Reversible Inactivation of Pancreatic Deoxyribonuclease A by Sodium Dodecyl Sulfate

REMOVAL OF COOH-TERMINAL RESIDUES FROM THE DENATURED PROTEIN BY CARBOXYPEPTIDASE A*

(Received for publication, October 8, 1974)

TA-HOll LIAO†
From The Rockefeller University, New York, New York 10021

SUMMARY

In the course of experiments on the role of the COOH-terminal residues in pancreatic deoxyribonuclease, we undertook to ascertain whether the presence of sodium dodecyl sulfate would render the normally unavailable terminus susceptible to hydrolysis by carboxypeptidase A. When DNase A is dissolved in 0.005% sodium dodecyl sulfate the protein becomes enzymically inactive when assayed against DNA in the same sodium dodecyl sulfate concentration. The loss of activity caused by treatment with sodium dodecyl sulfate for 1 hour at 45°C can be fully restored if the detergent-containing solution is diluted 10-fold into 6 M guanidinium chloride and then 10-fold into a pH 7.0 buffer, 10 mM in CaCl₂, prior to a 100-fold dilution for assay. The presence of Ca²⁺ is essential for the refolding process. If the same degree of dilution is made into sodium dodecyl sulfate-free buffer without the guanidinium chloride step, there is very little reversal of the inactivation. An almost complete loss of regenerable activity is caused by 1 hour of digestion by carboxypeptidase at 45°C in the presence of 0.03% sodium dodecyl sulfate. Although up to 6 amino acid residues can be removed from the COOH terminus, the loss of activity can be correlated with the removal of either 1 or 2 amino acid residues (-Leu-Thr) from the COOH-terminal sequence. Thus, DNase A is one of the several enzymes in which residues at the COOH terminus are essential to the active conformation. If the enzyme minus 2 to 6 terminal residues was mixed with a 15-residue COOH-terminal peptide (obtained by cyanogen bromide cleavage), only about 2% activity could be regenerated.

The present experiments began with the aim of studying the essentiality of residues near the COOH-terminal end of bovine pancreatic deoxyribonuclease A (deoxyribonuclease oligonucleotide 5'-phosphodiesterase, EC 3.1.4.5). DNase A is the major form of the enzyme in bovine pancreas (1); structural studies (2, 3) have provided, as a working hypothesis, a complete sequence for the 257 residues in the single chain and have demonstrated the positions of the two disulfide bridges and the carbohydrate side chain. Earlier experiments have shown that one of the disulfide bonds is essential for activity (3, 4) and that modification of His-118 (3, 5) or Tyr-62 (3, 6) results in complete inactivation of the enzyme. Controlled proteolysis by chymotrypsin (7) has led to a single cleavage of the chain between residues 178 and 179, without inactivation, and 8 amino acid residues could be removed by carboxypeptidase Y at this point of cleavage without loss of activity. The COOH terminus of the native enzyme is unavailable to carboxypeptidase action (7). We have undertaken to unfold the protein sufficiently by sodium dodecyl sulfate to render the COOH terminus susceptible to hydrolysis by carboxypeptidase A; this approach has led to a study of the reversibility of the denaturation by Na dodecyl-S0₄, the effects of other denaturing agents on DNase, and the consequence of removal of residues from the COOH terminus of the enzyme. Successful renaturation of several other enzymes from solution in 6 M urea after denaturation by Na dodecyl-S0₄ has been described by Weber and Kuter (8).

EXPERIMENTAL PROCEDURE

Materials—DNase A was prepared by chromatography on cellulose phosphate according to the procedure of Salnikow et al. (1), starting with DP grade bovine pancreatic DNase from Worthington. Traces of proteolytic activity were removed by affinity elution with Ca²⁺ from DEAE-cellulose (9). Calf thymus DNA and carboxypeptidase A (COADFP grade) were from Worthington. Reagents employed were Na dodecyl-S0₄ (Sigma), urea and guanidine HCl (ultrapure; Mann), sodium octyl sulfate, sodium deoxyl sulfate, sodium dodecyl sulfate, sodium tetradecyl sulfate, dodecyltrimethylammonium chloride and hexadecyltrimethylammonium bromide (Eastman), Brij-35 (Fisher), and Triton X-100 (Packard).

