BIOSYNTHETIC STUDY OF LEUHISTIN, A NEW INHIBITOR OF AMINOPEPTIDASE M

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As reported in our previous paper[1,2], leuhistin, a new inhibitor of aminopeptidase M, has been isolated from the culture broth of *Bacillus laterosporus* BMI156-14F1. The structure of leuhistin was determined to be (2*R*,3*S*)-3-amino-2-hydroxy-2-(1*H*-imidazol-4-ylmethyl)-5-methylhexanoic acid (Fig. 1). In this paper, we will report the biosynthetic pathway of leuhistin by the addition effects of several amino acids and incorporation of ^{14}C-labeled compounds.

L-[*U*-^{14}C]Leucine (333.0 mCi/mmol), L-[1-^{14}C]-leucine (54.4 mCi/mmol), L-[*U*-^{14}C] histidine (310 mCi/mmol), L-[1-^{14}C]histidine (52.2 mCi/mmol) and Aquazol-II were purchased from New England Nuclear, Boston, U.S.A. The seed culture was obtained as reported previously[1]. Two ml of the seed culture were inoculated into 110 ml of the basal medium consisting of glycerol 1.5%, Pharmamedia 1.0%, dry yeast 1.2% and $CaCO_3$ 0.2% (pH 7.0 before sterilization) in a 500-ml Erlenmeyer flask. Feeding compounds were added to the production medium before the pH adjustment. The results of the fermentation are shown in Tables 1 and 2.

The seed culture (2 ml) was inoculated into the medium consisting of glycerol 1.5%, Pharmamedia 1.0%, dry yeast 1.2%, L-leucine 0.2%, L-histidine·HCl 0.2% and $CaCO_3$ 0.2% (pH 7.0 before sterilization) in a 500-ml Erlenmeyer flask, and cultured 15 hours. ^{14}C-labeled compounds at 3 μCi were added and cultured for 48 hours. Fifty ml of culture filtrate were adsorbed on a 20-ml of Amberlite IRC-50 (free acid form) column, which was washed with water and eluted with 1 N ammonium hydroxide. The eluate was concentrated under reduced pressure to give brownish powder. The powder was dissolved in 1 ml of MeOH, and the solution was applied to a TLC plate of silica gel (E. Merck, Art. No. 5744) and developed with the solvent system: butanol - acetic acid - water (2 : 1 : 1). Leuhistin was visualized by spraying plates with 0.4% solution of ninhydrin in acetone. The area containing leuhistin was cut out from the plate and transferred to vials for measuring of radioactivity. Eight ml of Aquasol-II were added and the radioactivity of each sample was measured in a Beckman LS9800 liquid scintillation counter.

Taking account of the structure of leuhistin, amino acids and compounds related to L-histidine were tested. Addition of L-isoleucine, L-valine, L-ornithine·HCl, L-arginine·HCl and imidazole had essentially no effect. In contrast, addition of L-leucine and L-histidine·HCl had some effect, the production of leuhistin was enhanced 5-fold in the presence of 0.5% of L-leucine and 0.5% of L-histidine·HCl. The addition of D-forms of each amino acids had negative effects of production of leuhistin (Table 1). So the additive effects of L-forms of the two amino acids were studied in detail.

As summarized in Table 2, the addition of 1.0% of L-leucine and 0.2% of L-histidine·HCl afforded

Fig. 1. Structure of leuhistin.

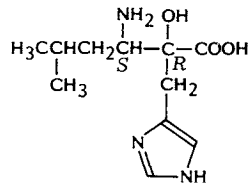

Table 1. Effect of D- or L-leucine and D- or L-histidine·HCl on production of leuhistin.

Histidine·HCl	Leucine		
	No addition	0.5% of D-Leu	0.5% of L-Leu
No addition	37[a]	22	55
0.5% of D-His	12	13	28
0.5% of L-His	46	22	180

[a] μg/ml.
Cultivation time: 65 hours.

Table 2. The relationship between the production of leuhistin and amount of L-leucine and L-histidine·HCl.

L-Histidine·HCl (%)	L-Leucine (%)				
	0	0.2	0.5	1.0	1.5
0	38[a]	55	64	75	84
0.2	72	126	152	215	190
0.5	66[b]	126[b]	191	175	133
1.0	54	134	103	103	75
1.5	74[b]	118	84	77	81[b]

[a] μg/ml.
Cultivation time: 64 hours ([b] 88 hours).

Fig. 2. Biosynthetic pathway of leuhistin.

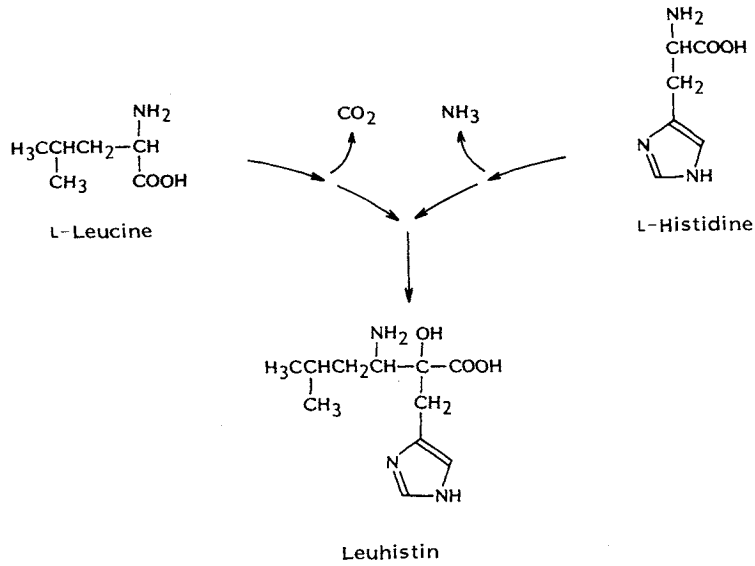

Table 3. Incorporation of ^{14}C-labeled compounds into leuhistin.

Labeled compounds	Incorporation (%)
L-[*U*-^{14}C]Leucine	1.80
L-[1-^{14}C]Leucine	0.16
L-[*U*-^{14}C]Histidine	2.17
L-[1-^{14}C]Histidine	2.55

the highest production of leuhistin (nearly six times the control value). These results indicate that L-leucine and L-histidine are precursors of leuhistin. In order to investigate the formation of the ^{13}C-^{13}C bond between the L-leucine moiety and the L-histidine moiety in leuhistin, the incorporation of ^{14}C-labeled compounds was examined. The percentage of incorporation of L-[1-^{14}C]leucine to leuhistin was less than 10% of that of L-[*U*-^{14}C]leucine, L-[*U*-^{14}C]histidine and L-[1-^{14}C]histidine (Table 3).

This result indicates that the carboxyl group of L-leucine was eliminated during the formation of leuhistin. Elimination of the carboxyl group of the *C*-terminal amino acid was demonstrated in the case of arphamenin[3]. The amino group of L-histidine may also be similarly eliminated.

These results suggest that leuhistin is formed from L-leucine and L-histidine, and that its biosynthesis includes the elimination of the carboxyl group from L-leucine and the amino group from L-histidine (Fig. 2).

References

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