Identification of the optical isomers of the amino acids in *Polysphondylium violaceum* acrasin

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Using optically specific enzymes on the hydrolysate of glorin, we show the structure of natural glorin to be:

N-propionyl–γ-L-glutamyl–L-ornithine–δ-lactam ethyl ester

Glorin structure *Optical isomer* Polysphondylium violaceum *acrasin*

1. INTRODUCTION

In [1] we identified the chemical structure of the acrasin of the cellular slime mold *Polyspondylium violaceum*. It is a dipeptide derivative made up of glutamic acid and ornithine (*N*-propionyl–γ-glutamyl–ornithine–δ-lactam ethyl ester) which we call glorin. The only missing information was whether the two amino acids existed in the L or the D form. We had the L–L form synthesized and its biological activity matched that of the natural glorin, but this still did not tell us which isomers were present in the glorin produced by the organism. Here we show, by the use of optically specific enzymes on the hydrolysate of glorin, that natural glorin unequivocally contains the L isomers of both glutamic acid and ornithine.

2. MATERIALS AND METHODS

The glorin used here was part of the material purified in [1]. The buffer solutions employed were 20 mM EPPS (pH 8.3), 20 mM MOPS (pH 7.5) and 20 mM sodium acetate (pH 5.0). The following chemicals were purchased from Sigma (St Louis MO): EPPS, MOPS, crystallized D-amino acid oxidase (DAO), L-amino acid oxidase type IV (LAO), L-glutamic decarboxylase type V (LGD), L-ornithine decarboxylase (LOD), D- and L-glutamic acid, and L-ornithine. D-Ornithine was obtained from the United States Biochemical Corp. and dansyl chloride from the Pierce Chemical Co. Before use, the $(NH_4)_2SO_4$ in the commercial DAO was removed by gel filtration through a small column of Sephadex G-25 equilibrated with the pH 7.5 MOPS buffer.

Glorin (24 μg) was hydrolyzed with 50 μl 5.5 N HCl at 105°C for 20 h in a sealed glass tube. The hydrolysate was equally divided into 5 test tubes (10 × 75 mm) and dried in a vacuum dessicator containing NaOH tables. The first of the 5 dried samples was dissolved in 50 μl pH 8.3 EPPS buffer containing 10 μg DAO; the second sample was dissolved in 50 μl pH 7.5 MOPS buffer containing 11 μg LAO; the third sample was dissolved in 50 μl pH 5.0 acetate buffer containing 5 μg LGD; the fourth sample was dissolved in 50 μl pH 5.0 acetate buffer containing 10 μg LOD; and the last sample was dissolved in 50 μl water. All 5 mixtures were capped with Parafilm and incubated at 30°C for 4 h, then evaporated to dryness under vacuum. The residue of each sample was dissolved in 30 μl 0.2 M $NaHCO_3$, followed by the addition of 30 μl 0.25% dansyl chloride in acetone. Each tube (covered with Parafilm) was then incubated at 30°C for 3 h to complete the dansylation.

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Control samples were prepared by treating the D- and L-forms of glutamic acid and ornithine (1.5×10^{-8} mol each) with the enzymes, followed by dansylation, under the same conditions as for the hydrolysate of glorin.

The dansylation products were analyzed by thin-layer chromatography (TLC) on Gelman ITLC Type SA sheets; 10 μl each of all dansylation mixtures prepared were spotted on one sheet, the sheet was developed with benzene/pyridine/acetic acid (80:20:2, by vol.) [2], then the spots were marked by fluorescence observed under a long-wavelength UV lamp.

Table 1

Treatment of glorin hydrolysate with optically specific enzymes

Sample	Enzyme[a]	Results[b]	
		Glu	Orn
Glorin hydrolysate	DAO	+ + +	+ + +
Glorin hydrolysate	LAO	+ + +	+
Glorin hydrolysate	LGD	−[c]	+ + +
Glorin hydrolysate	LOD	+ + +	+[d]
Glorin hydrolysate	None	+ + +	+ + +
L-Glu + D-Orn	DAO	+ + +	+
D-Glu + L-Orn	DAO	+ + +	+ + +
L-Glu + D-Orn	LAO	+ + +	+ + +
D-Glu + L-Orn	LAO	+ + +	+
L-Glu + D-Orn	LGD	−[c]	+ + +
D-Glu + L-Orn	LGD	+ + +	+ + +
L-Glu + D-Orn	LOD	+ + +	+ + +
D-Glu + L-Orn	LOD	+ + +	+[d]

[a] DAO, D-amino acid oxidase; LAO, L-amino acid oxidase; LGD, L-glutamic decarboxylase; LOD, L-ornithine decarboxylase

[b] According to the relative fluorescence intensity of dansyl derivatives after TLC as described in the text. R_f values: Glu 0.22, Orn 0.41. A sign of + + + indicates 4–5-times stronger fluorescence than +; a − sign means undetectable

[c] Fluorescent spot of the decarboxylation product of glutamic acid was at R_f 0.75

[d] Fluorescent spot of the decarboxylation product of ornithine was at R_f 0.87

3. RESULTS

Glorin was hydrolyzed with HCl, then the hydrolysate was treated with 4 kinds of optically specific enzymes to determine the configuration of the two amino acid constituents of glorin; i.e., glutamic acid and ornithine. The results are summarized in table 1, together with the results of control experiments carried out with the known samples of the optical isomers of glutamic acid and ornithine. The amount of ornithine in the glorin hydrolysate was greatly reduced by treating the hydrolysate with L-amino acid oxidase and with L-ornithine decarboxylase, but not with D-amino acid oxidase. Furthermore, the glutamic acid in the glorin hydrolysate was completely destroyed by treatment with L-glutamic decarboxylase. The glutamic acid in the hydrolysate and the control glutamic acid isomers were all found to be resistant to the action of the D- and L-amino acid oxidases.

These results clearly indicate that both the glutamic acid and ornithine in glorin are in the L-configuration. Thus, the structure of natural glorin is now established to be:

N-propionyl–γ-L-glutamyl–L-ornithine–δ-lactam ethyl ester

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