Amino Acid Analysis—Proteins or peptides (approximately 100 μg) were hydrolyzed in 0.2 ml of 6 N HCl for 24 hours in sealed evacuated tubes (10 X 75 mm) at 110°C. Amino acid analyses were performed on the nanomole scale with an analyzer (10) modified for use with 2.8 mm bore columns (11). The activity was measured by the hyperchromicity method of Kunitz (12), modified for use at pH 7 (9). The rate of hydrolysis was measured at 260 nm on a recording spectrophotometer.

1 The abbreviation used is: Na dodecyl-S0₄, sodium dodecyl sulfate.
photometer with calf thymus DNA (0.1 mg/ml) in 0.1 M Tris-HCl, pH 7.0, 5 mM in MgCl₂, at 25⁰. One unit of DNase activity causes an increase of 1.0/min/cm in absorbance.

**Gel Filtration of Na Dodecyl-SO₄-treated DNases**—The enzyme (3 mg in 1 ml) inactivated by 0.025% Na dodecyl-SO₄ was added to a Sephadex G-100 column (1.5 × 90 cm) equilibrated with Na dodecyl-SO₄-free 0.1 M Tris-HCl, pH 8.0.

**Hydrolysis of DNase by Carboxypeptidase A**—DNase (3 mg) was incubated with 80 μg of carboxypeptidase A in 1 ml of 0.1 M Tris-HCl, pH 8.0, 0.025% (w/v) in Na dodecyl-SO₄, for 1 hour at 45⁰. For analysis of the amino acids liberated, an aliquot (approximately 100 μl) was mixed with 2 volumes of 0.2 N citrate buffer, pH 2.2 (10), and heated at 100⁰ for 1 min to remove most of the protein as a precipitate; after centrifugation, the supernatant solution was applied to the amino acid analyzer.

**Preparation of CNBr-6 (Residues 215 to 267) from DNase A**—The conditions for the reaction of DNase A with CNBr were similar to those used in the structural study (3). The mixture of CNBr peptides was gel-filtered through a Sephadex G-100 column with 50% acetic acid as eluent. Peptides CNBr-1 (the NH₂-terminal peptide) and CNBr-5 (the COOH-terminal peptide) were partially separated; the material isolated from the right half of the peak contained CNBr-3 and 23% (on a molar basis) CNBr-1, as judged from amino acid analysis.

**RESULTS**

**Effect of Na Dodecyl-SO₄ on DNase**—When DNase is exposed to 0.005% Na dodecyl-SO₄ at 25⁰ for 1 min (Fig. 1) there is complete loss of enzymic activity. Under these conditions, in which the same Na dodecyl-SO₄ concentration is maintained in the substrate solution, 50% inactivation is obtained with 0.0025% Na dodecyl-SO₄. However, as indicated in the inset in Fig. 1, there is a further change with time; 0.0025% Na dodecyl-SO₄ leads to 82% inactivation at 4 hours.

Gottesfeld et al. (13) have shown that the inhibition of DNase action by hydroxybiphenyls arises from combination with the DNA rather than with the enzyme. In order to determine whether such an effect was occurring in the present experiments, solutions of DNase A with or without Na dodecyl-SO₄ were assayed in media with or without the detergent. The recorder tracings are shown in Fig. 2. In one of the controls (Curve 1b), both the DNase and the DNA solutions were 0.7 mM in EDTA (the same molarity as 0.01% Na dodecyl-SO₄) in order to be sure that any slight effect of Na dodecyl-SO₄ on the bivalent metal concentration in the medium would not be critical. When Na dodecyl-SO₄ was present only in the enzyme solution (Curve 2) the initial rate was decreased, but the activity increased with time, which indicated that the enzyme was recovering when the Na dodecyl-SO₄ concentration was diluted 100-fold after only 1 to 2 min of exposure to the detergent. When the Na dodecyl-SO₄ was present initially only in the substrate solution (Curve 3), the initial rate of catalysis was rapid, but dropped almost to zero within 2 to 3 min in the 0.01% Na dodecyl-SO₄. Curve 4 was a second control at one-tenth the DNase concentration used for Curve 1. When the Na dodecyl-SO₄ was present in both solutions (Curve 5), there was no activity at all.

