The Role of Lysine-41 of Ribonuclease A in the Interaction with RNase Inhibitor from Human Placenta*

Peter Blackburn and Jose G. Gavilanes
From The Rockefeller University, New York, New York 10021

3-N-Carboxymethyl-His-12 and 1-N-carboxymethyl-His-119-RNase A bind to the naturally occurring RNase inhibitor, isolated from human placenta, 1.3 and 3.6 times, respectively, more strongly than does native RNase A.

Near-ultraviolet circular dichroism measurements indicate that the conformational change which occurs upon carboxymethylation of either of the active site histidine residues appears different from that from which the protein undergoes upon binding of substrate or a substrate analogue.

Specific carboxymethylation of Lys-41 of RNase A decreased the strength of the interaction between the enzyme and the RNase inhibitor to about 12% of the initial value. The near-UV CD spectra of Cm-Lys-41-RNase A and of acetimidyl-RNase A (9.3 lysines modified) and carbamylated RNase A (3.0 lysines modified), which also have weaker interactions with RNase inhibitor of 25% and 10%, respectively, show a negative [O] at 275 nm but are altered in the positive [O] at 240 nm. The CD measurements suggest that one or more tyrosine residues of RNase A may be involved in the interaction with inhibitor.

The effects of pH and salt concentration suggest that a major part of the protein-protein interaction is probably through nonpolar forces. The strengths of interactions between the inhibitor and pancreatic RNases from several species were very similar. Since Tyr-92 is the only tyrosine residue retained in all of the species studied, this residue may have a key role in the nonpolar interaction.

The data presented herein suggest that the interaction between RNase A and the inhibitor involves the positively charged ε-NH₂ group of Lys-41 of RNase A. This interaction could result in the inactivation of the enzyme.

Normal mammalian tissues contain in their cytoplasm an inhibitor of neutral ribonuclease activity (1), as do some nonmammalian tissues (2-4). The equilibrium between the RNase and its inhibitor may have a role in the regulated turnover of cytoplasmic RNA species and thereby affect protein synthesis (5-9). The RNase inhibitors found in the high molecular weight proteins (18, 19).

In 1980, the addition of human placental RNase inhibitor (16, 17) to the wheat germ and rabbit reticulocyte lysate in vitro translation systems increases the synthesis of larger molecular weight proteins (18, 19).

The results of competition binding assays performed with a number of derivatives of RNase A, produced by either proteolytic or chemical modifications, were reported earlier (20). The removal of the active site residues His-12 and His-119, and the auxiliary residues Lys-7, Phe-120, Asp-121, and Ser-129 did not affect the binding with RNase inhibitor. These findings were consistent with the noncompetitive nature of the inhibition (16). It was also demonstrated that the four arginine residues of RNase A are not involved in binding to the RNase inhibitor. However, modifications made to lysines indicated that these residues were important for the interaction. Loss of the positive charges on 3 lysine residues by carbamylation decreased the interaction to 10% of the initial value, yet complete amidination of RNase A only decreased the interaction to 25%. Furthermore, RNase bound to the inhibitor protected the enzyme from inactivation by reagents which are known to inactivate RNase A by reaction at Lys-41. Thus, Lys-41 may be involved in the interaction with the RNase inhibitor.

In this communication, the effects of chemical derivatization of specific residues of RNase A on its ability to interact with RNase inhibitor are reported. CD measurements made on these derivatives of RNase A have permitted a more detailed evaluation of the effects of these chemical modifications on the interaction between the enzyme and its inhibitor.

EXPERIMENTAL PROCEDURES

Materials—Substances and sources were as follows: RNase A (RAF grade) phosphate-free (Worthington Biochemical Corp.); cyclic 2',3'-CMP, 2'-CMP, 3'-CMP (Sigma), yeast ribosomal RNA (Sigma type VI, from Torula yeast), treated as described previously (16); iodoacetate (Eastman), bromoacetate (Aldrich), methyl acetimidate-HCl (Fierce), cross-linked polymethylacrylic acid resin, Bio-Rex 70 (Bio-Rad). All other reagents were of analytical grade.

