Chemistry

M. M. Botvinnik and S. M. Abaeva

1957

SovietRxiv

Abstract

Full Text

Chemistry

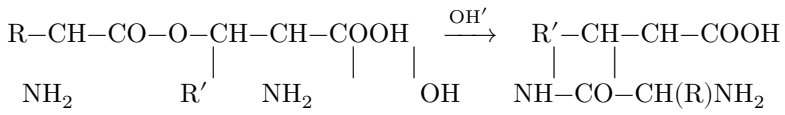
M. M. Botvinnik and S. M. Abaeva

STUDY OF THE PROPERTIES OF O-PEPTIDES OF -HYDROXYAMINO ACIDS

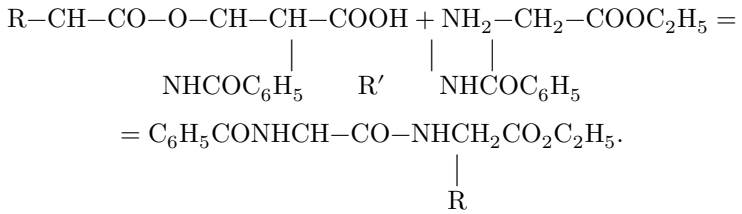
ON THE ENZYMATIC SYNTHESIS OF PEPTIDES FROM O-PEPTIDES OF -HYDROXYAMINO ACIDS

(Presented by Academician A. N. Nesmeyanov, September 13, 1956)

O-peptides (esters) of -hydroxyamino acids are characterized by the fact that their alcohol residue contains an amino group, and they are capable of intramolecular aminolysis, which proceeds spontaneously (1):



We have shown that such a rearrangement can also proceed intermolecularly. In the interaction of O-peptides of N-benzoylserine with ammonia and with ethyl glycinate, amides and peptides of the corresponding benzoyl derivatives of amino acids are formed:



Thus, in this reaction O-peptides are carriers of amino-acid residues and take part in the synthesis of new peptides. It may be assumed, although as yet there is no direct evidence for this, that a similar reaction may also occur in nature.

In the present work it has been established that this reaction proceeds especially readily under the action of chymotrypsin.

The experiments were carried out with N-benzoyl-O-(benzoyl-D,L-phenylalanyl)-D,L-serine (I), N-benzoyl-O-hippurylserine (II), and N-benzoyl-O-(benzoyl-D,L-phenylalanyl)-D,L-threonine (III). Ethyl glycinate served as the amino-acid

acceptor. In the presence of chymotrypsin, the experiment with the phenylalanine derivatives proceeded very rapidly, and after 10–15 min a precipitate of ethyl benzoyl-L-phenylalanylglycinate separated. The experiment with the hippuric acid derivatives ([2]) proceeded slowly, and ethyl benzoylglycylglycinate was isolated only after concentration of the solution, in 15% yield. In control experiments without addition of the enzyme, after prolonged standing only a small amount of the salt of the N-benzoyl-O-peptide and ethyl glycinate separated. When chymotrypsin was added to the control experiment, the reaction proceeded normally.

In order to verify that the interaction of the O-peptide with ethyl glycinate occurs under the action of the enzyme, an experiment was carried out in the presence of a chymotrypsin inhibitor—diisopropyl fluorophosphate. In this case the reaction did not proceed. After addition of a fresh portion of chymotrypsin, rapid separation of ethyl benzoylphenylalanylglycinate was observed.

A similar experiment with crystalline trypsin led to the same results.

Discussing the question of the pathways of peptide synthesis, Brenner ([2]) proposed that amino-acid esters may serve as intermediates in this process. He showed that esters of methionine and threonine are converted, under the action of chymotrypsin, into esters of the corresponding peptides. An analogous observation was made by Tauber ([3]) with the ethyl ester of phenylalanine, and in 1954 I. L. Kaganova and V. N. Orekhovich ([4]) carried out a systematic study of this reaction. Their experiments demonstrated the enzymatic synthesis of peptides from amino-acid esters. However, the investigations described were carried out with amino-acid esters whose formation in nature is unlikely. By contrast, the ester O-peptides of -hydroxyamino acids examined in the present work may serve as analogues of compounds formed in the process of protein degradation and resynthesis.

Experimental Part

1. Reaction of N-benzoyl-O-(benzoyl-DL-phenylalanyl)-D,L-serine with ethyl glycinate.

a) In the presence of chymotrypsin*. 500 mg of N-benzoyl-O-benzoylphenylalanylserine were dissolved in hot alcohol, neutralized with the calculated amount of 1 N sodium hydroxide, and mixed, in the presence of 2 ml of phosphate buffer (pH 7.9), with a solution of 700 mg of ethyl glycinate hydrochloride in an equivalent amount of 1 N alkali. To the reaction mixture (pH 8.1) were added 3–5 mg of chymotrypsin. After 3–5 min a precipitate began to separate, consisting of long needles. After 8–10 min it was filtered off and recrystallized from aqueous alcohol. Yield of ethyl benzoyl-L-phenylalanylglycinate: 80 mg, 24% calculated on the racemate and 50% calculated on the L-antipode of phenylalanine. M.p. 148–149°. $[\alpha]_D^{18} = -53.7 \pm 2.2^\circ$.