These results show that the effect of Na dodecyl-SO₄ is on

![Fig. 1. Inactivation of DNase by Na dodecyl-SO₄ (SDS). The determination of the sensitivity of DNase to immediate inactivation by different concentrations of Na dodecyl-SO₄ at 25⁰ is given in the large curve. To DNase (10 μg/ml) in 0.1 M Tris-HCl, pH 7.0, a few microliters of 0.25% Na dodecyl-SO₄ were added to give the desired concentration of the detergent; within approximately 1 min after mixing, 10 μl of the solution were pipetted into 1 ml of DNA (0.1 mg/ml) in 0.1 M Tris-HCl, pH 7.0, 5 mM in MgCl₂, having the same concentration of Na dodecyl-SO₄. Assay was by the hyperchromicity method (9, 12). For the time study (inset), the DNase was incubated in the presence of 0.025% Na dodecyl-SO₄ for the specified time prior to assay.](http://www.jbc.org/)

![Fig. 2. Effect of adding Na dodecyl-SO₄ to the enzyme or to the substrate. The recorder tracings in the hyperchromicity assay were obtained after adding 10 μl of DNase A solution in 0.1 M Tris-HCl buffer, pH 7.0, to 1 ml of DNA solution (0.1 mg/ml) in the same buffer, 5 mM in MgCl₂. The DNase was exposed to the Na dodecyl-SO₄ for only 1 to 2 min before the addition to substrate. The initial peak of absorbance was recorded as the two solutions were mixed.](http://www.jbc.org/)
FIG. 3. Difference spectra between Na dodecyl-SO₄-free and Na dodecyl-SO₄-bound DNase. The spectra were taken on an Aminco spectrophotometer, model DW-2, at 0.05 absorbance full scale, 1-cm path length. Curve 1 represents a control with 0.25 mg/ml DNase in 0.1 M Tris-HCl, pH 7.0, in each cell. Curve 2 was taken after the solution in the sample position had been incubated with 0.01% Na dodecyl-SO₄ for 1 hour at 25°. A blank with 0.01% Na dodecyl-SO₄ did not show appreciable absorbance. Inset, the absorption spectrum of DNase without Na dodecyl-SO₄. The molar extinction coefficients were calculated by assuming a molecular weight of 30,000 (3).

FIG. 4. Time course of inactivation of DNase exposed to Na dodecyl-SO₄ (SDS) but assayed without Na dodecyl-SO₄ in the substrate solution. DNase (3.0 mg/ml) was incubated in 0.1 M Tris- HCl, pH 7.0, 0.025% Na dodecyl-SO₄ at 25° and 45°. The top curve represents a control without Na dodecyl-SO₄ at 25°. At each time interval an aliquot (10 μl) was diluted 100-fold with 0.1 M Tris-HCl, pH 7.0, 10 mM in CaCl₂, and within a few minutes an aliquot was diluted 100 fold into the Na dodecyl-SO₄-free assay medium at 25°.

Effects of Guanidinium Chloride on DNase A—Full reversibility of the denaturation of the enzyme by Na dodecyl-SO₄ has been attained through experiments with guanidinium chloride. When DNase is exposed to various concentrations of guanidinium chloride (up to 8 M) for as long as 1 hour and then is diluted into CaCl₂-containing buffer, the activity remains unchanged (Fig. 5). If DNase that had been inhibited by Na dodecyl-SO₄ was first diluted 10-fold into 8 M guanidinium chloride and then 100-fold for assay, full activity was regained. The presence of Ca²⁺ is essential for this regeneration; if CaCl₂ was omitted there was no regain of activity. When 6 M sodium chloride was substituted for 6 M guanidinium chloride, no activity was regenerated.

The dashed line in Fig. 5 shows the recovery of activity gained through dilution of Na dodecyl-SO₄-treated DNase with intermediate concentrations of guanidinium chloride. About 6 M guanidinium chloride is needed to permit nearly complete reversal of the inhibition by Na dodecyl-SO₄.

Effects of Other Detergents—Jones et al. (15) and Blinkhorn and Jones (16) have shown that anionic detergents are more
**TABLE I**

**Effects of different detergents on the activity of DNase A**

The enzyme and DNA solutions, dissolved as defined for Fig. 2, were incubated with the detergents at room temperature for approximately 1 min before mixing for assay. The amount of DNase A was 0.1 μg/assay.

