LYSINE- AND ARGININE-CONTAINING POLYPEPTIDES OF REGULAR STRUCTURE AND THEIR BACTERICIDAL PROPERTIES

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Synthetic polypeptides and copolymers containing the basic amino acids ornithine and lysine are powerful antimicrobial agents. The influence of this type of compounds on such organisms as Escherichia coli and Staphylococcus aureus apparently depends on their surface-active properties. In this respect, their biological activity may be compared with the activity of the natural antibiotics gramicidin C and tyrocidine [1]. The bactericidal properties of polymers with regular and irregular sequences of ornithine and leucine residues have been reported previously [2] (Table 1). At a concentration of 5-10 mg/ml, these polymers exhibited a bactericidal action on a culture of Staphylococcus aureus. The replacement of the ornithine residues in the regular polymer H(L-Leu−L-Orn−L-Leu)-OH by arginine residues by means of guanidination did not lead to a change in the biological properties of the polymer. However, this does not permit the conclusion that the arginine polymers are identical in their antibacterial properties with the ornithine polymers, since the guanidination of the polymer described took place to the extent of only 75%.

We have obtained more fundamental results by testing arginine-containing polypeptides synthesized biologically. The polypeptides have regular structures with the following amino acid sequences (−Gly−L-Ala−L-Arg−) (I), (−L-Lys−L-Arg−L-Ala−) (II), (−L-Lys−L-Arg−Gly−) (III).

Biological tests showed that polypeptide (I) exhibits bactericidal action on a culture of Staphylococcus aureus at a minimum concentration of 2 mg/ml, while polypeptides (II) and (III) did not possess bactericidal properties at this concentration. On this basis, it may be assumed that the bactericidal properties of the polypeptides are apparently closely connected with the nature of the amino acids of which they are composed and their sequence (see Table 1).

The polypeptide (−Gly−L-Ala−L-Arg−) was obtained by the polycondensation of the hydrobromide of the 2,4,5-trichlorophenyl ester of the tripeptide in dimethylformamide (DMFA) with 2.5 equivalents of

* Deceased.

TABLE 1. Inhibition of Size of Staphylococcus aureus Culture by Polypeptides of Regular Structure

Polypeptide	*M*	Minimum inhibitor concn., mg/ml
L-leucyl, L-ornithyl*	100 000	5—10
Poly (L-Leu-L-Orn-L-Leu)*	10 000	5—10
Poly (L-Leu-L-Arg-L-Leu)*	4000	5—10
Poly (Gly-L-Ala-L-Arg-)	10 000	2
Poly (L-Lys-L-Arg-L-Ala)	5312	≤10 Not active
Poly (L-Lys-L-Arg-Gly)	7000	≤10 Not active

*See [2].

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$N(C_2H_5)_3$. The hydrobromide of the tripeptide was synthesized by the carbodiimide method in the following way:

$$Z\text{-Gly-L-Ala-OH} \xrightarrow{+HBr\cdot N^G\text{-}NO_2\text{-L-Arg-}OPhCl_3} Z\text{-Gly-L-Ala-}N^G\text{-}$$

$$\text{-}NO_2\text{-L-Arg-}OPhCl_3 \xrightarrow{HBr/CH_3COOH} HBr\cdot\text{Gly-L-Ala-}N^G\text{-}NO_2\text{-L-Arg-}OPhCl_3.$$

The removal of the protective group (N^G-NO_2- in the case of arginine) from the polypeptide was effected over Pd for 20 h. Polypeptides with the sequences (—L-Lys—L-Arg—L-Ala) and (—L-Lys—L-Arg—Gly) were formed in the polycondensation of the hydrochlorides of the corresponding tripeptides, the hydrochlorides having been obtained by the mixed-anhydride method.

$$\overset{\alpha}{N}\text{-BOC-}\overset{\varepsilon}{N}\text{-Z-L-Lys-}N^G\text{-}NO_2\text{-L-Arg-OH} \xrightarrow{+HBr\cdot X\text{-}OPhCl_3} \overset{\alpha}{N}\text{-BOC-}\overset{\varepsilon}{N}\text{-}$$

$$\text{-Z-L-Lys-}N^G\text{-}NO_2\text{-L-Arg-X-}OPhCl_3 \xrightarrow{HCl/C_2H_5OH} HCl\cdot\overset{\varepsilon}{N}\text{-L-Lys-}$$

$$\text{-}N^G\text{-}NO_2\text{-L-Arg-X-}OPhCl_3,$$

where X represents L-Ala or Gly; Z represents a benzyloxycarbonyl group; BOC represents a tertiary-butyloxycarbonyl group; and $-OPhCl_3$ represents a trichlorophenyl ether group.