Carboxymethylation of RNase A at Histidine Residues—The reaction was carried out by a modification of the procedure of Crestfield et al. (21). To 20 mg of RNase A in 1.0 ml of 0.1 M Na acetate buffer, pH 5.5, there was added 30 mg of iodoacetate acid dissolved in 1.0 ml of 0.1 M Na acetate buffer, adjusted to pH 5.5 with NaOH. The alklylation was carried out for 17 h in the dark at 37°C. The sample was applied directly to a column (1.6 × 23 cm) of Bio-Rex 70 equilibrated in 0.2 M Na phosphate buffer, pH 6.48, and then eluted with 0.266 M NaCl as described by Crestfield et al. (22). The fractions which contained Cm-His-12-RNase A and Cm-His-119-RNase A were pooled separately, lyophilized to dryness in a Speedvac Concentrator (Savant), and desalted on a column (1.6 × 25 cm) of Sephadex G-50 (fine) equilibrated with 5% acetic acid. The desalted samples were lyophilized and stored at -10°C. Under these alklylation conditions, chromatography of the reaction products indicated that all of the RNase A was modified.

Carboxymethylation of RNase A at Lysine Residues—The alklylation was carried out by a modification of the procedure of Heinrikson

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A 2-fold molar excess of bromoacetic acid dissolved in 1.0 ml of 0.05 M Tris buffer, adjusted to pH 8.5, was added to 20 mg of RNase A dissolved in 1.0 ml of the same pH 8.5 buffer. The reaction was performed in the dark at 37°C for 22 h. The sample was adjusted to pH 6.5 with 5% acetic acid and applied directly to the Bio-Rex 70 column described previously and eluted with 0.266 M NaCl. Fractions of 1.0 ml were collected and their absorption at 230 nm was measured. The proteins in all major peaks were submitted to amino acid analysis. The fractions which contained Cm-Lys-41-RNase A were pooled and desalted as described earlier. A portion of this sample was rechromatographed on a column (0.9 x 12 cm) of Bio-Rex 70 under the conditions described above and was found to give a single symmetrical peak which eluted at the position of Cm-Lys-41-RNase A (23). The results of amino acid analyses demonstrated the loss of 1.05 lysine residues; histidine recovery was quantitative. The derivative was further characterized by peptide mapping of a tryptic hydrolysate of the sample by high pressure liquid chromatography using a Lichrosorb RF-18 (10 µm particle size) silica-based column (4.6 x 250 mm). Detection of the peptides was performed by measurement of relative fluorescence of peptides after reaction with fluorescamine (24, 25), with on-line detection as described by Bohlen et al. (26).

One other major product of the alkylation reaction was similarly characterized by amino acid analysis and peptide maps. The derivatives were desalted as described earlier and stored at -10°C.

Carbamylatlon and Acetimidylation of Lysine Residues of RNase A—These modifications were performed with sodium cyanate and methyl acetimidate, as reported previously (20).

Human Placental RNase Inhibitor (16)—The protein was prepared as described previously (17). The RNase inhibitor used in these experiments was stored frozen at -10°C in a 20 mM Tris-HCl buffer, pH 7.5, 1 mM in EDTA, 5 mM in dithiothreitol, and 15% (v/v) in glycerol. The stock solution of inhibitor contained 400 µg/ml of protein.

RNase, RNase Inhibitor, and Competition Assays—Activities toward cyclic 2',3'-CMP were measured spectrophotometrically (27-29) with a Zeiss PMQ II spectrophotometer (17). Competition assays were conducted, as described earlier (20), to determine R50 values of each of the derivatives of RNase A studied. The R50 is defined as the molar ratio of derivative to RNase A which gives 50% reversal of the inhibition of RNase A by the inhibitor; the strength of binding can be expressed as the reciprocal 1/R50, or in per cent as 100 x the reciprocal.

