Artificial ribonucleases

5.* Synthesis and ribonuclease activity of tripeptides composed of amino acids involved in catalytic centers of natural ribonucleases

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The characteristic features of the spatial arrangement of the main functional groups involved in catalytic centers of ribonucleases and nucleases were revealed by computer analysis of the catalytic centers of these enzymes. Based on the results of computer simulation, tripeptides containing Lys, Arg, His or Hia, Thr, and Asn in different combinations were synthesized. In these tripeptides, the distances between the corresponding functional groups are equal to those observed in natural enzymes. The efficacy of RNA cleavage with Arg- and His-containing tripeptides depends on their structure and correlates with the overall positive charge of these compounds. Of all the tripeptides under consideration, compounds bearing the overall charge of +4 exhibit the highest ribonuclease activity.

Key words: artificial ribonucleases, RNase active site, tripeptides.

Low-molecular-weight "chemical" ribonucleases hold promise as tools for studying the structures of RNAs and RNA-protein complexes, as reactive groups in conjugates intended for cleavage of particular RNAs, and as therapeuticals inactivating virus genome RNAs or certain mRNAs. One of approaches, which allow one to construct compounds capable of cleaving RNA, is based on the design of mimetics of natural enzymes by organic chemistry methods.

Earlier,[1] it has been demonstrated that di- and tripeptides containing the lysine and histidine or histamine residues possess RNase activity. Among these compounds, Lys-His methyl ester (2L2) bearing a charge of +2 exhibits the highest activity. Cleavage of the phosphodiester bond in dinucleoside monophosphate CpA with the latter compound demonstrated[2] that adenine and cytidine 2´,3´-cyclophosphates are produced in this reaction, as in hydrolysis of RNA with RNase A. Conjugates of short peptides which mimic elements of the active site of RNase A with acridine (Acr) as an intercalator, which provides binding of the constructions to RNA, were synthesized.[3] The lysine residue was used as a linker. Experiments on the replacement of the N-terminal histidine residue with another amino acid demonstrated that the presence of histidine is necessary for such conjugates to manifest ribonuclease activity. Tetrapeptides His-Gly-His-Lys(Acr)NH_2 and His-Pro-His-Lys(Acr)NH_2 exhibit the highest ribonuclease activity. Synthetic peptides consisting of alternating hydrophobic (alanine, leucine) and basic (lysine, arginine) amino acid residues belong to yet another group of peptides showing ribonuclease activity.[4] It was hypothesized[4] that a regular (α-helical or β-folded) structure is prerequisite for such peptides to perform efficient RNA hydrolysis, because the alignment of the RNA polynucleotide chain between two parallel series of positively charged amino acid residues is of major importance in catalysis of RNA cleavage.

In the present study, we carried out comparative analysis of the structures of the catalytic centers of a series of ribonucleases and nucleases, synthesized tripeptides that mimic fragments of the catalytic centers of these enzymes, and examined the activity of these tripeptides in the cleavage of a synthetic 21-mer oligoribonucleotide under physiological conditions.

Experimentally

In the present work, dicyclohexylcarbodiimide (DCC), L-histidine dihydrochloride (Chemapol, Poland), histamine dihydrochloride (Sigma, USA), N_ω-nitro-L-arginine methyl ester dihydrochloride, *N*-hydroxysuccinimide (NHS-OH) (Fluka, Switzerland), N_α-Boc-N_ε-(Z(2Cl))-L-lysine, N_α-Boc-N_ω-nitro-L-arginine, N_α-Boc-*O*-Bzl-L-threonine, N_α-Boc-L-asparagine (FisherBiotech, USA), Boc_2O (Aldrich, USA), RNase T1 (Boehringer Mannheim, Germany), T4 polynucleotide kinase (Fermentas, Lithuania), and [γ-^{32}P]-ATP (Biosan, Russia) were used. The 21-mer oligonucleotide ON21

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(5′-UCGAAUUUCCACAGAAUUCGU-3′) was synthesized by M. N. Repkova (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences). Triethylamine, trifluoroacetic acid, and dry methanol, ethanol, diethyl ether, ethyl acetate, and DMF were purified according to standard procedures.[5] Thin-layer chromatography was performed on DC-Alufolien Kieselgel 60 F_{254} plates (Merck, Germany) using the following solvent systems: A, *tert*-butyl alcohol—ethyl methyl ketone—formic acid—water, 40 : 30 : 15 : 15; B, dichloromethane—methanol, 1 : 1. The ^{1}H NMR spectra were recorded on a Bruker WP-200-SY spectrometer (Germany). The chemical shifts were measured in the δ scale. All biochemical assays were carried out with the use of Milli-Q water (Millipore, USA).

