A number of properties of partially purified RNase A inhibitors from mammalian tissues, in particular from rat liver, have been described (1–4). Primarily as a consequence of the difficulties encountered in the extraction and purification of the protein, there have been very few studies on the structural details of the interaction of the inhibitor with the enzyme. With a crude preparation of the RNase inhibitor from rat liver (a high speed supernatant fraction), Roth and Hurley (5) examined the effects of a number of modifications of RNase A on its interaction with their inhibitor preparation. In this report we examine the interaction of RNase A with human placental RNase inhibitor; two general approaches have been undertaken. Specific proteolytic cleavage of the enzyme molecule designed to remove parts of the active site were performed to evaluate the contribution of this domain for binding of the RNase inhibitor. Secondly, specific chemical modifications of groups on the enzyme were performed to assess their role in the inhibitor-RNase interaction. The derivatives of RNase A were then assayed for inhibitor binding by competition with RNase A in the cyclic 2',3'-CMP assay described in the preceding report (7).

**EXPERIMENTAL PROCEDURES**

**Preparation of des-Derivatives of RNase A—Des-(119–124)-RNase was prepared from des-(121–124)-RNase by digestion with carboxypeptidase A (9) according to Lin (10). Carboxypeptidase A (2 mg) was added to 1 mg of des-(121–124)-RNase, in 1.0 ml of N-tris(hydroxymethyl)aminomethane-HCl, pH 7.6. Digestion was carried out at 45°C for 20 min. After 5, 10, 15, and 20 min, 50-μl samples were removed to a polyethylene centrifuge tube (400 μl capacity) that contained 10 μl of 50% sulfosalicylic acid. After the mixtures were kept for 30 min at 4°C, 50 μl of water were added to each of the suspensions and the samples were centrifuged for 5 min in a Beckman Microfuge model 152. From each supernatant, 75 μl were removed to a similar tube that contained 5 μl of 4% NaOH. After 15 min at room temperature, the samples were centrifuged again for 5 min and 40 μl of each supernatant were taken for amino acid analysis; the release of phenylalanine and histidine was determined to be 95% of the theoretical value.

**Denatured, Reduced S-Carboxamidomethyl RNase A—About 1 mg of the enzyme was incubated for 1 h at 50°C in 1 ml of 0.1 M Tris-HCl buffer, pH 8.5, 8 μl in guanidine chloride, 1 mM in EDTA, and 50 mM in dithiothreitol. Then, 0.1 ml of 1.2 M iodoacetamide in 0.1 M Tris-HCl, pH 8.5, 1 mM in EDTA was added to the reaction mixture. Alkylation was carried out for 3 h at 50°C in the dark. The sample was desalted on a column (0.9 × 13 cm) of Sephadex G-50 (fine grade) equilibrated in 5% acetic acid and the resulting protein solution was lyophilized.

**RNase A Carboxymethylated at Histidine Residues**—The alkylation was conducted according to Crestfield et al. (11). To 1 mg of RNase A in 0.9 ml of 0.5 M Na acetate buffer, pH 5.5, there was added 0.1 ml of 1 M iodoacetamide in 0.1 M Tris-HCl, pH 8.5. After 30 min at 25°C the reaction mixture was dialyzed against 50 volumes of buffer.
0.1 ml of 0.8 M iodoacetate dissolved in 0.2 M Na acetate buffer, adjusted to pH 5.5 with sodium hydroxide. Alklylation was carried out for 5 h at 37°C. The protein was desalted and lyophilized as described above.

Modification of Arginine Residues—The reaction with butanedione was performed under the conditions described by Rutherford (19). A fresh solution of the reagent was prepared by the addition of 10 μl of 2,3-butanedione to 1.0 ml of 0.1 M Na borate buffer, pH 9.0. The resulting solution had a pH of 8.3 and was adjusted to pH 7.8 with NaOH. To 0.1 mg of RNase A in 0.1 ml of 0.1 M Na borate buffer, pH 7.8, there was added 0.9 ml of the reagent solution. The reaction was carried out overnight at 37°C. The mixture was acidified by the addition of 0.1 ml of 15% acetic acid. Samples of 50 μl were removed for amino acid analysis.