Amino Acid Analysis (30, 31)—The analyses were performed with a Durrum D-500 amino acid analyzer to determine the concentration and extent of modification of all the derivatives used in this study. Where necessary, recoveries of amino acids (lysine in carboxymethyl- and acetimidyl-RNases) were corrected to zero time hydrolysis.

Circular Dichroism Spectra—The spectra were obtained with a Cary 60 spectropolarimeter equipped with a 6001 attachment for CD measurements. Cylindrical quartz cells of 1.0 cm path length were used in the near-ultraviolet region at protein concentrations of 0.5 to 1.0 mg/ml. All spectra were recorded at 25°C in 5.1 M Tris/acetate buffer, pH 6.5, 1 mM in EDTA (the same buffer used for competition assays). Ellipticity values, in degrees cm² dmol⁻¹ are calculated on a mean residue molecular weight basis of 110 for RNase A.

RESULTS

Interaction of Cm-His-RNase A with RNase Inhibitor—Carboxymethylation of the active site histidines of RNase A with iodoacetate at pH 5.5 yields a 1:8 mixture of 1-N-Cm-His-12-RNase A and 3-N-Cm-His-19-RNase A (21, 22); the mixture was shown to interact more strongly with RNase inhibitor than did native RNase A (20). The results of competition assays with the chromatographically resolved isomers (22) are shown in Fig. 1, a and b. The Cm-His-12-RNase A (R50 = 0.78) interacted with RNase inhibitor slightly more strongly than did RNase A. The Cm-His-19-RNase A (R50 = 0.28) interacted with RNase inhibitor 3.6 times more strongly than did RNase A. The R50 values reported here agree well with the value of 0.34 reported previously for the nonresolved mixture (20). The predicted R50 for the 1:8 mixture of Cm-His-RNase A isomers is 0.33.

Carboxymethylation of RNase A at the active site histidines introduces a negative charge at the active site. Yang and Hummel (32) noted that Cm-His-RNase A was denatured more slowly than RNase A by urea. The pH dependence of the denaturation of Cm-His-119-RNase A was almost identical to that of RNase A in the presence of the substrate analogue pyrophosphate and suggested that similar stabilizing forces were involved. The possibility that the introduction of a negative charge at the active site of RNase A by carboxymethylation induces a conformational change in the enzyme similar to that induced by the interaction with substrate was thus investigated. The CD spectrum of native RNase A was compared with the spectra of Cm-His-12-RNase A and Cm-His-19-RNase A, as shown in Fig. 2. The near-ultraviolet CD spectra of native RNase A and the enzyme in the presence of 2',3'-CMP and 3',5'-CMP are shown in Fig. 3.

The near-ultraviolet CD spectrum of native RNase A is in good agreement with those reported by others (33-35). After carboxymethylation of the active site histidines, the CD spectra differ both from one another and from that of native RNase A. Both the Cm-His-12 and Cm-His-19 derivatives...
with RNase inhibitor was reduced to 10% (20). The near-ultraviolet CD spectra of RNase A modified at 9 lysine residues with methyl acetimide and of RNase A modified at 3 lysine residues with cyanate are compared with that of native RNase A in Fig. 4. The spectra are essentially identical in the negative ellipticity exhibited at the 275-nm region; however, significant differences exist in the degree of positive ellipticity normally exhibited by RNase A at the 240-nm region. The acetimidylated RNase A exhibited a 60% reduction of this positive ellipticity; the derivative carbamyalted at only 3 lysines exhibited a 90% reduction.

The RNase inhibitor, when bound to RNase A, protects the enzyme from inactivation by reagents which react preferentially with Lys-41 of the enzyme (20). To explore the possibility that Lys-41 may have a role in the interaction of RNase A with RNase inhibitor, RNase A was carbamylated at Lys-41 (23) as described under "Experimental Procedures."