Synthesis of tripeptides. Synthesis of *N*-hydroxysuccinimide esters of protected amino acids[6] (general procedure). *N*-Hydroxysuccinimide (1.1 mmol, 127 mg) was added to a solution of *N*- or *N*,*O*-protected amino acid (1 mmol) in dry ethyl acetate (3 mL). The solution was cooled to 0 °C and then DCC (1.1 mmol, 226 mg) was added with stirring. The temperature of the reaction mixture was gradually raised to ≈20 °C in 2 h and the reaction mixture was stirred at ≈20 °C for 0.5 h. The precipitate of dicyclohexylurea was filtered off and the filtrate was concentrated. The oily residue was dissolved in dry ethyl acetate (2—3 mL), the precipitate of dicyclohexylurea was again filtered off, and the solution was concentrated *in vacuo* to afford the activated ester, which was used in reactions without additional purification.

Synthesis of peptides (general procedure). A solution of *N*-hydroxysuccinimide ester (1 mmol) in dry ethyl acetate (2 mL) was added with stirring to a suspension of an appropriately protected amino acid hydrochloride/dihydrochloride, histamine, or dipeptide (1 mmol) and triethylamine (1.1 mmol, 153 μL) in dry DMF (2 mL). The reaction mixture was stirred for 12 h. The precipitate that formed was filtered off and washed with dry ethyl acetate (2×10 mL). The combined filtrates were concentrated *in vacuo* and a 10% Na_2CO_3 solution (5 mL) was added to the oily residue. The reaction mixture was kept at 10 °C for 2 h and extracted with ethyl acetate (5×5 mL). The organic extracts were combined and dried with Na_2SO_4. The solvent was removed *in vacuo*.

Removal of the Boc group. ***A.*** A Boc-protected amino acid or peptide (1 mmol) was dissolved in CF_3COOH (1 mL per Boc group). The reaction mixture was kept at 40—50 °C for 2 h and then concentrated with ethanol (3×10 mL). The residue was dried *in vacuo*.

B. A Boc-protected peptide or amino acid (1 mmol) was dissolved in dry MeOH (1 mL) and 4 *M* methanolic HCl (1 mL per Boc group) was added. The reaction mixture was kept at 20 °C for 2 h and concentrated several times with ethanol. The residue was dried *in vacuo* to afford dipeptides, which were used in subsequent reactions without additional purification.

Removal of *N*,*O*-protective groups in tripeptides. After removal of the Boc protection, tripeptides (0.3—0.5 mmol) were dissolved in methanol (10 mL) and subjected to hydrogenolysis in the presence of 5% Pd/C (100 mg). The course of deprotection was monitored by TLC. After completion of the reaction, the catalyst was filtered off and the solvent was removed *in vacuo*. The final purification of the tripeptides was carried out by reversed-phase chromatography (1.5×25-cm Preparative C18 column (Waters, USA), 55—105 μm, the methanol concentration gradient (0→100%) in 0.05% CF_3COOH, the flow rate was 2—3 mL min^{-1}).

The structures of the target tripeptides were confirmed by ^{1}H NMR spectroscopy and elemental analysis of salts with trifluroacetic acid.

Cleavage of RNA in the presence of tripeptides. The [^{32}P] label was introduced at the 5′-terminus of the 21-mer oligonucleotide (ON21) using [γ-^{32}P]-ATP and T4 polynucleotide kinase according to known procedures.[7] Cleavage of the RNA substrate with tripeptides was carried out at 37 °C for 24 h. The standard reaction mixture (10 μL) contained a 50 *mM* imidazole or 50 *mM* Tris-HCl buffer, pH 7.0, 0.2 *M* KCl, 0.1 *mM* EDTA, an RNA carrier (0.1 mg mL^{-1}) (*Escherichia coli* total tRNA), and tripeptides (10^{-3} mol L^{-1}). The reaction was terminated by adding a 2% $LiClO_4$ solution in acetone (100 μL). The precipitate of ON21 and its fragments was separated by centrifugation (14000 rpm, 4 °C, 15 min), washed with acetone (300 μL), and dissolved in a loading buffer (5 μL) (4 *M* urea, 0.025% Bromophenol Blue, and 0.025% xylene cyanole). The cleavage products were analyzed by electrophoresis in a 15% polyacrylamide gel containing 8 *M* urea and a TBEx1 buffer (0.9 *M* Tris-borate, pH 8.0, 2 *mM* EDTA). After electrophoresis, the gel was dried *in vacuo* and autoradiographed on a RENEKS film. The autoradiograph was digitized with the use of the Gel-Pro Analyzer program (Media Cybernetics, 1993-97). The cleavage sites of the oligonucleotide were identified by comparing with the results of partial hydrolysis with RNase T1 in denaturing conditions[7] and in a 2 *M* imidazole buffer, pH 7.0, at 90 °C.[8] The degree of cleavage (%) was determined as the ratio of the radioactivity of the cleavage products to the total radioactivity of the sample applied onto the lane.

