain, AR65. After approximately 30 hr of incubation, however, a population begins to grow up. A loopful of culture was streaked onto LB agar, and subsequently one colony was picked and purified. This strain, AR182, was then examined for growth and enzymatic properties. It grows as well as the parent strain on SN medium but does not grow in SP medium. When assayed for the proline-degrading enzymes, there was no detectable level of proline oxidase nor of P5C dehydrogenase activity.

Two more independently isolated baikiain-resistant mutants were selected from a constitutive population in the following way. Two tubes of SN medium (5 ml/tube) supplemented with the analogue to a final concentration of 5 mM were inoculated with 0.15 ml of an overnight culture of a constitutive strain, one with AR65 and the other with AR63. The tubes were incubated at 37 C until turbid. A loopful of culture from each tube was streaked onto LB agar, and subsequently one colony from each was picked and purified.

All three of the resistant strains, AR182, AR183, and AR185, grow normally on SN medium but do not grow on SP medium. All are pleiotropic-negative since they fail to show any detectable level of either proline oxidase or P5C dehydrogenase activity.

Linkage analysis of baikiain-resistant strains. By means of transduction, genetic crosses were performed to determine the linkage relationship between the sites of the pleiotropic-negative mutations and the consti-

tutive mutations. In each cross, phage particles propagated on the wild-type strain served as donor, and one of the baikiain-resistant strains, unable to utilize proline, served as recipient. Recombinants were selected on SP agar and then spot-assayed for the constitutive phenotype.

The results (Table 6) indicate that for strain AR182 (*putC3, P108*) the site of the mutation resulting in a pleiotropic-negative phenotype (*putP108*) is 82% co-transduced with *putC3*. For the other two strains, this site is 100% co-transduced with the constitutive site (either *putC3* or *putC1*). The three mutations resulting in the inability to degrade proline are each linked by transduction to mutations producing a constitutive phenotype, and are thus linked to the *put* region (5). In the latter two crosses (crosses 2 and 3) between presumptive double mutants (since a back mutantiontion would confer an inducible, rather than pleiotropic-negative, phenotype) and the wild-type strain, the constitutive mutation was not recovered from almost 200 recombinants per cross. No revertants were observed on either of the two control plates containing each of the recipients without phage. These two results suggest but do not prove (since there was no selection for a crossover between the two sites) that for strains AR183 and AR185 the pleiotropic-negative phenotype might result from a deletion mutation which covers the site that results in constitutivity.

DISCUSSION

An analogue of proline, baikiain, induces *S. typhimurium* to form the enzymes required for proline degradation. The natural effector of the synthesis of these enzymes appears to be proline (5), although P5C, the product of the initial reaction, also induces, perhaps by reduction to proline via the biosynthetic enzyme P5C reductase.

The inducer baikiain is a substrate for the first enzyme in the pathway, proline oxidase. This suggests that baikiain might induce only by virtue of its conversion to an oxidized derivative. Were the derivative the effector, baikiain would not induce P5C dehydrogenase in a strain lacking proline oxidase activity. We have only one mutant strain in our collection that is deficient in this enzyme activity while retaining P5C dehydrogenase activity. Since its basal level is very low and since baikiain is a poor inducer, it is not possible, by the assay used, to determine whether or not the analogue induced P5C dehydrogenase in this strain.

The oxidized derivative of baikiain, forming a pink complex with o-AB, was not identified. By analogy to the oxidation product of proline, one might expect 1,4,5,6-dehydro-L-pipecolic acid to be the oxidation product. Furthermore, a compound forming a colored complex with o-AB suggests a Δ^1-pyrroline or Δ^1-piperideine (12). Δ^1-Piperideine-6-carboxylate (1,6-dehydro-pipecolic acid) gives an orange-yellow color with o-AB (3). Thus, a likely possibility is 1,4,5,6-dehydro-pipecolic acid. The product of proline oxidase activity with baikiain as a substrate should be purified, analyzed, and tested directly for its effects on proline metabolism.

For both proline-degrading enzymes, baikiain gives only 10% of the level obtained when proline is used as inducer. This poor induction could be explained by the fact that baikiain is metabolized. Or it could be the result of an indirect mechanism in which baikiain interferes with the regulation of proline biosynthesis so as to cause an over-production; the excess proline would then act as the inducer. α-Methylhistidine which induces the histidine-degrading enzymes by such a mechanism elicits only 10% of the level induced by histidine (11). A third possibility is that baikiain does not bind to the repressor as well as does the natural effector.