	Found, %:	C 67.76; H 6.42; N 7.92
$C_{20}H_{22}O_4N_2$.	Calculated, %:	C 67.79; H 6.21; N 7.92

After prolonged standing of the mother liquor, or after the addition of a fresh portion of enzyme, no further isolation of ethyl benzoylphenylalanylglycinate was observed.

b) Control experiment without enzyme. 400 mg of N-benzoyl-O-benzoyl-D,L-phenylalanylserine were dissolved in hot alcohol, neutralized with the calculated amount of 1 N alkali, and mixed, in the presence of 2 ml of phosphate buffer (pH 7.9), with a solution of 560 mg of ethyl glycinate hydrochloride in an equivalent amount of alkali. The slightly cloudy reaction mixture did not change over several hours. After 18 h a salt of N-benzoyl-O-benzoylphenylalanylserine and ethyl glycinate was isolated. M.p. 147–148°. Readily soluble in water. The ninhydrin reaction was positive.

	Found, %:	NH_2 2.52 (amino nitrogen by Van Slyke)
$C_{30}H_{33}O_8N_3$.	Calculated, %:	NH_2 2.48

Upon addition of chymotrypsin to the mother liquor, ethyl benzoyl-L-phenylalanylglycinate precipitated.

c) In the presence of diisopropyl fluorophosphate. 300 mg of N-benzoyl-O-(benzoyl-DL-phenylalanyl)-serine were reacted with 420 mg of ethyl glycinate hydrochloride as described above. To the reaction mixture were added 3–5 mg of chymotrypsin that had been previously mixed (2 h) with 2 ml of phosphate buffer and a drop of diisopropyl fluorophosphate. The solution remained clear for 18 h. Addition of a new portion of enzyme

---

* For the characteristics of chymotrypsin, see ([6]).

led to the slow (3 hours) precipitation of small amounts of the dipeptide ester. Yield of ethyl benzoyl-D,L-phenylalanylglycinate, 15 mg. M.p. 146–147°.

c) **In the presence of crystalline trypsin.** 100 mg of N-benzoyl-O-(benzoyl-DL-phenylalanyl)-serine was reacted with ethyl glycinate as described above. To the solution 3 mg of trypsin was added. After 3–4 hours a precipitate began to appear, which was filtered off after 18 hours. Ethyl benzoyl-L-phenylalanylglycinate melted at 145–146°. Yield 10 mg.

2. **Reaction of N-benzoyl-O-(benzoyl-DL-phenylalanyl)-D,L-threonine with ethyl glycinate in the presence of chymotrypsin.**

The reaction was carried out with 250 mg of N-benzoyl-O-(benzoyl-D,L-phenylalanyl)-DL-threonine and 250 mg of glycine ester hydrochloride under

the conditions described above. After 10 min, 25 mg of ethyl benzoyl-L-phenylalanylglycinate was isolated. M.p. 148–148.5°. The preparation gives no depression in a mixed melting-point test with the substance isolated in experiments with the O-peptide of serine.

3. **Reaction of N-benzoyl-O-hippuryl-D,L-serine with ethyl glycinate.**

a) **In the presence of chymotrypsin.** 350 mg of N-benzoyl-O-hippurylserine, dissolved in alcohol, was treated with 1 ml of 1 N NaOH, mixed in the presence of 2 ml of phosphate buffer with a solution of ~70.0 mg of ethyl glycinate hydrochloride in 5 ml of NaOH, and 3–5 mg of chymotrypsin was added. After 24 hours the reaction mixture was concentrated to a small volume, and the precipitated solid was recrystallized from water. M.p. of ethyl benzoylglycylglycinate 114–116°. Literature value 117° (5). Yield 20 mg.

b) **Control experiment without enzyme.** 170 mg of N-benzoyl-O-hippurylserine and 350 mg of ethyl glycinate were treated as indicated above, but without addition of enzyme. After concentration of the solution (after 24 hours), the salt of N-benzoyl-O-hippurylserine and ethyl glycinate was isolated.

Conclusions

Using as examples the N-benzoyl derivatives of O-benzoyl-D,L-phenylalanyl-D,L-serine, O-benzoyl-D,L-phenylalanyl-D,L-threonine, and O-hippuryl-D,L-serine, it has been shown that O-peptides of -hydroxyamino acids can serve as carriers of an amino acid residue in the enzymatic synthesis of peptides.

We express our gratitude to Prof. S. R. Mardashev and M. P. Chernikov for providing crystalline trypsin and chymotrypsin.

Moscow State University
named after M. V. Lomonosov

Received
7 IX 1956

References

1. M. Bergmann, A. Miekeley, *Zs. physiol. Chem.*, **140**, 128 (1924); M. M. Botvinnik, S. M. Avaeva, E. A. Mistryukov, *DAN*, **82**, 727 (1952); *ZhOKh*, **24**, 2084 (1954); P. Desnuelle, A. Casal, *Biochim. et Biophys. Acta*, **2**, 64 (1948); D. F. Elliott, *Biochem. J.*, **50**, 542 (1952).

2. M. Brenner, H. R. Müller, R. W. Pfister, *Helv. Chim. Acta*, **33**, 668 (1950); M. Brenner, E. Sailer, K. Rüfenacht, *Helv. Chim. Acta*, **34**, 2098

(1951).

3. H. Tauber, *J. Am. Chem. Soc.*, **72**, 847 (1952).

4. I. L. Kaganova, V. N. Orekhovich, *DAN*, **95**, 1259 (1954).

5. Th. Curtius, *J. Prakt. Chem.*, (2) **70**, 78 (1904).

6. M. P. Chernikov, *Ukr. Biochem. J.*, **27**, 94 (1955).

Note: Figure translations are in progress. See original paper for figures.