Results and Discussion

The catalytic centers of RNases and nucleases involve histidine, lysine, arginine, aspartic and glutamic acids and their amides, as well as hydroxy amino acid residues.[9—12] An approach developed in the study[13] allowed one to reveal the role of amino acid residues in the catalytic centers of enzymes based on comparative structure-function analysis of these centers. We used this approach to perform structure-function analysis of the active centers of RNases A and T1, nucleases S and Sm, and binase. We chose a minimum set of amino acid residues involved in the catalytic centers responsible for the activation of a nucleophilic species (B:), protonation of the oxygen atom of the leaving group (AH), an increase in the electrophilicity of the phosphorus atom, and stabilization of the transition state (S1, S2) whose role in the catalytic process has been thoroughly investigated.[9—12] The scheme of measurements of the distances between amino acid residues is presented in Fig. 1, *a*. The minimum structure of the active site of RNase A is shown in Fig. 1, *b*. It is commonly accepted[14] that RNase A functions by the acid-base catalysis mechanism, according to which the acid protonates the leaving group and the base deprotonates the newly formed nucleophile. In the step of trans-

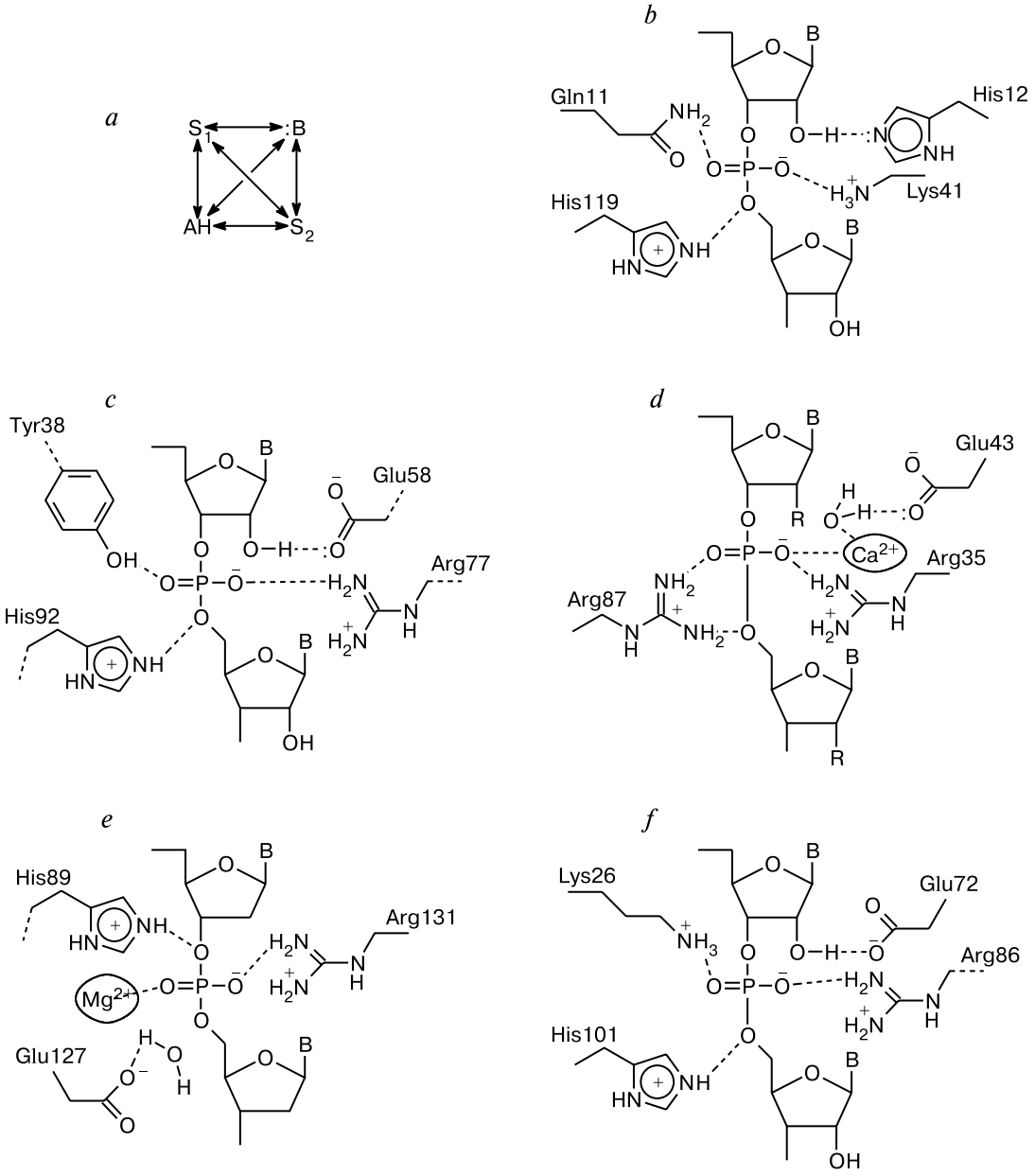

Fig. 1. Minimum catalytic centers of ribonucleases and nucleases: *a*, the scheme of measurements of the distances between the functional groups in the catalytic centers of enzymes (AH is acid, :B is base; S_1 and S_2 are groups reducing the electron density on phosphate); *b*, RNase A; *c*, RNases T1; *d*, nuclease S; *e*, nuclease Sm; *f*, binase. The enzyme—RNA substrate interaction sites are indicated by dashed lines.