Modification of arginine residues with cyclohexanedione was performed according to P. B. Pathy and Smith (13, 14). To 1 mg of RNase A in 0.1 ml of 0.1 M Na borate buffer, pH 9.0, there was added 0.9 ml of a freshly prepared solution of reagent that contained 10 μg of 1,2-cyclohexanedione dissolved in 1.0 ml of the pH 9.0 borate buffer. The reaction mixture was kept at 37°C overnight, and then acidified with 0.1 ml of 15% acetic acid. Samples of 50 μl were removed for amino acid analysis.

Amidation of RNase A—The amidation of lysine residues was performed with methyl acetimidate·HCl (15-18) or methyl p-hydroxybenzimidylmethyl carbonate·HCl (19) according to the general procedure suggested by Brown and Kent (20, 21). To 1 mg of RNase A in 1.0 ml of 0.2 M Na bicarbonate buffer, pH 8.5, sufficient reagent was added, as the solid, to give final concentrations of 0.1 M or 0.02 M, respectively. After 30 min at 37°C, a similar addition of methyl acetimidate was made and the reaction was continued for a further 2 h. For the amidation with methyl p-hydroxybenzimidylmethyl carbonate, the second addition of reagent was made after 1 h and the reaction was continued overnight. Both reactions were terminated by the addition of 0.1 ml of 15% acetic acid. Samples of 50 μl were removed for amino acid analysis. The proteins were desalted as described above and lyophilized.

Carbamoylation of RNase A—The carbamoylation was performed essentially as described by Stark et al. (22) at 37°C in 0.2 M Na bicarbonate buffer, pH 8.5, with sodium cyanate added, as the solid, to give final concentrations of 0.1 M or 0.02 M, respectively. After 30 min at 37°C, a similar addition of sodium cyanate was made and the reaction was continued for a further 2 h. The reaction mixture was kept at 37°C overnight, and then acidified with 0.1 ml of 15% acetic acid. Samples of 50 μl were removed for amino acid analysis. The proteins were desalted as described above.

RNase Inhibitor Analysis—The preparation of RNase S-protein was prepared as described in the accompanying communication (7). The RNase inhibitor used in these experiments was stored at -10°C in 20 mM Tris-HCl buffer, pH 7.5, 1 mM EDTA, 5 mM dithiothreitol, 0.15 M in NaCl, and 15% (v/v) in glycerol.

RNase, RNase Inhibitor, and Competition Assays—Activities toward RNase A were measured spectrophotometrically as described in the preceding publication (7). Assays with yeast ribosomal RNA as substrate were performed as described previously (6). Competition assays were conducted with various concentrations of RNase derivative, at fixed concentrations of RNase A (1 μg/ml), and sufficient RNase inhibitor to give approximately 50% inhibition of the activity of 1 μg of RNase A in the cyclic 2',3'-CMP assay. The assays were performed by first mixing the RNase derivative with RNase inhibitor in the assay cuvette for 5 min at 25°C in the presence of the substrate. After a stable base-line was recorded at 266 nm, 1 μg of RNase A was added in 10 μl of solution to start the reaction. Control assays were performed in which RNase inhibitor was omitted. The molar ratio of RNase derivative to RNase A which gave 50% reversal of the inhibition of RNase A produced by the RNase inhibitor is defined as the R0 value of that derivative. An R0 value of 1.0 indicates that the derivative binds to the RNase inhibitor with the same affinity as does native RNase A. With such a derivative, the observed RNase A activity would increase from 50% of a noninhibited RNase A control to 75% of the control.

Amino Acid Analysis—The analyses (23, 24) were performed with a Durrum D-500 amino acid analyzer to determine concentration, purity, and extent of modification of all of the derivatives used in this study. In general approximately 50 μg of protein sample were hydrolyzed with 0.5 ml of 6 N HCl at 110°C for 20 h (for concentration measurements), 48, 72, and 96 h in Pyrex tubes (10 X 75 mm) sealed after evacuation to below 25 milliliter. Hydrolysates of S-carboxamidomethylated RNase and 2,3-butanedione and 1,2-cyclohexanedione (13) modified RNases were performed with the addition of 20 μl of 2-mercaptoethanol. Quantitative recoveries of S-carboxymethyllysine and no regeneration of arginine were observed when hydrolysis was performed under these conditions for up to 96 h.