| Detergent (0.01%, w/v) | Relative activity % |
|------------------------|---------------------|
| Control (H₂O)          | 100                 |
| Sodium octyl sulfate (Cs) | 100               |
| Sodium dodecyl sulfate (Cd₁₂) | 93               |
| Sodium dodecyl sulfate (Cd₂₃) | 0               |
| Sodium tetradecyl sulfate (Cd₁₄) | 0               |
| Dodecyltrimethylammonium chloride (Cl₃₈) | 78               |
| Hexadecyltrimethylammonium bromide (Cl₄₆) | 23               |
| Brij-35                | 101                 |
| Triton X-100           | 100                 |

effective than cationic ones in inhibiting the action of RNase A. Nozaki et al. (17) have also shown that with tetradecyltrimethylammonium chloride a 10-fold higher concentration is required for the denaturation of serum albumin than is needed with Na dodecyl-SO₄. The results in Table I show that neutral detergents, such as Brij-35 and Triton X-100, do not have any effect on RNase A. With the alkyl sulfates, the length of the hydrophobic side chain is important; there is little effect with the Cs and Cl₀ analogs of Na dodecyl-SO₄. Quaternary ammonium bases are much less effective than the acidic detergents.

**Action of Carboxypeptidase A on Na Dodecyl-SO₄-treated DNase A**—With the knowledge that fully active DNase can be recovered from Na dodecyl- SO₄-treated DNase A, it was possible to study the effect upon activity of the removal of COOH-terminal residues from the enzyme by carboxypeptidase A. Guidotti (18) has shown that carboxypeptidase A is active in the presence of 1.6% Na dodecyl-SO₄, and he used the enzyme in the presence of the detergent to remove COOH-terminal residues from the α and β chains of hemoglobin. With DNase in 0.03% Na dodecyl-SO₄, the action of carboxypeptidase A was slow at 25° but appreciable at 45° (Fig. 6). The action of carboxypeptidase A can be expected to stop at Pro-251 in the COOH-terminal sequence -Pro-Val-Glu-Val-Thr-Leu-Thr (3). Also plotted in Fig. 6 is the percentage loss of activity accompanying this hydrolysis after dilution into guanidinium chloride to give full opportunity for the native Na dodecyl-SO₄-treated protein to regain activity. The activity is very sensitive to the removal of the COOH-terminal residues; the loss of activity parallels very closely the removal of leucine, indicating that the removal of only 2 residues leads to almost complete inactivation of DNase A.

This conclusion is valid, of course, only if there has been no proteolysis in other parts of the DNase chain. The product of carboxypeptidase A treatment was examined by gel filtration in 6 M guanidinium chloride (Sephadex G-100 column, 1.5 x 90 cm; flow rate, 3 ml/hour). The protein material had the same elution volume as DNase A in the control and no separation of fragments was observed. In the control and the carboxypeptidase A experiments the recoveries of protein were about equal, based upon absorbance at 280 nm. In order to be sure that —S—S— bridges were not holding proteolyzed fragments together, gel electrophoresis (19) in the presence of Na dodecyl-SO₄ and mercaptoethanol was performed; the control and the carboxypeptidase A-treated DNase gave bands in identical positions.

**TABLE II**

**Enzymic activities of products of action of carboxypeptidase A on DNase in Na dodecyl-SO₄ solution**

All activities on the Na dodecyl-SO₄-treated proteins were measured after exposure to 6 M guanidinium chloride and dilution into pH 7.0 buffer, 10 mM in CaCl₂, in order to facilitate refolding to the active conformation. The gel filtration step involved approximately a 24-hour exposure to the 6 M guanidinium chloride, which may be the cause of a measurable loss of activity.

| Sample                        | Specific activity | Yield |
|-------------------------------|-------------------|-------|
| Starting material             | 903a              | %     |
| Peak tube of control DNase A  | 516a              |       |
| Peak tube of carboxypeptidase A-treated DNase A | 30a             |       |
| After dialysis to remove guanidinium Cl⁻ Control DNase A | | |
| Supernatant                   | 670               | 49.6  |
| Precipitate                   | 334               | 32.1  |
| Carboxypeptidase A-treated DNase A |         | 10.7  |
| Supernatant                   | 134               | 76.2  |
| Precipitate                   | 14                | 65.5  |

* Weight of protein in solution was based upon absorbance of the solution at 280 nm and the assumption that a 1-mg/ml solution had an absorbance of 1.23.

