CHARACTERIZATION OF RECOMBINANT AMINOACYLASE FROM *ESCHERICHIA COLI*

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Introduction

Aminoacylases – (N-acylamino acid amidohydrolase, EC 3.5.1,14) catalyzes N-acylated amino asids to yield the acyl group and the corresponding amino acid. Acylases are widespread in nature. This enzyme is found in the kidneys of animals and is produced by some fungi, yeast and other microorganisms. Most isolated aminoacylases show a substrate specificity towards N-acetyl-L-amino acids, including ones isolated from hog kidney [1], *Aspergillus oryzae* [2], *Alcaligenes denitrificans* DA181 [3], *Bacillus stearotermophilus* [4], *Lactococcus lactis* MG1363 [5], and *Burkholderia* sp. strain LP5_18B [6]. In addition, the genes for enzymes such as those from *Bacillus stearotermophilus, Lactococcus lactis* MG1363 and *Burkholderia* sp. strain LP5_18B have recently been analyzed. In practice, aminoacylases are widely used on an industrial scale to obtain optically active amino acids [7].

The aim of this work is to study the substrate specificity of the recombinant inracellular aminoacylase from *Escherichia coli* LGE 36 and some of its properties.

Materials and methods

Recombinant strain-producer of aminoacylase *E. coli* LGE 36 [8] was used in this work. The cells were grown in M9 minimal medium with supplements at 37^0C. The cells were disintegrated by sonication in 100 mM Na, K-phosphate buffer, pH7,0, containing 0,2mM $CoCI_2$ and 1 mM phenylmethylsulfonyl fluoride (PMSF). The cell debris was removed by centrifugation and the resulting supernatant was used for experiments.

The aminoacylase activity was determined by the modified method of Gade and Brown [9]. In the reaction medium of 200 µl of final volume, containing 100 mM Na, K-phosphate buffer, pH7,0, 0,2mM $CoCI_2$, 40 mM N-acetyl-D, L-methionine and enzyme in the required amount, at 37^0C. The unit of acylase activity was defined as the amount of the enzyme catalyzing the formation of 1 µmol of L-amino acid per min. Protein was determined by method of Lowry [10].

All chemicals were purchased from Sigma-Aldrich and Reanal.

Results and Discussion

Earlier we have developed the method for obtaining (isolation and purification) the recombinant intracellular aminoacylase of *E. coli* by twofold ion exchange chromatography on DEAE-Cellulose [11]. The investigated enzyme has shown that it is a dimer, composed of two identical subunits with the molecular mass of 42 kDa for each. It has a pH optimum of 7,0.

The substrate specificity of the aminoacylases from various soucers has been briefly described, but we have reexamined the specificity of the enzyme for a wide variety of N-acyl amino asids and derivatives. A number of N-acyled amino acids were tested as possible substrates for aminoacylase preparations. Table 1 summarizes the substrate specificity of the recombinant intracellular aminoacylase from *E. coli* LGE36. The enzyme from *E. coli* catalyzed the hydrolysis of most of the α-N-acetyl-L-amino acids. The recombinant aminoacylase showed high activities, particullarly towards N-acylated amino acids, such as N- acetyl- L-methionine, N- acetyl- L- alanine, N- acetyl- L-valine, N-acetyl-L-leucine, while activities for N-acetyl-L-serine was low. It had very low activity for N-acetyl -L-aspartic acid and N-acetyl-L-glutamic acid. Under the conditions employed only α-N-acetyl-L-ornithine and α-N-acetyl-L-lysine were found to be deacetylated at a higher rate. The relative activity of hydrolysis of α-N-acetyl-L-lysine is threefold efficient, whereas that of α-N-acetyl-L-ornithine is fivefold better than N-acetyl-L-methionine substrate. Perhaps, the best substrates for the aminoacylase are α-N-acetyl- L-ornithine and α-N- acetyl-L-lysine.

The enzyme exhibits absolute stereospecificity for acylated L-amino acids, being unable to hydrolyze N-acyl-D-amino acids. The rate of deacylation of N-acylated amino acid substrates is sensitive to the nature of the amino acid, with amino acids containing aromatic side chains, for example, N-acetyl-L-phenylalanine showing no activity. When incubated with this enzyme the chloroacetyl derivatives of

phenylalanine were moderately hydrolyzed. Besides acyl amino acids, hydrolytic activity (peptidase activity) of the recombinant aminoacylase from *E. coli* towards dipepdides such as glycyl-L-methionine was also studied. As a result, it was revealed that glycyl-L-methionine was rapidly hydrolyzed.