The recoveries of lysine from carboxamidomethylated RNase and acetylated-RNase were corrected to zero-time hydrolysis. The half lives of the modified lysine derivatives during hydrolysis were 52 h and 94 h, respectively. No release of free lysine was observed upon amino acid analysis of the methyl p-hydroxybenzimidamide-modified RNase hydrolyzed for up to 96 h in 6 N HCl. No ninhydrin-positive peak which would correspond to p-hydroxybenzimidyllysine or its break down products was eluted from the column. The basic and aromatic p-hydroxybenzimidyllysine was not expected to elute from the column of the Durrum D-500 as used under the routine conditions for analysis of protein hydrolysates.

RESULTS

Interaction of Protease-modified RNase A with RNase Inhibitor A number of RNase A derivatives, derived by specific proteolytic modifications which remove residues at the NH2- or COOH-terminal regions of the enzyme, were studied in order to examine the contribution of the residues to the binding of RNase inhibitor. RNase A is selectively cleaved after alanine 20 by subtilisin (25, 26) to yield RNase S, which can be dissociated into the NH2-terminal S-peptide, residues (1 to 20), which contains His-12 of the active center, and the S-protein, residues (21 to 124), which contains His-119. The results of competition binding assays, performed as described under "Experimental Procedures," with various concentrations of these derivatives are shown in Fig. 1. The preparation of RNase S, which retained 99% of the activity of RNase A (Table I) interacted with placental RNase inhibitor to the same extent as RNase A. RNase S-protein, which had only 0.4% of the enzymic activity of RNase A toward cyclic 2',3'-CMP, in competition assays, had an R0 value of 1.0, and thus interacted with the RNase inhibitor to the same extent as does RNase A. No interaction of RNase S-peptide with the RNase inhibitor was detectable up to a 100-fold molar excess of S-peptide over RNase A. The RNase inhibitor binding domain therefore resides in the S-protein portion of the RNase A molecule; the catalytic site residue His-12 is not essential for binding of the RNase inhibitor.

Proteolysis of RNase A with pepsin removes four amino acids from the COOH terminus of the molecule (8); the product des-(121-124)-RNase retains about 1% the activity of RNase A toward cyclic 2',3'-CMP (9). Further removal of Phe-120 and the catalytic site residue His-119 with carboxypeptidase A yields des-(119-124)-RNase which is enzymically inactive. Both derivatives interacted with RNase inhibitor by guest on September 22, 2017http://www.jbc.org/ Downloaded from
residues, Phe-120 to Val-124 are not required for RNase-metabolism, the catalytic site residue His-119 and the five other residues is consistent with the observations of Patthy and inactivation of RNase A upon modification of its arginine of more than 95% of the activity toward cyclic 2',3'-CMP. The structural analogue 1,2-cyclohexanedione. Extensive modification of the enzyme was accompanied in both cases by loss of the enzyme molecule is thus required for binding to RNase A still interacted with the RNase inhibitor, but to a lesser same extent as RNase A. Cyclohexanedione-modified RNase -A interacted with RNase inhibitor to the extent that the intact three-dimensional structure of the enzyme molecule is thus required for binding to RNase inhibitor, as to be expected for a specific interaction between the two molecules.

Chemical modification of the guanidino groups of arginine residues was performed with 2,3-butanedione and its higher structural analogue 1,2-cyclohexanedione. Extensive modification of the enzyme was accompanied in both cases by loss of more than 95% of the activity toward cyclic 2',3'-CMP. The inactivation of RNase A upon modification of its arginine residues is consistent with the observations of Patthy and Smith (14). Yankelev (27), reported that 45% of the enzymic activity of RNase A was retained upon modification of the enzyme with oligomers of 2,3-butanedione. The butanedione-modified RNase A interacted with RNase inhibitor to the same extent as RNase A. Cyclohexanedione-modified RNase A still interacted with the RNase inhibitor, but to a lesser degree (\(R_0 = 2.0\)) than does RNase A. The four guanidino groups of RNase A, in arginine residues 10, 33, 39, and 85 (28), are thus not essential for the binding of RNase A to the inhibitor.