esterification, the His-12 (:B) residue exists in the deprotonated form and accepts the proton from the 2′-OH group of ribose, while the His-119 (AH) residue protonates the O(5′) atom of the leaving group. In the step of hydrolysis of cyclophosphate, His-119 deprotonates the water molecule, and His-12 protonates the O(2′) atom of the leaving group. According to the results of X-ray diffraction analysis, Lys-41 interacts with the 2′-OH group of ribose, thus facilitating deprotonation of the latter with His-12. In addition, Gln-11 (S1) and Lys-41 (S2) also interact with the oxygen atoms of phosphate, thus stabilizing the transition state.[14]

Original data on the organization of the catalytic centers of the enzymes were obtained from the SWISS-PROT database (http://www.expasy.ch). The minimum structures of the catalytic centers of RNase T1 [EC 3.1.27.3],[15,16] staphylococcal nuclease S [EC 3.1.31.1],[17,18] extracellular endonuclease Sm [EC 3.1.4.9],[19] and binase (G-specific ribonuclease) [EC 3.1.27.3][20,21] are shown in Fig. 1, *c*—*f*. The distances

Table 1. Average structural parameters of the catalytic centers of natural ribonucleases and nucleases*

Functional domains	RNase A	RNase T1	Binase	Nuclease S	Nuclease Sm	Average values
AH—:B	**6.7**	**6.5**	**7.2**	**8.8**	**6.3**	**7.1±0.80**
:B—S_1	4.0	6.7	5.9	7.7	4.0	5.6
:B—S_2	**5.3**	**5.9**	**5.5**	**4.4**	**6.5**	**5.5±2.3**
AH—S_1	**6.4**	**3.9**	**5.9**	**5.5**	**4.4**	**5.2±1.6**
AH—S_2	8.91	6.00	5.46	7.34	9.91	7.5
S_1—S_2	4.46	3.35	7.48	3.75	9.31	5.7
PDB refcode	1RBCL, 1RBEL, 1SSAL, 1SRNL	2GSP, 1RGA, 3BU4, 6RNT	1GOU, 1GOV, 1GOY, 1BUJ	1EYO, 1STH, 1STG	1G8T, 1QAE, 1QLO, 1SMN	

* The average distances (Å), which were determined from analysis of several (three—four) structures, are given. The structural parameters characterized by a high degree of correlation are printed in bold type.

between the corresponding functional groups were determined using the SwissPdbViewer program (version 3.51) based on the data available in the Protein Data Bank (PDB, http://www.rcsb.org). The average distances between the reactive groups of the amino-acid residues involved in the catalytic centers of the enzymes are given in Table 1.

The enzymes under consideration cleave various substrates (ribonucleases cleave RNA, nucleases S and Sm cleave RNA and DNA). Hydrolysis affords various products, *viz.*, 5′-phosphorylated nucleotides in the case of nuclease Sm and 3′-phosphorylated nucleotides in the other cases. However, three conserved distances, *viz.*, AH—:B, AH—S_1, and :B—S_2 (7.1, 5.5, and 5.2 Å, respectively), are present in the catalytic centers of all the enzymes under study (see Table 1). The presence of conserved domains in the active sites of the enzymes suggests that the required structural parameters can be achieved by synthesizing short peptides consisting of amino acids, which contain carboxy, imidazole, guanidinium, amino, or hydroxy groups in the side chains. The three-dimensional structures of a series of short peptides consisting of 3—4 amino-acid residues were analyzed using the Hyper Chem 6.0 program.[22] We found that the distances between the functional groups in the side chains of amino acids are similar to those in natural enzymes and can be attained already in tripeptides with particular structures. Figure 2 exemplifies the structures of the tripeptides His-Lys-Arg(OMe) and Lys-Thr-Hia in which the distances between the corresponding functional groups are similar to conserved structural elements of the catalytic centers of natural ribonucleases. Based on the results of computer simulation, we chose the following six tripeptides: Lys-His-Arg(OMe) (KHR), His-Lys-Arg(OMe) (HKR), Arg-Lys-Hia (RKHa), Asn-Lys-Hia (NKHa), Lys-Asn-Hia (KNHa), and Lys-Thr-Hia (KTHa).