b Weights of protein and yields were calculated on the basis of amino acid analyses. The activities (after solution in 6 M guanidinium chloride and dilution into CaCl₂-containing buffer) and the amino acid compositions of the gel-filtered proteins were compared. There was a significant decrease in activity of the control after the gel filtration (Table II) which may indicate that prolonged (24 hours) exposure to 6 M guanidinium chloride is not innocuous. For amino acid analyses.
The amino acid analysis of the proteins, the 6 M guanidinium chloride of the eluant used for the gel filtration was released by dialysis against water. If a fine precipitate formed, it was removed by centrifugation and resuspended in water for the pipetting of aliquots of the suspension. In the control with DNase, the major proportion of the protein remained water-soluble; the carboxypeptidase A-treated protein, however, became largely water-insoluble. The specific activities of the fractions indicated a 98% inactivation of the protein remained after solubilization and resuspended in water for the pipetting of aliquots of the suspension. In the control with DNase, the major proportion of the protein remained water-soluble; the carboxypeptidase A-treated protein, however, became largely water-insoluble. The specific activities of the fractions indicated a 98% inactivation of the insoluble carboxypeptidase A-treated protein. If a fine precipitate formed, it was removed by centrifugation and resuspended in water for the pipetting of aliquots of the suspension.

The amino acid analyses summarized in Column 4 of Table III indicate that the insoluble carboxypeptidase A-treated protein almost completely lacks the last 2 COOH-terminal residues (Val1-Leu0.8) and that about one-half of the molecules have 2 amino acid residues present in the supernatant after carboxypeptidase A action and heat treatment were also measured. Tryptophan and half-cystine were not determined. The results are expressed as residues per molecule; theoretical values are in parentheses (from Ref. 3). The COOH-terminal sequence of DNase A is -Pro-Val-Glu-Val-Thr-Leu-Thr.

### Table III

| Amino acid | 1. Starting material | 2. Control DNase A supernatant | 3. CPA-treated DNase A precipitate | 4. Free amino acids after CPA treatment | 5. Average of Columns 1 and 2 minus Column 1 |
|------------|----------------------|--------------------------------|----------------------------------|--------------------------------------|-----------------------------------------|
| Lysine     | 8.4 (9)              | 8.6                            | 8.3                              |                                      |                                         |
| Histidine  | 11.0 (11)            | 9.6                            | 9.8                              |                                      |                                         |
| Arginine   | 11.0 (11)            | 10.5                           | 10.9                             |                                      |                                         |
| Aspartic acid | 31.3 (32)         | 31.8                           | 31.1                             |                                      |                                         |
| Threonine  | 14.7 (15)            | 14.6                           | 13.0                             | 1.6                                  | 1.6                                     |
| Serine     | 28.5 (30)            | 28.7                           | 28.8                             | 0.7                                  | 1.0                                     |
| Glutamic acid | 19.0 (19)         | 19.5                           | 18.2                             |                                      |                                         |
| Proline    | 9.7 (9)              | 9.4                            | 9.3                              |                                      |                                         |
| Glycine    | 9.1 (9)              | 9.3                            | 9.2                              |                                      |                                         |
| Alanine    | 21.7 (22)            | 21.4                           | 21.3                             |                                      |                                         |
| Valine     | 23.1 (24)            | 21.0                           | 19.6                             | 1.2                                  | 1.5                                     |
| Methionine | 3.9 (3)              | 4.0                            | 4.0                              |                                      |                                         |
| Isoleucine | 9.5 (11)             | 9.9                            | 9.6                              |                                      |                                         |
| Leucine    | 22.8 (23)            | 22.7                           | 21.5                             | 0.8                                  | 1.2                                     |
| Tyrosine   | 15.0 (15)            | 14.9                           | 14.7                             |                                      |                                         |
| Phenylalanine | 11.4 (11)         | 11.4                           | 11.4                             |                                      |                                         |
| Tryptophan | (3)                  |                                |                                  |                                      |                                         |
| Half-cystine | (4)                |                                |                                  |                                      |                                         |

a CPA, carboxypeptidase A.
b Corrections were made for destruction during acid hydrolysis for 24 hours at 110°, threonine, 5%; serine, 10%.
c The low values result from the Val-Val and Ile-Val bonds present in the sequence which are only partially hydrolyzed in 24 hours.

**Discussion**

Enzymes vary widely in the effect of the binding of Na dodecyl-SO₄ upon activity (24). DNase A is one of the most sensitive, since inactivation is complete of 0.005% Na dodecyl-SO₄. The aim of reversing this inactivation was achieved only by introducing the transient exposure to 6 M guanidinium chloride; this step makes it possible to obtain, upon further dilution in the presence of Ca²⁺, the monomeric, active enzyme instead of inactive aggregates.