Carboxymethylation of histidine residues of RNase A with iodoacetate at pH 5.5 results in the modification of N-3 of His-12 or N-1 of His-119 in a ratio of approximately 1:8 (29). No attempt was made to resolve the two isomeric derivatives in this study. The carboxymethylation of 0.98 residues of histidine per molecule of enzyme resulted in a derivative with 2.7% residual activity toward cyclic 2',3'-CMP. This derivative, in competition binding assays, interacted with RNase inhibitor approximately 3 times more strongly (\(R_0 = 0.34\)) than did RNase A.

Modifications to the \(\alpha\)-NH\(_2\) group and 10 \(\epsilon\)-NH\(_2\) groups of lysine residues of RNase A were made with methyl acetimidate-HCl and its higher structural analogue, methyl \(p\)-hydroxybenzimidate-HCl. The resultant amide derivatives are not changed in charge at neutral pH. Modifications of amino groups, with loss of the positive charge, were effected with cyanate. Under the conditions described under "Experimental Procedures," complete modification of RNase A lysine residues was obtained with methyl \(p\)-hydroxybenzimidate (Table II). Quantitative modification of lysine residues with methyl acetimidate was more difficult to achieve (20, 21) In both

### Table I

| Derivative          | Enzymic activity* | Interaction with inhibitor* |
|---------------------|-------------------|-----------------------------|
| RNase des-(121-124) | 1.2               | 1.0                         |
| RNase des-(119-124) | 0                 | 1.0                         |
| RNase S-protein     | 0.4               | 1.0                         |
| RNase S-peptide     | 0                 | >10                         |
| RNase S             | 90                | 1.0                         |

* Enzymic assays were performed with cyclic 2',3'-CMP (7).

### Table II

| Residues modified | Reagent                 | No. of residues modified | Enzymic activity* | Interaction with inhibitor* |
|-------------------|-------------------------|--------------------------|-------------------|-----------------------------|
| Cysteine          | Reduced and carboxamidomethylated | 8                        | 0                 | >10                         |
| Arginine          | Butanedione             | 4.0                      | 5.1               | 1.0                         |
| Arginine          | Cyclohexanedione        | 3.34                     | 3.2               | 2.2                         |
| Histidine         | Iodoacetate            | 0.98                     | 2.7               | 0.34                        |
| Lysine            | Methyl acetimidate      | 9.1                      | 0.87              | 3.7                         |
| Lysine            | Methyl \(p\)-hydroxybenzimidate | 10                      | 0                 | 4.0                         |
| Cyanate           |                         | 6.6                      | 0                 | >10                         |

* Amino acid analyses were performed as described under "Experimental Procedures."
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Fig. 4 (left). Rate of inactivation of RNase A and extent of lysines modified with methyl acetimidate. Reagent was added as the solid, to give a final concentration of 0.1 M, to 1 mg of RNase A in 1 ml of 0.2 M Na bicarbonate buffer, pH 8.5, 1 mM in EDTA. The reaction was performed at 37°C. Samples of 50 μl were removed at the indicated times and each was acidified with 20 μl of 15% acetic acid to terminate the reaction. Enzymic activity was then determined by the cyclic 2',3'-CMP assay (7). Amino acid analyses were performed as described under "Experimental Procedures." instances, complete amidination of RNase A led to a decrease, but did not abolish, the interaction of the derivative with RNase inhibitor. Methyl acetimidate- and methyl p-hydroxybenzimidamide-modified RNase A had similar RSo values of 3.7 and 4.0, respectively.