Synthesis of tripeptides. Tripeptides were synthesized in solution from commercially available α-Boc-protected amino acids according to a protocol based on the use of

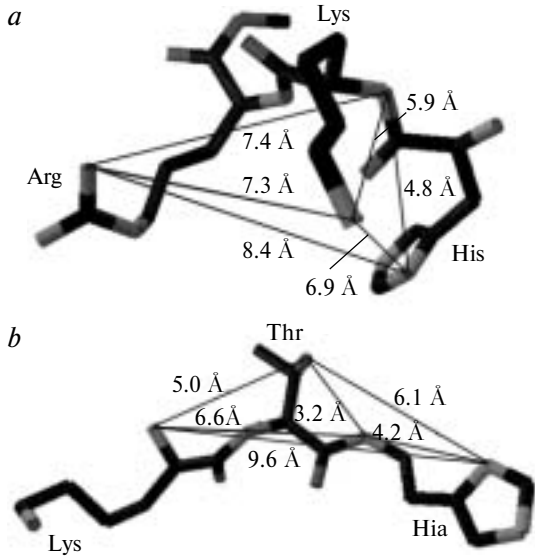

Fig. 2. Three-dimensional structures of the synthetic tripeptides HKR (*a*) and KTHa (*b*) calculated using the Hyper Chem Pro 6.0 program (Hypercube, Inc., 2000). Semiempirical optimization of the molecules was carried out using the PM3 Hamiltonian followed by molecular mechanical simulation with the MM+ force field.

activated esters. The standard procedure for the preparation of *N*-hydroxysuccinimide esters[23] of α-Boc-protected amino acids was modified as follows: a solution of a protected amino acid and *N*-hydroxysuccinimide was cooled to 0—5 °C and then a cooled solution of dicyclohexylcarbodiimide was added. This procedure allowed us to increase the yield of the target product, *viz.*, *N*-hydroxysuccinimide ester of α-Boc-protected L-amino acid, to 90—95%. Activated esters prepared according to this procedure were used in condensation without additional purification. The functional groups in the side chains of amino acids of the tripeptides were deprotected by catalytic hydrogenolysis. Histamine was used in the synthesis of peptides without protection of the imidazole ring. The

schemes of the synthesis of tripeptides and their main characteristics are given in Scheme 1 and Table 2. The purities of intermediates were monitored by TLC and ^{1}H NMR spectroscopy. The structures of the tripeptides synthesized were confirmed by elemental analysis and ^{1}H NMR spectroscopy (see Table 2).

Study of hydrolytic activity. The ability of the peptides to cleave RNA was assayed using the 5′-[^{32}P]-labeled synthetic oligoribonucleotide (21-mer) (hereinafter, ON21). The secondary structure of this oligonucleotide is a hairpin (Fig. 3, *a*), whose stem is identical to the amino-acceptor stem of the yeast tRNA^{Phe}, and the sequence of the UCCACAG loop is identical to the fragment 59—65 of the same tRNA, which is particularly sensitive to cleavage with natural and chemical ribonucleases.[24] Treatment of RNA with tripeptides was carried out in physiological conditions and the cleavage products were analyzed by electrophoresis in polyacrylamide gel.

A typical autoradiograph of the gel after separation of the products of ON21 cleavage with tripeptides is shown in Fig. 3, *b*. It can be seen that certain peptides cleave RNA. The tripeptides KHR, HKR, RKHa, and KTHa at a concentration of 10^{-3} *M* in a 50 *mM* imidazole buffer efficiently cleave ON21 at the bonds in two CpA fragments located in the loop, *viz.*, C10-A11 and C12-A13, whereas the peptides NKHa and KNHa are virtually in-