Carbamylmethylation of RNase A with bromoacetate at pH 8.5 yields a number of products other than Cm-Lys-41-RNase A (23). Among these, two other products, both enzymically inactive derivatives, were eluted as overlapping peaks from the column (1.6 × 23-cm) of Bio-Rex 70 before the peak of Cm-Lys-41-RNase A and were designated as Derivatives I and II. Those fractions from the front of the peak which contained Derivative I were pooled and characterized after rechromatography on a column (0.9 × 55 cm) of Bio-Rex 70 and the interaction of the derivative with RNase inhibitor studied. Similar attempts to purify Derivative II were unsuccessful.

The CD measurements of RNase A made in the presence of 2'-CMP and 3'-CMP show that the enzyme undergoes a conformational change upon interaction with substrate. However, the conformational change induced by the binding of 2'-CMP is different from that induced by the binding of 3'-CMP. The difference spectrum obtained in the presence of 2'-CMP is very similar to that reported by Meyer et al. (36). The conformational changes induced in the enzyme molecule by carboxymethylation of the active site histidines are thus different from those induced by the interaction of the enzyme with the substrate.

Interaction of Lysine-modified RNase A with RNase Inhibitor—RNase A acetimidylated on more than 90% of its lysine residues still interacts with RNase inhibitor, but only 25% as strongly as does RNase A (20). After carbamylation of only 3 of the 10 lysine residues of RNase A, the interaction exhibit reduced negative ellipticity in the 275-nm region. The positive ellipticity exhibited at 240 nm is very similar for the native enzyme and Cm-His-12-RNase A; however, Cm-His-119-RNase A exhibits an increased positive ellipticity in the 240-nm region. The nature of the conformational change thus depends upon which active site histidine is alkylated.

The near-ultraviolet CD spectra of native RNase A, RNase A acetimidylated on 9.3 of 10 lysine residues, and RNase A carbamylated on 3 lysine residues; the spectra were recorded at pH 6.5 as described under "Experimental Procedures."

Fig. 3. The near-ultraviolet CD spectra of native RNase A alone, and in the presence of 0.1 mM 2'-CMP or 0.1 mM 3'-CMP; the spectra were recorded at pH 6.5 as described under "Experimental Procedures."

Fig. 4. The near-ultraviolet CD spectra of native RNase A, RNase A acetimidylated on 9.3 of 10 lysine residues, and RNase A carbamylated on 3 lysine residues; the spectra were recorded at pH 6.5 as described under "Experimental Procedures."

Fig. 5. High pressure liquid chromatography of tryp tic digests of performic acid-oxidized (a) RNase A and (b) Derivative I (Cm-Lys-1, Cm-Lys-41 RNase A). About 200 μg of protein were incubated in 1.0 ml of 0.1 M Na borate buffer, pH 8.0, at 37°C with 1% (w/w) tosylphenylalanylnylcarboxymethyl ketone-treated trypsin for 4 h, and for a further 12 h with a second addition of 1% (w/w) tosylphenylalaninyl chloromethyl ketone-treated trypsin. Samples which contained ~50 μg of protein digest were acidified with 0.4 M formate/pyridine buffer, pH 3.25, and chromatographed on a column (4.6 × 250 mm) of Lichrosorb RP-18 (10 μg particle size). The column was eluted at 34 ml h⁻¹ with 0.4 M formate/pyridine buffer, pH 3.25, for 30 min, followed by a linear gradient of 0 to 30% n-proyl alcohol in 90 ml of the same buffer. Fractions of 2.5 ml were collected, and a portion (15%) of the column effluent was directed to the fluorescence monitoring system. Peptide nomenclature: I through 14 refer to the tryptic peptides in order of their sequence from the NH₂ terminus to the COOH terminus of RNase A (37, 38). The dotted lines at the positions of peptides 1 and 7 + 8 in (b) indicate the relative fluorescence expected for these peptides in a digest of RNase A.
The results of reverse phase chromatography of tryptic digests of performic acid-oxidized RNase A and Derivative I are shown in Fig. 5. The tryptic peptides from RNase A were identified from the results of amino acid analyses on fractions which contained the respective peptides. Trypsin does not cleave performic acid-oxidized RNase A after Lys-41 (37). The peptide map of Derivative I shown in Fig. 5b demonstrates that peptide 8, which includes Lys-41, and peptide 1, which includes Lys-1 and Lys-7, both exhibit reduced fluorescence after carboxymethylation. Peptides 2 and 9 are present in unmodified quantities; this result means that Lys-7 and Lys-61 were not modified, otherwise trypsin would not have cleaved after these residues. Therefore, Lys-1 and Lys-41 must be the residues modified in peptide 1 and peptide 8, respectively. Probably carboxymethylation has occurred on the alpha-NH2 group of Lys-1. Similarly, peptide 8 of the tryptic digest of Cm-Lys-41-RNase A exhibited reduced fluorescence. These data were consistent with the results of amino acid analyses which demonstrated that only 1 lysine residue and 2 lysine residues were lost from Cm-Lys-41-RNase A, respectively. The interaction of Cm-Lys-41-RNase A with RNase inhibitor is shown in Fig. 6a. The results of competition assays demonstrate that this single modification decreased the interaction of the enzyme with RNase inhibitor to about 12% of the initial value. CD measurements made on this derivative (Fig. 7) demonstrate that Cm-Lys-41-RNase A has similar spectral properties to those of native enzyme in the 275-nm region. However, the positive ellipticity exhibited by RNase A at 240 nm has been reduced nearly to zero. The results of competition assays with Derivative I are shown in Fig. 6b. This derivative had a 1/Rm of 0.08 and thus interacts with RNase inhibitor 1.4 times less strongly than does Cm-Lys-41-RNase A (1/Rm = 0.12).