Carbamylation of RNase A with cyanate, however, completely abolished the interaction with RNase inhibitor after 6 lysine residues were modified. Partial carbamylation of native RNase A generates derivatives with decreased enzymic activity dependent upon the extent of modification. The results of competition assays with these derivatives are difficult to interpret, since more than one species of enzyme contributes to the hydrolysis of the cyclic 2',3'-CMP substrate. To study the effect of carbamylation of RNase A on its ability to bind to RNase inhibitor, carbamylation of carboxymethyl-His-RNase A was chosen for study. The carboxymethyl derivative binds to the RNase inhibitor, is nearly enzymically inactive, and thus facilitates the interpretation of competition assay results. The effect of progressive carbamylation of this derivative on its ability to interact with RNase inhibitor was determined by competition binding assays. The change in RSo value with extent of carbamylation is plotted in Fig. 3. After only 3 lysine residues were carbamylated, 90% of the ability of the derivative to bind to RNase inhibitor was lost. We have noted that upon storage of the carbamylated derivatives there was a slow (days) decrease in their ability to interact with RNase inhibitor.

The loss of RNase activity upon amidination of its lysine residues is shown in Fig. 4. In 0.2 M Na bicarbonate buffer, pH 8.5, with 0.1 M methyl acetimidate, a rapid inactivation of RNase A occurs. The rate of inactivation exceeds the rate of overall modification of lysine residues; amidination of 2 to 3 lysine residues per molecule of enzyme was accompanied by a 70 to 80% loss of activity toward cyclic 2',3'-CMP. This result is consistent with the observation by Heinrikson (30), that alkylation of Lys-41, situated near the active site, proceeds preferentially and results in the inactivation of the enzyme. No selective modification of lysine residues of RNase A was observed on amidination at pH 10 (18).

The rates of inactivation of RNase A upon amidination with methyl acetimidate in 0.2 M Na bicarbonate buffer, pH 8.5, and 0.2 M Heps' buffer, pH 7.5, are compared with the effect of amidination of the RNase A-inhibitor complex (Fig. 5). The rate of inactivation of RNase A is less at pH 7.5; hydrolysis of the reagent at this pH limited the rate and extent of enzyme modification. However, at pH 7.5, no appreciable loss of RNase activity occurred when the enzyme was bound to the RNase inhibitor. Also, no inactivation of the inhibitor occurred under these conditions. In 0.2 M bicarbonate, pH 8.5, it was not possible to study the protective effect of the inhibitor on the amidination of the enzyme because, under these conditions, the RNase inhibitor was rapidly inactivated prior to the addition of reagent.

Amidination of RNase A with methyl p-hydroxybenzimidamide in 0.2 M Heps' buffer, at pH 7.5 and pH 8.5, with resultant loss of enzymic activity along with the effect of this imidoester on the RNase-inhibitor complex is shown in Fig. 6. The rate of inactivation of RNase A occurred less rapidly at pH 7.5 than at pH 8.5. However, the extent of inactivation upon prolonged incubation with reagent was approximately the same. Amidination of the RNase A-inhibitor complex with methyl p-hydroxybenzimidamide did not appreciably inactivate either the enzyme or the inhibitor. In contrast to the situation in bicarbonate buffer, no inactivation of the enzyme-bound inhibitor occurred at pH 8.5 in 0.2 M Heps' buffer.

Discussion

RNase A is noncompetitively inhibited by the human placental RNase inhibitor (6) and by the rat liver RNase inhibitor (31). The present experiments show that several specific modifications of the active site of the enzyme do not decrease the binding of the protein to the RNase inhibitor, a finding which

1 The abbreviation used is: Heps, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.
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is consistent with the noncompetitive nature of the interaction.

The loss of Asp-121 in des-(121-124)-RNase is thought to raise the pKₐ of His-119 and results in the almost complete inactivation of the enzyme (10). The pKₐ for cyclic 2',3'-CMP of this derivative is only slightly altered; however, there is a 13-fold decrease in the ability to bind 2'-CMP, possibly as a result of the loss of Ser-123 which may participate in a hydrogen bond with the phosphate group of the substrate (32). Removal of Phe-120 results in the complete loss of enzymatic activity. Phe-120 is thought to maintain the correct orientation and charge distribution of the catalytic site histidine residues, are essential for binding of the RNase inhibitor.