The results on the importance of residues at the COOH terminus of DNase add this enzyme to the list of proteins that possess this property; the group includes ribonuclease (25, 26), aldolases (27, 28), isocitrate lyase (29), and thymidylate synthetase (30). The COOH-terminal residues are the last to be added in the biosynthetic process and in many instances they have a determining effect upon the folding of the chain into the active conformation.

The chances for regeneration of major activity through replacement by adsorption of a COOH-terminal peptide would probably be increased if a longer terminal segment had been removed from DNase; with ribonuclease (20) the optimum regain is obtained when 6 amino acid residues have been removed from the COOH terminus.

Acknowledgments—These experiments were conducted with encouragement from Dr. Stanford Moore and Dr. William H. Stein and with the technical assistance of Miss Lucy Palmer and Mrs. Anne Fischer.

**References**

1. Salmikow, J., Moore, S., and Stein, W. H. (1970) J. Biol. Chem. 245, 5085-5090
2. Salmikow, J., Liao, T.-H., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 1480-1488
3. Liao, T.-H., Salmikow, J., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 1480-1405
4. Price, P. A., Stein, W. H., and Moore, S. (1969) J. Biol. Chem. 244, 929-932
5. Price, P. A., Moore, S., and Stein, W. H. (1969) J. Biol. Chem. 244, 924-928
6. Hugli, T. E., and Stein, W. H. (1971) J. Biol. Chem. 246, 7191-7200
7. Hugli, T. E. (1973) J. Biol. Chem. 248, 1712-1718
8. Weber, K., and Kuter, D. J. (1971) J. Biol. Chem. 246, 4504-4509
9. Liao, T.-H. (1974) J. Biol. Chem. 249, 2354-2359
10. Spackman, D. H., Stein, W. H., and Moore, S. (1958) Anal. Chem. 30, 1190-1206
11. Liao, T.-H., Robinson, G. W., and Salmikow, J. (1973) Anal. Chem. 45, 2286-2289
12. Kunits, M. (1950) J. Gen. Physiol. 33, 349-362
13. Gottesfeld, J. M., Adams, N. H., El-Badry, A. M., Moses, V., and Calvin, M. (1971) Biochim. Biophys. Acta 228, 365-386
14. Zimmerman, S. B., and Coleman, N. F. (1971) J. Biol. Chem. 246, 309–317
15. Jones, M. N., Skinner, H. A., Tipping, E., and Wilkinson, A. (1973) Biochem. J. 135, 231–236
16. Blinkhorn, C., and Jones, M. N. (1973) Biochem. J. 135, 547–549
17. Nozaki, Y., Reynolds, J. A., and Tanford, C. (1974) J. Biol. Chem. 249, 4452–4459
18. Guidotti, G. (1969) Biochim. Biophys. Acta 22, 117–119
19. Weber, K., and Osborn, M. (1961) J. Biol. Chem. 244, 4406–4412
20. Lin, M. C., Gutte, B., Moore, S., and Merrifield, R. B. (1970) J. Biol. Chem. 245, 5169–5170
21. Gutte, B., Lin, M. C., Caldi, D. G., and Merrifield, R. B. (1972) J. Biol. Chem. 247, 4763–4767
22. Lin, M. C., Gutte, B., Caldi, D. G., Moore, S., and Merrifield, R. B. (1972) J. Biol. Chem. 247, 4768–4774
23. Hayashi, R., Moore, S., and Merrifield, R. B. (1973) J. Biol. Chem. 248, 3889–3892
24. Nelson, C. A. (1971) J. Biol. Chem. 246, 3895–3901
25. Anfinsen, C. B. (1955) J. Biol. Chem. 221, 405–412
26. Lin, M. C. (1970) J. Biol. Chem. 245, 6726–6731
27. Drechsler, E. R., Boyer, P. D., and Kowalsky, A. G. (1959) J. Biol. Chem. 234, 2627–2634
28. Rutter, W. J., Richards, O. C., and Woodfin, B. M. (1961) J. Biol. Chem. 236, 3193–3197
29. McFadden, B. A., Rao, G. R., Cohen, A. L., and Roche, T. E. (1968) Biochemistry 7, 3574–3582
30. Aull, J. L., Loeble, R. B., and Dunlap, R. B. (1974) J. Biol. Chem. 249, 1167–1172
Reversible inactivation of pancreatic deoxyribonuclease A by sodium dodecyl sulfate. Removal of COOH-terminal residues from the denatured protein by carboxypeptidase A.

T Liao

J. Biol. Chem. 1975, 250:3831-3836.