Scheme 1

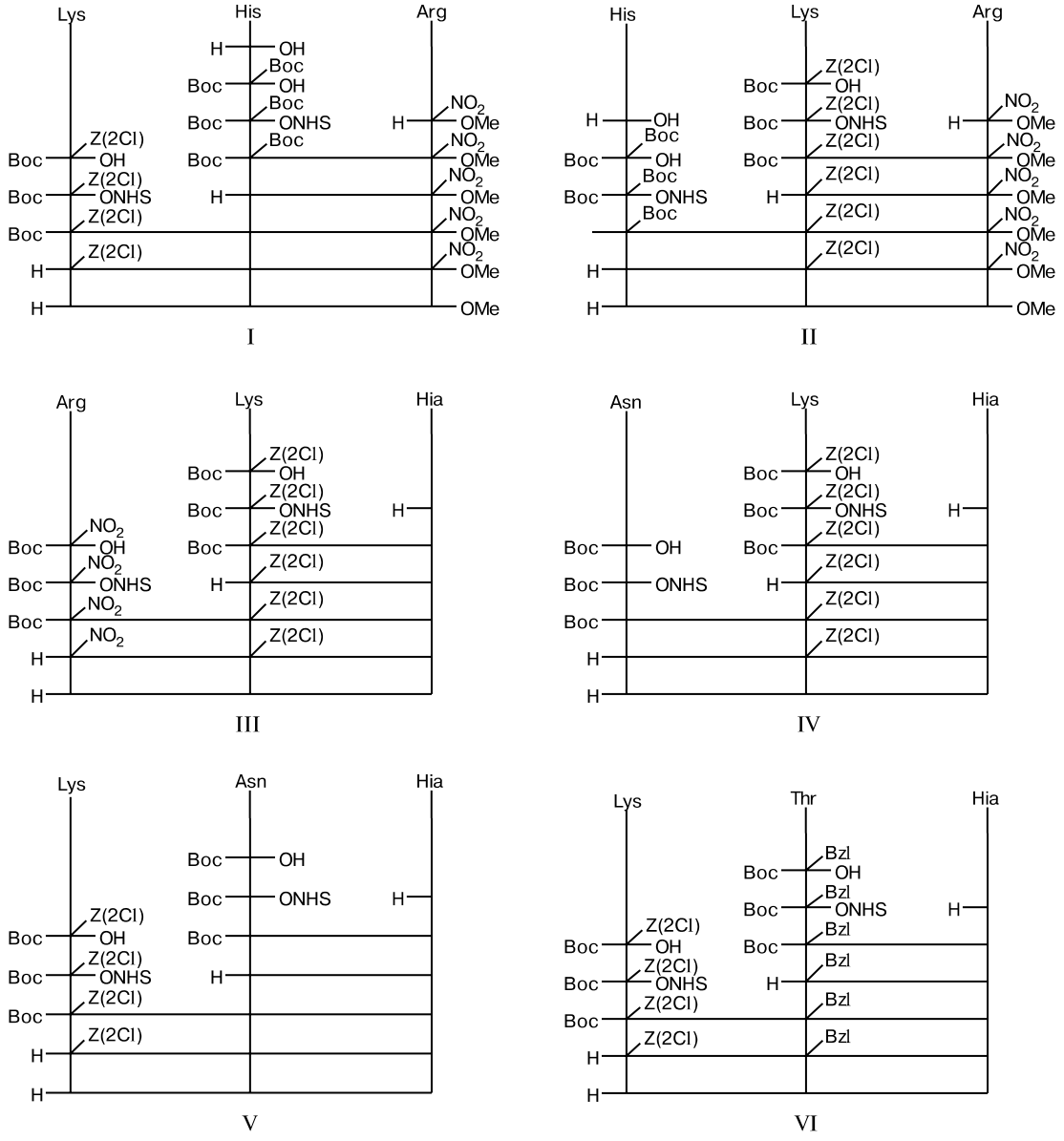

Table 2. Physicochemical characteristics of the tripeptides synthesized

Tripeptide	Yield (%)	R_f	^{1}H NMR (D_2O) δ (*J*/Hz)	Found / Calculated (%)		Molecular formula*
				C	H	
I	39	0.35 (B)	1.25 (m, 2 H, $CHCH_2C\underline{H}_2^{Lys}$); 1.50 (m, 2 H, $CHCH_2C\underline{H}_2^{Arg}$); 1.95 (m, 6 H, $CHC\underline{H}_2^{Arg}$, $NH_2CH_2C\underline{H}_2^{Lys}$, $CHC\underline{H}_2^{Lys}$); 2.65 (m, 2 H, $NHC\underline{H}_2^{Arg}$); 3.20 (m, 4 H, CH_2-im, $NH_2C\underline{H}_2^{Lys}$); 3.75 (m, 3 H, OMe); 4.53 (m, 1 H, CH^{Arg}); 4.58 (m, 1 H, CH^{Lys}); 4.92 (m, 1 H, CH^{His}); 7.30 (s, 1 H, H(5)); 8.25 (s, 1 H, H(2))	38.70 38.50	5.78 5.76	$C_{23}H_{37}F_6N_9O_8 \cdot 2H_2O$
II	51	0.36 (A)	1.50 (m, 10 H, $CHCH_2C\underline{H}_2^{Lys}$, $NH_2CH_2C\underline{H}_2^{Lys}$, $CHCH_2C\underline{H}_2^{Arg}$, $CHC\underline{H}_2^{Arg}$, $CHC\underline{H}_2^{Lys}$); 3.15 (m, 4 H, CH_2-im, CH_2^{Hia}); 3.37 (t, 2 H, $NHC\underline{H}_2^{Arg}$, *J* = 6.5); 3.39 (t, 2 H, $NHC\underline{H}_2^{Lys}$, *J* = 5.5); 3.90 (s, 3 H, OMe); 4.50 (m, 2 H, CH^{Lys}, CH^{Arg}); 4.90 (m, 1 H, CH^{His}); 7.48 (s, 1 H, H(5)); 8.64 (s, 1 H, H(2))	37.71 37.74	5.39 4.81	$C_{25}H_{38}F_9N_9O_{10}$
III	40	0.35 (A)	1.50 (m, 2 H, $CHCH_2C\underline{H}_2^{Lys}$); 1.79 (m, 6 H, $NH_2CH_2C\underline{H}_2^{Lys}$, $CHCH_2C\underline{H}_2^{Arg}$, $CHC\underline{H}_2^{Arg}$); 2.01 (m, 2 H, $CHC\underline{H}_2^{Lys}$); 3.07 (m, 4 H, CH_2-im, $NHC\underline{H}_2^{Arg}$); 3.34 (m, 2 H, $NHC\underline{H}_2^{Arg}$); 3.63 (m, 2 H, CH_2^{Hia}); 4.12 (m, 1 H, CH^{Lys}); 4.43 (m, 1 H, CH^{Arg}); 7.33 (s, 1 H, H(5)); 8.64 (s, 1 H, H(2))	38.01 38.24	5.88 5.96	$C_{21}H_{35}F_6N_9O_6 \cdot 2H_2O$
IV	41	0.33 (A)	1.50 (m, 2 H, $CHCH_2C\underline{H}_2^{Lys}$); 1.85 (m, 4 H, $NH_2CH_2C\underline{H}_2^{Lys}$, $CHC\underline{H}_2^{Lys}$); 3.10 (m, 6 H, CH_2^{Asn}, CH_2-im, $NH_2C\underline{H}_2^{Lys}$); 3.63 (t, 2 H, CH_2^{Hia}, *J* = 6.5); 4.35 (t, 1 H, CH^{Asn}, *J* = 6); 4.45 (t, 1 H, CH^{Lys}, *J* = 6); 7.40 (s, 1 H, H(5)); 8.74 (s, 1 H, H(2))	35.91 36.27	4.59 4.35	$C_{21}H_{30}F_9N_7O_9$
V	25	0.23 (A)	1.44 (m, 2 H, $CHCH_2C\underline{H}_2^{Lys}$); 1.70 (t, 2 H, $NH_2CH_2C\underline{H}_2^{Lys}$, *J* = 6.5); 1.89 (m, 2 H, $CHC\underline{H}_2^{Lys}$); 2.73 (m, 2 H, $NH_2C\underline{H}_2^{Lys}$); 2.95 (m, 4 H, CH_2^{Asn}, CH_2-im); 3.52 (m, 2 H, CH_2^{Hia}); 4.11 (m, 2 H, CH^{Asn}, CH^{Lys}); 7.25 (s, 1 H, H(5)); 8.58 (s, 1 H, H(2))	36.18 36.12	6.50 6.87	$C_{15}H_{27}N_7O_3 \cdot 2H_2O$