**The Effect of Tyrosine Substitution on RNase Inhibitor Binding**—To examine the role of the tyrosine residues of RNase A in the interaction with the RNase inhibitor, pancreatic RNases from other species with known amino acid substitutions, which include some of the tyrosine residues found in the bovine enzyme (39), were examined for their ability to interact with the RNase inhibitor. The mammalian pancreatic RNases are aptly suited for this type of approach. Beintema and his co-workers (39-41) have studied the sequence of many such enzymes and have demonstrated the degree of sequence conservation which they exhibit. The species examined and the nature of the substitutions at the various tyrosine positions of bovine RNase A are shown in Table I.

The specific activities of the enzymes toward cyclic 2',3'-CMP are shown in Table II; while generally similar to that of bovine RNase A, there are some differences. Sufficient amounts of each of the enzymes to give the equivalent activity of 1 pg of bovine RNase A in the cyclic 2',3'-CMP assay were incubated with various concentrations of the RNase inhibitor. The results of these inhibitor titration assays are shown in Fig. 8. The per cent inhibition of each enzyme was linear with respect to RNase inhibitor concentration, as had been found previously for bovine RNase A (17). From the titration data, that amount of inhibitor which would inhibit 1 pg of each of the enzymes by 50% relative to the value for bovine RNase A was calculated and presented in Table II. In accord with the strong interaction between bovine RNase A and the human placental RNase inhibitor, very little

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**Table I.** Tyrosine residues in ribonucleases of the pancreatic type

| Residue number | Bovine | Mouse* | Dromedary* | Hamster | Rat | Pig | BS* |
|----------------|--------|--------|------------|---------|-----|-----|-----|
| 25             | Tyr    | Tyr    | Tyr        | Tyr     | Tyr | Tyr | Tyr |
| 73             | Tyr    | Tyr    | His        | Tyr     | His | Ser | Asn |
| 76             | Tyr    | Ser    | His        | Ser     | Ser | Ser | Ser |
| 92             | Tyr    | Tyr    | Tyr        | Tyr     | Tyr | Tyr | Tyr |
| 97             | Tyr    | Tyr    | Tyr        | Tyr     | Tyr | Tyr | Tyr |
| 115            | Tyr    | Tyr    | Phe        | Tyr     | Pro | Ser | Ser |

*Has an additional Tyr at 102.

