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M. M. Botvinnik, A. P. Andreeva

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Abstract

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M. M. Botvinnik, A. P. Andreeva

Interaction of N-Benzoyl-O-(benzoylphenylalanine-C^{14})-serine with Ribonuclease

(Presented by Academician A. N. Nesmeyanov, 22 January 1960)

In our previous communication ([1]) it was shown that N-benzoyl-O-(benzoylphenylalanine-C^{14})-serine

$$\begin{array}{c} CH_2-CH-COOH \\ \quad\quad | \quad\quad | \\ O \quad\quad NHCOC_6H_5 \\ \quad\quad\quad | \\ C^*OCH(CH_2C_6H_5)NHCOC_6H_5 \end{array}$$

reacts in the presence of chymotrypsin with insulin and blood-serum albumin with transfer of benzoylphenylalanine-C^{14} to the protein.

In the present work an analogous study was carried out with ribonuclease. The reaction was conducted under the same conditions as before. Experimen-

Table 1

Interaction of N-benzoyl-O-(benzoylphenylalanine-C^{14})-serine with ribonuclease

Experiment No.	Ratio of O–N-peptide components : protein in moles	O–N-peptide, mg	Protein, mg	Initial activity per sample, imp/min	Incorporation of label: imp/min per 5 mg protein after TCA*	Incorporation of label: imp/min per 5 mg protein after 0.1 N NaOH	O,N-peptide equiv. per 1 g protein per h	O,N-peptide equiv. per molecule protein**	Enzyme
1	0.4 : 1	0.4	30	8 790	99	110	2.12	0.029	Chymotrypsin
2	0.4 : 1	0.4	30	15 688	130	98	1.08	0.014	»
1a	0.4 : 1	0.4	30	8 790	175	169	3.25	0.044	Without enzyme
2a	0.4 : 1	0.4	30	15 688	122	90	0.99	0.012	» »
3	1 : 1	1	30	17 760	230	67	1.63	0.022	Chymotrypsin
4	1 : 1	1	30	21 975	97	69	1.36	0.018	»
3a	1 : 1	1	30	17 760	263	110	2.67	0.036	Without enzyme
4a	1 : 1	1	30	21 975	375	430	8.47	0.10	» »
	1 : 1	1	30	39 222	410	334	3.69	0.05	» »
6	3 : 1	3	30	117 666	525	531	5.82	0.079	Chymotrypsin
6a	3 : 1	3	30	117 666	974	948	10.7	0.14	Without enzyme
7	5 : 1	5	30	196 110	250	283	3.13	0.042	Chymotrypsin
8	5 : 1	5	30	180 000	885	470	5.66	0.076	»
9	5 : 1	5	30	140 900	400	396	6.09	0.066	»
7a	5 : 1	5	30	196 110	1358	820	9.06	0.121	Without enzyme

Experiment No.	Ratio of O–N-peptide components : protein in moles	O–N-peptide, mg	Protein, mg	Initial activity per sample, imp/min*	Incorporation of label: imp/min per 5 mg protein after TCA*	Incorporation of label: imp/min per 5 mg protein after 0.1 N NaOH	O,N-peptide equiv. per 1 g protein per h	O,N-peptide equiv. per molecule protein**	Enzyme
7b	5 : 1	5	30	196 110	1004	888	9.82	0.131	» »
8a	5 : 1	5	30	180 000	845	638	7.69	0.104	» »
9a	5 : 1	5	30	140 900	538	492	7.56	0.082	» »
10	5 : 1	5	30	143 850	986	686	10.34	0.143	Chymotrypsin, inactivated by boiling
10a	5 : 1	5	30	143 850	842	614	9.25	0.128	Chymotrypsin, inactivated by boiling
11	5 : 1	5	30	124 250	485	359	6.25	0.081	Without enzyme

* Pulses are given with background taken into account.
** Molecular weight of ribonuclease 13500.

mental data are presented in Table 1. As in the preceding experiments, treatment with alkali does not remove the label from the protein. However, in contrast to the behavior of other proteins, addition of chymotrypsin to the reaction mixture did not activate the process of transfer of benzoylphenylalanine onto the protein, but in most cases inhibited it. This course of the reaction led to the assumption that ribonuclease is capable of activating the process by itself, while chymotrypsin in this reaction is a kind of inhibitor. These assumptions

were confirmed by the following experiments. In experiments 10 and 10a (Table 1), chymotrypsin inactivated by boiling was used. As a result, the number of imp/min per 5 mg of protein increased from 396 to 614-686. In experiments 12, 13, and 14 (Table 2), ribonuclease inactivated by oxidation was used.

