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OXIDATION OF TRIPEPTIDES CONTAINING HYDROXYAMINO ACID RESIDUE BY PERIODIC ACID

By SETSURO FUJII and KIKUO ARAKAWA

(From the Department of Medical Chemistry and the 3rd Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka)

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Some experiments of treating proteins with periodic acid have been tried by some investigators (*1-5*), but a little has been known about the details of its reaction mechanism.

In our previous reports, we have investigated the action of periodic acid on amino acids (*6*) and dipeptides (*7*) to confirm the existence of an analogous oxidative action on either hydroxyamino acids and hydroxyamino acid residue contained in peptide. Accordig to the results, not only hydroxyamino acid but also non-hydroxyamino acids were oxidized by periodic acid at higher temperature. But dipeptides containing hydroxyamino acid residue were more rapid in its oxidation velocities than that without the residue.

Present communication deals with the oxidation velocities of tripeptides by periodic acid comparing with that of dipeptides reported previously.

EXPERIMENTAL

I. Synthesis of Materials

Only new peptides among the synthesized are described below as to the details. And all of them were confirmed to have only one spot respectively by paperchromatography before they were used in oxidation experiment.

DL-Serylglycylglycine was synthesized by azide method. As carbobenzoxy-DL-serylglycine hydrazide was not obtainable by the reaction of carbobenzoxy-DL-serylglycine benzylester (*7*) and hydrazine hydrate, it was restarted from its methylester.

*Carbobenzoxy-*DL-*Serylglycine Methyl Ester*; m.p., 91-93°

$C_{14}H_{18}O_6N_2$ (310.30) Calculated: N 9.03
Found: „ 8.97

*Carbobenzoxy-*DL-*Serylglycine Hydrazide*; m.p., 189°

$C_{13}H_{18}O_5N_4$ (310.31) Calculated: N 18.08
Found: „ 18.21

*Carbobenzoxy-*DL-*Serylglycylglycine Benzyl Ester*; m.p., 134°

$C_{22}H_{25}O_7N_3$ (443.44) Calculated: N 9.69
Found: „ 9.65

DL-*Serylglycylglycine*; m.p. (decomposition), 185–188°

$C_7H_{13}O_5N_3$ (219.20) Calculated: N 19.17
Found: „ 19.08

Glycyl-DL-serylglycine was also synthesized by the azide method.

Carbobenzoxy-Glycyl-DL-*Serine Methyl Ester*; m.p., 63°

$C_{14}H_{18}O_6N_2$ (310.30) Calculated: N 9.03
Found: „ 9.11

Carbobenzoxy-Glycyl-DL-*Serine Hydrazide*; m.p., 183°

$C_{13}H_{19}O_5N_4$ (310.31) Calculated: N 18.04
Found: „ 18.21

Carbobenzoxy-Glycyl-DL-*Serylglycine Benzyl Ester*; m.p., 161°

$C_{22}H_{25}O_7N_3$ (443.44) Calculated: N 9.69
Found: „ 9.39

Glycyl-DL-*Serylglycine*; m.p., 201°

$C_7H_{13}O_5N_3$ (219.20) Calculated: N 19.15
Found: „ 18.96

Glycyl-DL-threonylglycine was synthesized by the mixed anhydride method (*8*).

DL-*Threonine Ethyl Ester Hydrochloride*—4 g. of DL-threonine was suspended in 40 fold of ethanol and saturated with dry hydrogen chloride at room temperature. After standing overnight at 0°, ethanol and hydrogen chloride were evaporated. Crystalline needle were obtained and recrystallized from ethanol-ether, yield 4.8 g., m.p. 119°.

$C_6H_{14}O_3Cl$ (183.64) Calculated: N 19.30
Found: „ 19.13

Carbobenzoxy-Glycyl-DL-*Threonine Ethyl Ester*—To 3.4 g. of carbobenzoxyglycine and 1.64 g. of triethylamine dissolved in 30 ml. of toluene was added 2.22 g. of *iso*-butylchlorocarbonate under the stirring and cooling at −5°. After 5 minutes, 3 g. of DL-threonine ethyl ester hydrochloride and 1.64 g. of triethylamine dissolved in 25 ml. chloroform was added to the mixture and stood for 2 hours at 0° and overnight at 8°. The mixture was washed with water and bicarbonate solution and then dried over sodium sulfate. As it began to be turbid during the drying, it was evaporated after 4 hours and added petroleum ether. 2.1 g. of the precipitate melted at 98°.

$C_{16}H_{22}O_6N_2$ (338.35) Calculated: N 8.28
Found: „ 8.25

Carbobenzoxy-Glycyl-DL-*Threonine Hydrazide*; m.p., 182°

$C_{14}H_{20}O_5N_4$ (324.33) Calculated: N 17.28
Found: „ 17.44

Carbobenzoxy-Glycyl-DL-*Threonylglycine BenzylEster*; m.p., 137°

$C_{23}H_{27}O_7N_3$ (457.47) Calculated: N 9.19
Found: „ 9.11

Glycyl-DL-*Threonylglycine Monohydrate*; m.p. (decomposition), 214–216°

		N	C	H
$C_8H_{17}O_6N_3$ (251.24)	Calculated:	16.73	38.20	6.81
	Found:	16.77	38.03	6.60

Glycylglycyl-DL-threonine was synthesized by the use of *N,N'*-dicyclohexylcarbodiimide (*9*).