The protective groups ($N^\varepsilon-Z$ in the case of lysine and N^G-NO_2 in the case of arginine) were eliminated from the peptides by successive hydrobrominolysis (45 min) and hydrogenation over Pd (7-10 h). Sephadex G-25 (medium) was used for freeing the polypeptides from low-molecular-weight impurities.

EXPERIMENTAL

The hydrobromide of the 2,4,5-trichlorophenyl ester of Gly—L-Ala—N^G-NO_2—L-Arg (I) has been obtained previously and is described in our previous paper [3].

The Hydrochloride of the 2,4,5-Trichlorophenyl Ester of $N^\varepsilon-Z-$Lys—N^G-NO_2—L-Arg—L-Ala (IIa). With stirring, 0.29 ml of $N(C_2H_5)_3$ and, at —15°C, 0.3 ml of isobutyl chloroformate (IBCF) were added to a solution of 1.3 g (0.002 mole) of $N^\alpha-BOC-N^\varepsilon-Z-$L-Lys—$N^G-NO_2$—Arg in tetrahydrofuran (THF). The mixture was stirred for another 20-25 min and then 1.3 g (0.003 mole) of HBr · Ala—$OPhCl_3$ in THF and 0.5 ml of $N(C_2H_5)_3$ were added. The mixture was stirred at —15°C for 1 h and at 20°C for 2 h and was left for 12 h. The solvent was distilled off and the residue was dissolved in $CHCl_3$ and was washed successively with water, 10% citric acid, and 0.5 N $NaHCO_3$ and was dried over $MgSO_4$. After the distillation of the $CHCl_3$, the product was dried and treated with petroleum ether. The yield of the tripeptide $N^\alpha-BOC-N^\varepsilon-Z-$L-Lys — N^G-NO_2—L-Arg — L-Ala—$OPhCl_3$ was 1.2 g (66%); $C_{34}H_{45}N_8O_{10}Cl_3$, mp 94°C (decomp.); R_f 0.37 [system 1: H_2O-CH_3COOH-n-C_4H_9OH (30 : 10 : 100) on silica gel, chromogenic agent iodine]; R_f 0.76 [system 2: sec-C_4H_9OH—3% NH_4OH (100 : 44); chromogenic agent iodine]; $[\alpha]_D^{22}$ —24.4° (c 0.82; THF).

To 1.2 g (0.0014 mole) of the product obtained was added 5.4 ml of 3 N HCl/CH_3OH. The mixture was kept at 20°C for 35 min, and after this the solvent was distilled off and the product was precipitated from methanol with ether. Yield 1 g (99%); R_f 0.58 (system 2).

The hydrochloride of the 2,4,5-trichlorophenyl ester of $N^\varepsilon-Z-$L-Lys—N^G-NO_2—Arg—Gly was synthesized in the same way as (IIa). Yield 0.8 g (90%), R_f 0.64 (system 2).

Polycondensation. A solution of 1 g (0.0013 mole) of (Ia) in 1.2 g of absolute DMFA was treated with 0.32 g of $N(C_2H_5)_3$, and the mixture was left at room temperature for 5 days. Then 6-7 ml of absolute methanol was added, and the precipitate that deposited was filtered off and washed with ether. Yield 0.55 g. After the removal of the protective groups by van Slyke's method, the molecular weight of polypeptide (I) was 10,000 (n = 27).

The polypeptides (II) with mol. wt. 5312 (n = 14) and (III) with mol. wt. 7000 (n = 20) were obtained in the same way.

Investigation of the Bactericidal Action of the Substances Synthesized. A weighed sample of the polypeptide under examination (20 mg) was dissolved in 5 ml of distilled water. Then the following dilutions of the polypeptides with meat-peptone broth to concentrations of 1, 0.5, 0.25 mg/ml, etc. were prepared. Fresh Staphylococcus aureus with all the well-defined signs of pathogenicity was used as the strain to be inhibited. The experiments were performed with an agar culture incubated in the thermostat for 18 h at 37°C. The microbial suspension was diluted with physiological solution to a concentration of 20,000 microbial cells per ml and was added in 0.2-ml portions to the working solutions and also to a control culture (meat-peptone broth and the given concentration of the culture).

The inoculates were incubated in the thermostat at 37°C for 18 h, taking into account the growth of the culture being incubated.

SUMMARY

1. Polypeptides of regular structure with the amino acid sequences (–Gly–L-Ala–L-Arg–), (–L-Lys–L-Arg–L-Ala–), (–L-Lys–L-Arg–Gly) have been synthesized.

2. It has been established that the polypeptide (–Gly–L-Ala–L-Arg) possesses a bactericidal action which exceeds that of the known polypeptide (–L-Leu–L-Orn–Leu–).

LITERATURE CITED

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