Table 1. **Substrate specificity of the intracellular recombinant aminoacylase from *E. coli* LGE 36.**

Substrate	Relative activity*, %
N-Acetyl-D,L-methionine	100
N-Acetyl-D-methionine	0
N-Acetyl-D,L- alanine	64
N-Acetyl-L -alanine	70
N-Acetyl-D -alanine	0
N-Acetyl-D,L-valine	60
N -Acetyl-D-valine	0
N-Acetyl-D,L-leucine	80
N-Acetyl-D-leucine	0
N-Acetyl-D,L-serine	35
N-Acetyl-L-aspartic acid	30
N-Acetyl -L-glutamic acd	15
a-N-Acetyl-L-lysine	300
a-N-Acetyl-L-ornithine	500
N-Acetyl-L-phenylalanine	0
N-Chloroacetyl-L-phenylalanine	28
Glycyl-L-methionine	200

*100% of relative activity corresponded to 1050 U/mg of the specific activity of the enzyme.

A number of studies have reported data on the substrate specificity of aminoacylases from various sources. Aminoacylases such as hog kidney [1] and bovine liver [12] show a very narrow range of substrate specificity. Aminoacylase from *Aspergillus oryzae* [2], which is produced industrially, shows substrate specificity for N-acetyl -L-phenylalanine, N-acetyl -L-methionine, N-acetyl -L-tryptophan, N-acetyl -L-alanine, but its specific activity is very low. Aminoacylases from *Alcaligenes denitrificans* DA181 [3] and *Pseudomonas maltophila* B1 [12] show high specific activity, but the substrate specificity is narrow. The enzyme from *A. denitrificans* DA181 preferentially hydrolyses N-acetyl -L-alanine and N-acetyl -L-valine. The enzymes from *B. stearotermophillus* and *L. lactis* MG1363 have substrate specificity towards N-acetyl -L-amino acids with hydrophobic amino acid residues. Obtained data indicated that the recombinant intracellular aminoacylases from *E. coli* show wider substrate specificity than those from the other sources, as described above. The investigated enzyme effectively catalyzes the hydrolysis of most N-acetyl -L-amino acids, including those of basic amino asid. Among the best substrates, we find acetyl derivatives of two basic amino acids – L-ornithine and L- lysine.

Conclusions

In this study, we report on characterization of the recombinant intracellular aminoacylase from *E. coli* LGE36 with high levels of aminoacylase activity and wide substrate specificity. The *E. coli* aminoacylase showed higher activity towards α-N-acetyl-L-ornithine and α-N-acetyl-L-lysine rather than towards N-acetyl-L-methionine. The comparison of the substrate specificity of the recombinant intracellular aminoacylase from *E. coli* with other members of the aminoacylase family suggests an origin of the obtained enzyme.

References:

1. Birnbaum S.M., Levintow L., Kingsley R.B. and Greenstein J.P. 1952, *J.Biol.Chem*.,194,455-470.
2. Gentzen I, Loffler H.G. and Schneider F. 1980 Z. Naturforsch., 35 c, 544-550.
3. Yang Y.B., Hu H.L., Chang M.C., Li H. and TsaiY.C., 1994*, Biosci. Biotechnol. Biochem*., 58, 204-205.
4. Sakanyan V., Desmarez L., Legrain C., Charlier D., Mett I., Kochikya A., Savchenko A., Boyen A., Falmagne P., Pierard A. and Glansdorff N.1993, *Appl. Environ. Micbiol*., 59, 3878-3888.
5. Curley P., Van Der Does c., Driessen A. J., and Van Sinderen D. 2003, *Arch.Microbiol*., 179, 402-408.
6. Takakura Ya. and Asano Ya. 2019, *Biosci. Biotechnol. Biochem*., v. 83, No. 10, 1964-1973.
7. Aggarwal S., Sahni S. 2012*, International Conference on Enviromental, Biochemical and Biotechnology IPCBEE*, 41, 18-22.
8. Mett I. L., Mett A.L., Kochikyan A.V., Sakanyan V. A. and Ivanov A.S. 1987, *USSR patent* 1, 510, 360.
9. Gade W., Brown J.L. 1981*, Biochem. Biophys. Acta*, 662, 86-93.
10. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951, *J.Biol. Chem.,* 193, 265-275.
11. Yepremyan H. S., Artsruni G.K. 2011*, Abstracts of the 6th Moscow Intarnational Congress "Biotechnology: state of the art and properties of development",* Moscow, March 16-20,128.
12. Wakayama M., Shiiba E., Sakai K., and Moriguchi M. 1998, *J. Ferment. Bioeng*., 85, 278-282.