*Carbobenzoxy-Glycylglycyl-*DL-*Threonine Ethyl Ester*—To the mixture of 0.00865 moles of carbobenzoxyglycylglycine, dicyclohexylcarbodiimide and 50 ml. of methylene chloride was added methylene chloride solution of 0.00865 moles DL-threonine ethyl ester hydrochloride and triethylamine. After standing overnight, the precipitate was filtered off and the filtrate was washed and dried over sodium sulfate. The solution was then evaporated and the residue was dissolved in small amount of ethyl acetate and the undissolved was filtered off. After addition of petroleum ether, precipitate was recrystalized from ethyl acetate and petroleum ether three times and melted at 119–120°. Yield 2.1 g.

$C_{18}H_{25}O_7N_3$ (395.40) Calculated: N 10.63
Found: „ 10.60

*Carbobenzoxy-Glycylglycyl-*DL-*Threonine*—0.005 moles of carbobenzoxy-glycylglycyl-DL-threonine ethylester dissolved in 40 ml. of methanol was treated with equivalent amount of 2 *N* sodium hydroxide solution for thirty minutes at room temperature. After the acidification with hydrochloric acid, the precipitate was washed with cold water. Yield 1.4 g., m.p. 196°.

$C_{18}H_{21}O_7N_3$ (367.35) Calculated: N 11.44
Found: „ 11.38

*Glycylglycyl-*DL-*Threonine Monohydrate*—After the hydrogenaton of the above in the mixture of glacial acetic acid, water and methanol with hydrogen gas and palladium black, the product was obtained in the technique as usual and melted at 150–154° (decomposition). Yield 0.6 g.

		N	C	H
$C_9H_{17}O_6N_3$ (251.24)	Calculated:	16.73	38.2	6.81
	Found:	16.45	37.8	6.73

II. Oxidation

Each 10 mg. of material was oxidized with 1 ml. of 0.5 *M* HIO_4, prepared from KIO_4 according to Jackson *et al.* (*10*), in a medium neutralized by addition of alkaline phosphate buffer. Oxidation velocity of tripeptide was determined by mesuring ammonia liberated from them in the same technique as our previous reports (*6*, *7*).

RESULTS AND DISCUSSION

Among the tripeptides investigated, only DL-serylglycylglycine was oxidized appreciably at room temperature as shown in Fig 1. The curve shows that the ammonia liberation from it becomes suddenly flat as soon as it reached one third of the amount from the total N. It was also disclosed by paperchromatography after hydrolysis by hydrochloric acid that the reaction mixture oxidized for 3 hours at room temperature contained no more any serine but glycine. It is evident from these results that the serine residue of DL-serylglycylglycine is almost selectively oxidizable by the action of periodic acid.

On the contrary, other tripeptides were hardly oxidized at 15° and a higher temperature was needed to liberate ammonia from them as shown in Fig. 2. In those cases, however, it was disclosed by paperchromatography that the hydroxyamino acid residue was still remained in the reaction mixture oxidized at 60° even after the liberation of ammonia in the amount

of one third calculated from the total N. Among them, glycylglycyl-DL-threonine, which had hydroxyamino acid residue in its C-terminal, was most resistant to periodic acid. Because the curve of it is slacker than that of previously reported glycyl-DL-threonine or glycyl-DL-serine (*8*), it is conceivable that the longer the chain of other residue combined with the amino group of hydroxyamino acid residue the more resistant to periodic acid.

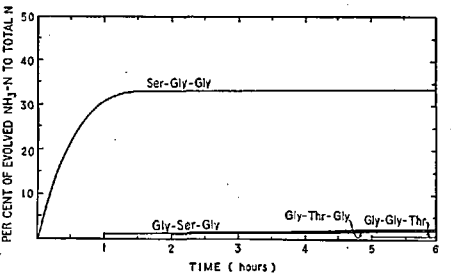

FIG. 1. Oxidation velocities of tripeptides by the action of neutral periodate at 15°.

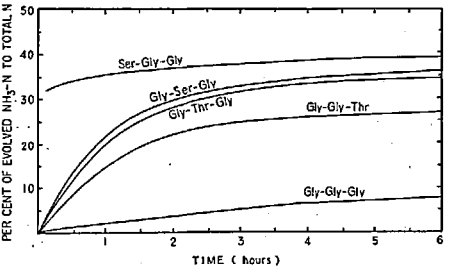

FIG. 2. Oxidation velocities of tripeptides by the action of neutral periodate at 60°.

10 mg. of material dissolved in 1 ml. of water and 20 ml. of *M*/25 phosphate buffer of pH 8.03 was oxidized with 1 ml. of 0.5 *M* HIO_4. After the oxidation, excess of HIO_4 was removed as barium salt. The filtrate and washings were combined and NH_3 from them was distilled and determined by the use of Parnas' apparatus.

Glycyl-DL-serylglycine and glycyl-DL-threonylglycine containing hydroxyamino acid residue in the middle were oxidized in almost equal velocities to those of glycyl-DL-serine and those of glycyl-DL-threonine reported previously. This fact may suggest that other residue combined with carboxyl

group of hydroxyamino acid residue do not inhibit the oxidation of tripeptide, and most of the inhibition is derived from other residue combined with amino group of hydroxyamino acid residue.

SUMMARY

1. Some new tripeptides were synthesized and oxidized by periodic acid in a neutral medium.

2. For the selective oxidation of hydroxyamino acid residue in tripeptide, its amino group must be free.

3. The length of the other residue combined with the amino group of a hydroxyamino acid residue was inverse to the oxidizability of the peptide.

4. Free or not free of its carboxyl group of a hydroxyamino acid residue was independent to the oxidizability of the peptide.

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