It is difficult to compare our results with those of Roth and Hurley (5) because of the different assay conditions and the absence of analytical data on some of their derivatives. The present study clearly confirms their qualitative observation that carbamylation of RNase A interferes with its combination with the RNase inhibitor. They also indicated that performic acid oxidized RNase A, and reduced and carbamylated RNase did not combine with RNase inhibitor.

Of the modifications which are thought to retain the native conformation of the enzyme, the cyanate reaction, which neutralizes the charges on lysine residues, yields the most marked decrease in the inhibitor-enzyme interaction. Derivatizations of the lysine residues by reactions that do not alter the charge have a much smaller effect. Since RNase A is a basic protein (pl 9.4, Richards and Wyckoff (34)) and the RNase inhibitor is an acidic protein (pl 4.7, Blackburn et al. (6)), charge effects are likely to be involved.

Roth and Hurley (5) found that RNase A, deamidated by treatment with nitrous acid, interacted with their RNase inhibitor, a finding somewhat contradictory to their findings upon carbamylation. They proposed that arginine residues were involved in the interaction; the present study indicates that the guanidino groups of arginine residues are not essential for RNase inhibitor binding.

Modification of the amino groups of RNase A inactivates the enzyme primarily as a result of modification of Lys-41 (30, 35-40). Modification of Lys-7 can also lead to a decrease in enzymatic activity (38, 39, 41, 42). Lys-41 is most reactive to a number of reagents as a result of its lower pKₐ, estimated to be 8.8 (40). The precise role that Lys-41 plays in the catalytic mechanism is unknown. It is apparently not directly involved in binding substrate (34, 43). After binding of substrate, however, it may have a catalytic role as a result of a changed conformation at the active site of the enzyme (44, 45). Since we obtain a major decrease in RNase inhibitor binding with the carbamylation of only 3 out of the 10 lysine residues, and since Lys-41 is known to be one of the most reactive lysine residues, we conclude that Lys-41 may be involved in the RNase inhibitor binding and results in the inactivation of the enzyme. This hypothesis is strengthened by the finding that the presence of the RNase inhibitor blocks reactions that inactivate the free enzyme at Lys-41. In addition, Lys-7 and Lys-66 are known to be normally readily available residues (40), but Lys-7 can be ruled out in this case because of the data with S-peptide and S-protein.

The unexpected finding that carbamylmethylation of His-119 or His-12 strengthens the binding to inhibitor may indicate that carbamylmethylation alters the region of the active site in such a way as to render the nearby Lys-41 and neighboring residues more readily available for participation in the binding process. Yang and Hummel (46) noted that carbamylmethyl-His-119- and His-12-RNase A are denatured much more slowly than RNase A by urea; the pH dependence of the denaturation of carbamylmethyl-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.