VI	35	0.35 (A)	1.22 (d, 3 H, Me, *J* = 3); 1.58 (m, 2 H, $CHCH_2C\underline{H}_2$); 1.82 (m, 2 H, $NH_2CH_2C\underline{H}_2$); 2.05 (m, 2 H, $CHC\underline{H}_2$); 3.10 (m, 4 H, $NH_2C\underline{H}_2$, CH_2-im); 3.65 (m, 3 H, $C\underline{H}_2CH_2$-im, CH^{Lys}); 4.18 (m, 2 H, $C\underline{H}Me$, $C\underline{H}^{Thr}$); 7.39 (s, 1 H, H(5)); 8.71 (s, 1 H, H(2))	39.76 40.14	5.96 5.32	$C_{19}H_{30}F_6N_6O_7$

* Salts with trifluoroacetic acid.

active (the degree of cleavage was 1—2%). The efficacy of ON21 cleavage with tripeptides depends on both the structures of the compounds and the nature of the buffer in which the reaction is performed. In an imidazole buffer, the RNA cleavage proceeds more efficiently than that in a Tris-HCl buffer (Table 3). Since all the tripeptides synthesized contain histidine or histamine, it can be assumed that, in combination with imidazole (a component of the buffer), imidazole pairs can occur with a certain probability in the vicinity of the bonds that are cleaved, as in the catalytic center of RNase A. An increase in the efficacy of RNA cleavage in an imidazole buffer compared to a Tris-HCl, HEPES, or potassium phosphate buffer has been observed earlier for DABCO-imidazole conjugates.[25] As can be seen from Table 3, tripeptides containing arginine residues (the histidine or histamine residue is present in all tripeptides) show the highest efficacy in RNA cleavage regardless of the buffer; HKR > KHR = RKHa ≫ ≫ KTHa (the degree of cleavage was 55, 31, and 10%, respectively). The fact that the arginine-containing tripeptides exhibit substantially higher ribonuclease activity compared to other tripeptides and the dipeptide KH (2L2) described earlier[1] can be attributed to an increase in the affinity of such tripeptides for RNA. As can be seen from Table 3, the ribonuclease activity of tripeptides correlates not only with their overall charge. For example, in the presence of the tripeptides NKHa and KNHa possessing the overall charge of +3, ON21 is only weakly cleaved (1—2%) in an imidazole buffer and is not cleaved at all in a Tris-HCl buffer, whereas the tripeptides KHR, HKR, and RKHa with an overall charge of +4 exhibit the highest activity. Earlier,[26] we have observed a more than 30-fold