**Table II.** Relative affinities of inhibitor for pancreatic RNases

| Species                  | Specific enzyme activity | Relative affinity of the inhibitor for RNase |
|--------------------------|--------------------------|---------------------------------------------|
| Bovine RNase A           | 1.00                      | 1.00                                        |
| Mouse                    | 0.24                      | 1.37                                        |
| Dromedary                | 1.18                      | 0.87                                        |
| Hamster                  | 1.36                      | 0.76                                        |
| Rat                      | 0.52                      | 0.96                                        |
| Pig                      | 0.47                      | 1.33                                        |
| Bovine seminal dimer     | 1.38                      | 0.19                                        |
| Bovine seminal monomer, car
boxamidomethylated          | 0.65                      | 1.83                                        |

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**Fig. 6.** Competition binding of RNase inhibitor with (a) RNase A carboxymethylated at Lys-41 and (b) RNase A carboxymethylated at Lys-1 and Lys-41 (see legend to Fig. 1).

**Fig. 7.** The near-ultraviolet CD spectra of native RNase A, and RNase A carboxymethylated at Lys-41; the spectra were recorded at pH 6.5 as described under "Experimental Procedures."
**DISCUSSION**

The results of the competition assays reported here with RNase A specifically alkylated at Lys-41 with bromoacetate clearly demonstrate that this residue plays an important role in the interaction between the enzyme and its naturally occurring inhibitor. When Lys-1 was also alkylated, there was little further effect on the interaction. This result is consistent with data reported previously (20), which demonstrated that the first 20 NH₂-terminal residues of RNase A are not important for the interaction with inhibitor.

The positive ellipticity exhibited at 240 nm in CD measurements of native RNase A has been attributed to the exposed tyrosine residues of the enzyme (33, 51-53). The negative ellipticity at 275 nm arises as a result of the contribution of the buried tyrosine residues, and in part of that of the exposed tyrosines (33, 53).

In the present experiments, the CD spectra of a number of RNase A derivatives which exhibit altered interaction with RNase inhibitor show that those derivatives which interact less strongly exhibit decreased positive ellipticity at 240 nm. Of those derivatives which were previously shown to have an unaltered interaction with the RNase inhibitor (20), RNase A (des-121-124) (34, 54), RNase A (des-119-124) (55), and RNase S (33) all have CD spectra very similar to that of native RNase A. Only RNase S-protein, which interacts with RNase inhibitor to the same extent as does native RNase A, has an altered CD spectrum which exhibits increased positive ellipticity at 240 nm (33). It has been shown by Li et al. (56) that Tyr-25, an abnormally titrating tyrosine of RNase A, is normalized in this derivative. Thus, normalization of an abnormal tyrosine can lead to an increase in the positive ellipticity at 240 nm.

Also in accordance with data presented here, CD measurements made on the dimeric bovine seminal plasma enzyme demonstrate that, unlike RNase A, the ellipticity in the 240-nm region is negative. Negative ellipticity in the 275-nm region is exhibited by the dimer but is less than that of RNase A. The S-carboxymethylated seminal RNase monomer has a CD spectrum very similar to that of RNase A, with positive ellipticity at 240 nm, implying exposure of tyrosyl residues upon dissociation of the dimeric seminal RNase (35).

The CD measurements suggest that one or more tyrosine residues of RNase A may be involved in the interaction with RNase inhibitor. Carboxymethylation of RNase A at His-119 apparently increases the exposure or normalizes a tyrosine residue, and this may account for the stronger interaction with RNase inhibitor exhibited by this derivative. Acetimidylation (retains positive charge), carbamylation (replaces positive charge with a neutral moiety), and carboxymethylation (replaces a positive charge with a negative charge) of lysine residues of RNase A result in a decreased exposure of the surface tyrosines of the enzyme. This effect apparently results primarily from modification of the ε-NH₂ group of Lys-41.