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REFERENCES
1. Roth, J. S. (1967) Methods Cancer Res. 3, 151-243
2. Shortman, K. (1961) Biochem. Biophys. Acta 51, 37-49
3. Grivna, A. M., Schoenmakers, J. G. G., van Kraakamp, M., Hilak, M., and Bloemendal, H. (1970) Biochim. Biophys. Acta 244, 55-62
4. Gagnon, C., and de Lamirande, G. (1973) Biochem. Biophys. Res. Commun. 51, 580-584
5. Roth, J. S., and Hurley, D. (1966) Biochem. J. 101, 112-119
6. Blackburn, P., Wilson, G., and Moore, S. (1977) J. Biol. Chem. 252, 5904-5910
7. Blackburn, P. (1979) J. Biol. Chem. 254, 12494-12467
8. Anfinsen, C. B. (1956) J. Biol. Chem. 221, 405-412
9. Roth, J. S., and Hurley, D. (1966) Methods Enzymol. 11, 155-166
10. Liu, M. C. (1970) J. Biol. Chem. 245, 6726-6731
11. Crestfield, A. M., Stein, W. H., and Moore, S. (1963) J. Biol. Chem. 238, 2413-2420
12. Rottman, K. F. (1973) Biochemistry 12, 3979-3983
13. Patthy, L., and Smith, E. L. (1975) J. Biol. Chem. 250, 567-564
14. Patthy, L., and Smith, E. L. (1975) J. Biol. Chem. 250, 565-569
15. Hunter, M. J., and Ludwig, M. L. (1962) J. Am. Chem. Soc. 84, 3491-3504
16. Ludwig, M. L., and Hunter, M. J. (1967) Methods Enzymol. 11, 596-604
17. Ludwig, M. L., and Hunter, M. J. (1972) Methods Enzymol. 25, 585-596
18. Reynolds, J. H. (1968) Biochemistry 7, 3131-3135
19. Wood, F. T., Wu, M. M., and Gerhart, J. C. (1975) Anal. Biochem. 69, 339-349
20. Bowd, D. T., and Kent, S. B. H. (1975) Biochem. Biophys. Res. Commun. 67, 126-132
21. Bowd, D. T., and Kent, S. B. H. (1975) Biochem. Biophys. Res. Commun. 67, 133-138
22. Stark, G. R., Stein, W. H., and Moore, S. (1960) J. Biol. Chem. 235, 3177-3181
23. Moore, S., and Stein, W. H. (1968) Methods Enzymol. 6, 819-831
24. Moore, S. (1968) J. Biol. Chem. 243, 6281-6283
25. Richards, F. M. (1959) C. R. Trav. Lab. Carlsberg 29, 322-329
26. Richards, F. M., and Vithayathil, P. J. (1959) J. Biol. Chem. 234, 1459-1465
27. Yankelev, J. A., Jr (1970) Biochemistry 9, 2434-2439
28. Smyth, D. G., Stein, W. H., and Moore, S. (1963) J. Biol. Chem. 238, 227-234
29. Cretan, A. M., Stein, W. H., and Moore, S. (1963) J. Biol. Chem. 238, 2421-2428
30. Heimrikson, R. L. (1966) J. Biol. Chem. 214, 1393-1405
31. Bartholomey, J., and Bauduin, P. (1977) Biochemistry 16, 675-683
32. Wyckoff, H. W., Hardman, K. D., Allewell, N. M., Ingami, T., Johnson, L. N., and Richards, F. M. (1967) J. Biol. Chem. 242, 2273-2283
33. Lin, M. C., Gutte, B., Caldi, D. G., Moore, S., and Merrifield, R. B. (1972) J. Biol. Chem. 247, 4769-4774
34. Richards, F. M., and Wyckoff, H. W. (1971) in The Enzymes (Boyer, P.D., ed) 3rd Ed, Vol. 4, pp. 647-806, Academic Press, New York
35. Hirs, C. H. W., Halmann, M., and Kycia, J. H. (1961) in Biological Structure and Function (Goodwin, T. W., and Lindberg, I., eds) Vol. I, pp. 41-57, Academic Press, New York
36. Hirs, C. H. W. (1962) Brookhaven Symp. Biol. 15, 154-183
37. Carty, R. P., and Hirs, C. H. W. (1968) J. Biol. Chem. 243, 5244-5253
38. Raetz, C. R. H., and Auld, D. S. (1972) Biochemistry 11, 2229-2236
39. Dudkin, S. M., Karabachyan, L. V., Borisova, S. N., Shlyapnikov, S. V., Karpisly, M. Y., and Geidarov, T. G. (1975) Biochim. Biophys. Acta 386, 275-282.
40. Brown, L. R., and Bradbury, J. H. (1976) Eur. J. Biochem. 68, 227-235.
41. Glick, D. M., and Barnard, E. A. (1970) Biochim. Biophys. Acta 214, 326-342.
42. Means, G. E., and Feeney, R. E. (1971) J. Biol. Chem. 246, 5532-5533.
43. Walter, B., and Wold, F. (1976) Biochemistry 15, 304-310.
44. Hammes, G. G., and Waltz, F. G. (1969) J. Amer. Chem. Soc. 91, 7175-7186.
45. Myer, Y. P., Barnard, E. A., and Pal, P. K. (1979) J. Biol. Chem. 254, 137-142.
46. Yang, S. T., and Hummel, J. P. (1964) J. Biol. Chem. 239, 3775-3780.
Ribonuclease inhibitor from human placenta: interaction with derivatives of ribonuclease A.
P Blackburn and B L Jailkhani

J. Biol. Chem. 1979, 254:12488-12493.