If baikiain induces by a direct mechanism, irrespective of whether the effector is baikiain itself or a derivative, poor induction might be expected. If baikiain is the effector, the fact that it is metabolized could result in low en-

TABLE 6. *Linkage of the mutation sites of baikiain-resistant strains to the put region*

Cross	Donor		Recipient		No. of transductants of unselected phenotype[a]			Percent cotrans-duction
	Strain	Phenotype	Strain	Phenotype	PutC3	PutC1	PutC+	
1	AR	C+,P+	AR182	C3,P108	32		142	82
2	AR	C+,P+	AR183	C1,P109		0	181	100
3	AR	C+,P+	AR185	C3,P111	0		182	100

[a] In all crosses the selected phenotype was PutP+.

zyme levels. However, one might then expect a high baikiain-induced level of P5C dehydrogenase in strain AR27, the proline oxidase-deficient strain. If a derivative is the effector, and assuming that baikiain is a relatively poor substrate of proline oxidase, the low level of induction could result from the low concentration of effector.

If induction by baikiain is a consequence of derepression, one expects to find high levels of the proline biosynthetic enzymes in cells grown in its presence. Baikiain does not derepress the synthesis of P5C reductase, the biosynthetic enzyme catalyzing the final step. It was not possible to assay the enzyme which presumably catalyzes the initial biosynthetic reaction. Proline was not detected in the culture medium of cells grown in the presence of baikiain. However, derepression cannot be excluded as the mechanism by which baikiain induces. Possibly, it could effect the synthesis of enough proline to induce the degradative enzymes but not enough to be excreted in an amount detectable by the method used.

Since baikiain inhibits P5C dehydrogenase, another mechanism of induction must be considered. Inhibition of this second enzyme could bring about an accumulation of P5C, produced from endogenous proline by the basal level of proline oxidase. The P5C would then serve as the inducer. This interpretation is not in agreement with a previous observation (5). Strains in which the conversion of P5C to glutamate is blocked by mutation do not synthesize proline oxidase constitutively. Therefore, this possible mechanism of induction can be ruled out.

When the initial kinetics of induction are examined after baikian is added, the increase in proline oxidase activity is apparent at 5 min after addition of baikiain. A 3-min lag is noticed when proline is the inducer. This slight difference could be the result of baikiain being taken up by the cells at a slightly slower rate than proline; or a small, basal-level amount of proline oxidase might convert baikiain to an effector which then will induce. The initial rate of proline oxidase induction by baikiain is almost as rapid as that with 10 mM proline as the inducer; however, the rate quickly decreases and levels off. This might be the result of the internal baikiain pool being depleted by the newly formed proline oxidase. However, a proline oxidase-deficient mutant (strain AR27) does not exhibit high P5C dehydrogenase activity when baikiain is added to the medium.

Other than inducing the proline-degrading enzymes, baikiain also inhibits the growth of strains constitutive for proline oxidase. This inhibition occurs in the presence or absence of P5C dehydrogenase activity. Proline overcomes inhibition.

Either baikiain or a derivative might have a specific effect on the regulation of proline biosynthesis resulting in a lower level of proline. The degradation of endogenous proline, mediated by the proline oxidase present in sufficient amount in constitutive strains but not in the baikiain-induced wild-type strain, would result in proline starvation. Addition of proline would therefore overcome this inhibition. However, it could be that exogenous proline simply prevents baikiain from getting into the cell.

Because baikiain is a substrate for proline oxidase, the production of a toxic derivative is a likely mechanism of growth inhibition. Proline would overcome inhibition because, assuming that proline oxidase has a greater affinity for proline than for baikiain, it would prevent conversion of baikiain, via proline oxidase, to the toxic compound. Or again, proline could simply prevent entry of baikiain into the cell.

Three strains resistant to baikiain were isolated from constitutive strains and characterized enzymatically. They are all of one phenotype, being pleiotropic-negative for the proline-degrading enzymes. The possibility that these resistant strains are defective in a permease required for the entry of both proline and baikiain can be eliminated since the parent strain is constitutive. Although the mechanism of toxicity is not known, the use of baikiain provides a powerful selection technique. It provides a simple and direct selection for strains unable to degrade proline. In addition, it allows the selection of strains with an inducible rather than constitutive phenotype.

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