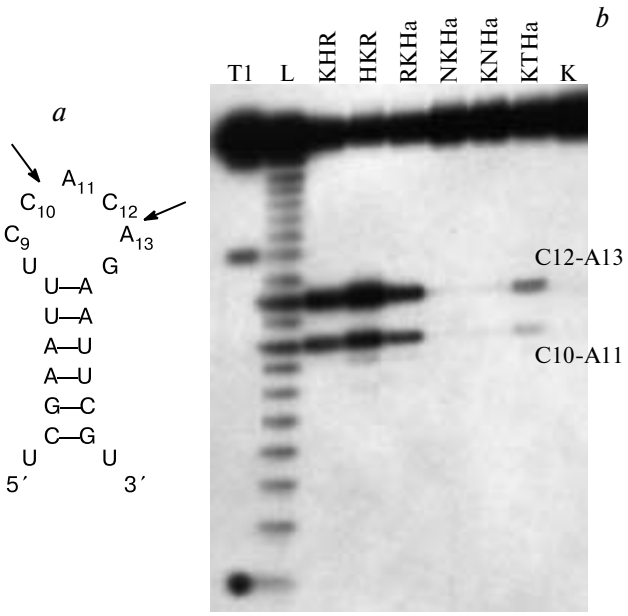

Fig. 3. Cleavage of ON21 with tripeptides. (*a*) The secondary structure of the 21-mer oligoribonucleotide (ON21). The bonds that are cleaved in the presence of tripeptides are indicated by arrows. (*b*) Analysis of the products of ON21 cleavage with tripeptides by electrophoresis in 15% denaturing polyacrylamide gel (PAAG). Lanes L and T1 correspond to random hydrolysis in an imidazole buffer and with RNase T1 in denaturing conditions, respectively. The ON21 cleavage with tripeptides is described in the Experimental section.

increase in the ribonuclease activity of DABCO-imidazole conjugates, when the overall positive charge of the molecules increases from +2 to +4.

In the series of the tripeptides synthesized, each tripeptide contains the lysine residue, which, depending on the position, contributes a charge of either +1 (in the middle) or +2 to the overall positive charge of the molecule. Formally, arginine possesses a positive charge of +1, but it can form a network of hydrogen bonds ("arginine fork") with the adjacent acceptor groups in RNA.[27] These acceptors can involve the oxygen atoms of phosphates, the 2′-OH group of ribose, and certain groups in bases (for example, the O(6) and N(7) atoms of guanine, the O(4) atom of uridine in the major groove, the N(3) atom of guanine or the O(2) atom of uridine in the minor groove). Of three arginine-containing tripeptides (KHR, HKR, and RKHa), the tripeptide HKR displays the highest activity. In this tripeptide, the histidine residue is most remote from the guanidinium group of arginine. The fact that the peptide RKHa exhibits lower (compared to HKR) ribonuclease activity (55 and 31%, respectively) is apparently associated with the replacement of the histidine residue by histamine. A decrease in ribonuclease activity of the conjugates by a factor 1.5 caused by the replacement of His by Hia has been observed earlier for DABCO-imidazole conjugates.[28]

To summarize, the results of the present study demonstrate that of the histidine- or histamine-containing compounds, which were synthesized in the present study and are characterized by a similar arrangement of the functional groups, only peptides containing the arginine residues possess pronounced ribonuclease activity. This fact is apparently associated with a high affinity of the arginine residue for RNA. The arginine residues are known to be involved in the formation of RNA-binding sites of such proteins as *tat*[29] and ribonuclease T1.[15,16] Presumably, the arginine residue in a tripeptide enhances the affinity of chemical ribonuclease for RNA, which is favorable for its more efficient cleavage. It cannot be ruled out that arginine is directly involved in the catalytic event.

Table 3. Efficacy of cleavage of the 21-mer oligoribonucleotide with the tripeptides synthesized and compound 2L2*

Compound	Efficacy of cleavage (%)*		*Q***
	I	II	
KHR	31	17	+4
HKR	55	8	+4
RKHa	31	6	+4
NKHa	1	0	+3
KNHa	2	0	+3
KTHa	10	4	+3
KHa (2L2)	13	9	+2

* The total degree (%) of ON21 cleavage during 24 h is given. The RNA cleavage was carried out in a 50 *mM* imidazole (I) or 50 *mM* Tris-HCl buffer (II), pH 7.0, 0.2 *M* KCl, 0.1 *mM* EDTA, an RNA carrier (0.1 mg mL^{-1}) in the presence of tripeptides at a concentration of 10^{-3} mol L^{-1}, at 37 °C.
** *Q* is the overall charge at pH 7.0.

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