From the x-ray studies of Richards and Wyckoff (57), four tyrosines are on the surface of the molecule; 3 of these residues, 73, 76, and 115, titrate normally and the remaining Tyr-92 titrates abnormally (58-61). The other two abnormally titrating tyrosine residues, 25 and 97, are buried in the interior of the molecule and are thus less likely to be involved directly in the binding of RNase inhibitor.

Among the various species of pancreatic RNases examined for RNase inhibitor binding, Tyr-76 of the bovine enzyme is a variable residue and is non-conservatively substituted (i.e. the substitution is not by an aromatic residue) in all of the other species examined. Tyr-73 is nonconservatively substituted in the RNases of two of the six species, the dromedary and the rat. Tyr-115 is nonconservatively substituted in the pig and the bovine seminal plasma RNases. None of these substitutions seriously affected the interaction with the RNase inhibitor. Thus, it is unlikely that these residues, 73, 76, and 115, have an important role in the binding to RNase inhibitor.

The fourth surface tyrosine, residue 92, is retained in all of the pancreatic RNases so far examined, with the exception of the guinea pig RNases A and B where it is conservatively substituted by phenylalanine (62). Tyrosine 92 is involved in a hydroxyl hydrogen bond with the carbonyl group of the amide bond between Lys-37 and Asp-38, residues which are very close to the active center residue Lys-41. This interaction raises the pK of tyrosine-92, whereas in horse pancreatic RNase because of steric constraints as a result of a deletion of residue 39, Tyr-92 cannot form a hydroxyl hydrogen bond with these residues (63, 64) and has a lower pK than Tyr-92 of bovine RNase A. The changes observed in the CD spectrum of RNase A upon modification of Lys-41, His-119, and His-12 can be explained by an effect on the environment of the surface tyrosine residues.

From study of the x-ray structure of the active center of RNase A (57), carboxymethylation of N of His-119 is most likely to result in an interaction with the e-NH₂ group of Lys-41 so as to neutralize the negative charge introduced by the carboxymethyl group. This interaction would necessitate a conformational shift involving the polypeptide backbone from residues Lys-37 through Val-43 and lead to a partial closure of the active site cleft. This conformational shift would in-
increase the distance between the peptide carbonyl of Lys-37 and the phenolic hydroxyl of Tyr-92. Disruption of the hydrogen bond between these two groups would occur and result in a conformational change involving Tyr-92. Thus, no change in [θ]$_{220}$ at 240 nm would be anticipated.

Carboxymethylation of N$_\text{H}$ of His-12 (in contrast to reaction at His-119) is likely to result in interactions with the eNH$_2$ group of His-119 which is likely to result in a conformational change involving Tyr-92. This interaction of Lys-41 could arise because there is a direct role in binding to the RNase inhibitor, and not as a result of a secondary effect on the surface tyrosine residues 73, 76, and 115.

The inhibition of RNase A by RNase inhibitor demonstrates very little variation with pH in the range of 5.5 to 8.5. Previously it has been shown that the inhibition is unaffected by salt concentrations up to 0.6 M NaCl (17). These data suggest that the major part of the protein-protein interaction between RNase A and RNase inhibitor is non-polar.

There are very few regions of the surface of RNase A which are conserved among the species investigated here. One of these consists of the active site cleft residues Lys-41, Pro-42, Val-43 and the loop region involving Lys-91, Tyr-92, Pro-93, and Pro-94. The active site histidine residues 12 and 119 and those residues involved in maintaining the correct charge and orientation of the histidine residues are not directly involved in the interaction with RNase inhibitor (20). Lys-41, a residue at or near the active site of the enzyme (57), is a key residue in the interaction with the RNase inhibitor. The e-NH$_2$ group of Lys-41 probably interacts with a negatively charged group of the RNase inhibitor. This ionic interaction would be stabilized by an apolar environment, provided by the interaction of the two proteins and which possibly involves the loop region including Tyr-92. This interaction of Lys-41 could result in the inactivation of the enzyme, since the activity of RNase A is sensitive to modification at Lys-41 (23, 65-69).

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Interaction of RNase A and Its Inhibitor

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