Table 2

Incorporation of label into oxidized RNase and RNase treated with urea

Experiment No.	Treatment before incubation	Component ratio O-N-peptide : protein	Initial activity, imp/min	Label incorporation, imp/min per 5 mg protein per h after TCA	Label incorporation, imp/min per 5 mg protein per h after 0.1 N NaOH	μeq peptide per 1 g protein	μeq peptide per 1 μeq protein	Enzyme
12	Preparation “a” of oxidized RNase	5 : 1	180 000	302	254	2.77	0.037	Chymotrypsin
12a	RNase	5 : 1	180 000	28	22	0.24	0.003	Without enzyme
13	Preparation “b” of oxidized RNase	5 : 1	169 400	682	305	3.9	0.053	Chymotrypsin
13a	RNase	5 : 1	169 400	20	12	0.15	0.02	Without enzyme
14	Preparation “c” of oxidized RNase	5 : 1	140 900	34	20	0.308	0.004	Without enzyme

Experiment No.	Treatment before incubation	Component ratio O–N-peptide : protein	Initial activity, imp/min	Label incorporation, imp/min per 5 mg protein per h after TCA	Label incorporation, imp/min per 5 mg protein per h after 0.1 N NaOH	μeq peptide per 1 g protein	μeq peptide per 1 μeq protein	Enzyme
14	RNase	5 : 1	140 900	39	18	0.277	0.0036	Without enzyme
14	RNase	5 : 1	140 900	35	20	0.308	0.004	Without enzyme
15	RNase treated with urea	5 : 1	135 000	320	318	5.11	0.07	Without enzyme
15	RNase treated with urea	5 : 1	135 000	200	170	2.7	0.036	Without enzyme
15	RNase treated with urea	5 : 1	124 250	197	157	2.73	0.037	Without enzyme
15	RNase treated with urea	5 : 1	124 250	193	137	2.38	0.032	Without enzyme

Under the action of the O-peptide, incorporation of the label almost did not occur. If, however, chymotrypsin is added to oxidized ribonuclease and the O-peptide, a sharp increase in the labeling of the protein is observed (experiments 12 and 13, Table 2).

Oxidation of ribonuclease was carried out with performic acid ([2]). Preparation "a" was precipitated with trichloroacetic acid, preparation "b" with acetone, and preparation "c" by lyophilization. In experiment No. 15, ribonuclease treated with urea was used. According to the literature, ribonuclease is only slightly

inactivated by urea ([3]), which was also reflected in the results. Ribonuclease oxidized with performic acid completely lost its ability to react with the O-peptide, while that treated with urea did so only partially (Table 2). The question of the nature of the bond between benzoylphenylalanine and ribonuclease requires further investigation.

Experimental Part

Crystalline ribonuclease (Hungary) was used in the experiments. The preparation was electrophoretically homogeneous. The interaction of ribonuclease with the O-peptide was carried out under the same conditions as previously ([1]). The electrophoretic mobility of the preparation did not change after incubation.

Oxidation of ribonuclease. 1. Preparation "a" ([2,3]). To a solution of 80 mg of ribonuclease in 0.8 ml of 98% performic acid was add-

add 0.02 ml of 30% H_2O_2. After 30 min, the protein is precipitated with a tenfold volume of 10% trichloroacetic acid; the precipitate is centrifuged, washed with 5% trichloroacetic acid, with alcohol and ether (1 : 3), with ether, and dried.

2. **Preparation "b"** [(2)]. Oxidation is carried out analogously to that described. The protein is precipitated with acetone; the precipitate is centrifuged, washed with acetone, and dried.
3. **Preparation "v"** [(3)]. 0.45 ml of 30% H_2O_2 is added to 9 ml of 98% formic acid and left for 1 hour at 20°; then the mixture is cooled to 0°. 5 ml of the cooled solution is added to 100 mg of ribonuclease in 5 ml of formic acid. After 2 hours (with cooling), the mixture is diluted with a tenfold volume of water and lyophilized.

Treatment of ribonuclease with urea[(4)]. Crystalline urea is added to the reaction mixture (O-peptide and ribonuclease) to bring the solution to 8 M concentration, and incubation is carried out under the same conditions as before.

Thus, it has been shown that N-benzoyl-O-(benzoylphenylalanyl-C^{14})-serine reacts with ribonuclease with transfer of benzoylphenylalanine to the protein. The reaction is inactivated by chymotrypsin.

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Moscow State University
named after M. V. Lomonosov

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