

Turkey Egg White Lysozyme

PREPARATION OF THE CRYSTALLINE ENZYME AND INVESTIGATION OF THE AMINO ACID SEQUENCE*

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SUMMARY

Crystalline turkey egg white lysozyme has been prepared by a simple procedure consisting of adsorption on carboxymethylcellulose followed by elution with $(\text{NH}_4)_2\text{CO}_3$ solution and crystallization from 5% NaCl solution. The twice crystallized enzyme gave a single peak when eluted from carboxymethylcellulose, and it migrated as a single band during disc electrophoresis. Separation of the tryptic peptides from reduced and *S*-carboxymethylated turkey egg white lysozyme has yielded peptides analogous to those of reduced and *S*-carboxymethylated chicken egg white lysozyme. It is found that the turkey enzyme amino acid sequence differs from that of the chicken lysozyme at a minimum of seven positions which are distributed over six of these tryptic peptides. The replacements detected from examination of these peptides are: Tyr for Phe₈, Leu for His₁₅, His for Gln₄₁, Lys for Arg₇₃, Ala for Val₉₉, Gly for Asp₁₀₁, and His for Gln₁₂₁ of the chicken egg white lysozyme. The apparent occurrence of glycine as Residue 101 of turkey egg white lysozyme is especially interesting inasmuch as the x-ray crystallographic investigation of chicken egg white lysozyme and its complexes with certain inhibitors has led to the view that the carboxyl group of Asp₁₀₁ is involved in binding substrates.

The recent x-ray crystallographic investigations (1, 2) of chicken egg white lysozyme have stimulated interest in the structures of lysozymes of different origin. Of especial pertinence to any consideration of the mechanism of lysozyme catalysis is the question of whether in other lysozymes substitutions occur for those amino acid residues that Phillips and co-workers have regarded as being involved either in substrate binding by chicken egg white lysozyme or in this enzyme's catalytic mechanism.

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nism. The present paper describes the preparation of crystalline, apparently homogeneous turkey egg white lysozyme together with an amino acid sequence determination for this protein.

EXPERIMENTAL PROCEDURE

Turkey Eggs—The eggs used in these experiments were from varieties known as Broad-breasted Bronze and Beltsville White and were obtained through the courtesy of Dr. Theodore H. Coleman and Dr. John H. Wolford of the Department of Poultry Science, Michigan State University. No attempt was made to separate these eggs according to variety.

Preparation of Crystalline Turkey Egg White Lysozyme—The first steps of the procedure, in which the basic egg white proteins were adsorbed on carboxymethylcellulose and the crude lysozyme was eluted with $(\text{NH}_4)_2\text{CO}_3$ solution, are identical with those of Canfield and McMurry's method for isolating goose egg white lysozyme (3). After freeze-drying the $(\text{NH}_4)_2\text{CO}_3$ eluate, the solid residue of the crude lysozyme was taken up in water by adjusting the pH to 5.5 with 1 M HCl. A small amount of insoluble material was removed from this solution by filtering, and the filtrate was freeze-dried. For crystallization, the freeze-dried filtrate was dissolved in water at a concentration of 50 mg per ml by adjusting the pH to 5.5 as before. Sodium chloride was added to this solution to a concentration of 5%, and the pH was brought to 8.0 with 1 M NaOH. After storing at 5° overnight, this solution was stirred, whereupon it immediately became cloudy. Crystallization then proceeded virtually to completion within 1 hour. The same crystallization procedure was followed for recrystallizing the enzyme.

Chromatography on Carboxymethylcellulose—The dimensions of the column were 2 × 22 cm for chromatography of 200 mg of the twice crystallized enzyme. Elution was carried out at a rate of 30 ml per hour with 1 liter of a linear gradient of aqueous $(\text{NH}_4)_2\text{CO}_3$ of from 0.05 to 0.4 M.

Disc Electrophoresis—Electrophoresis of the twice crystallized enzyme was carried out by the method of Reisfeld, Lewis, and Williams (4) at pH 4.5 for 45 min. The enzyme loads were 37.5 μg and 200 μg (overload).

Preparation of RCm¹-Turkey Egg White Lysozyme—The twice crystallized enzyme was reduced and the thiol groups formed were carboxymethylated by the procedure of Crestfield, Moore, and Stein (5) except that urea and salts were removed at the end

¹ The abbreviation used is: RCm¹-, reduced and *S*-carboxymethylated.

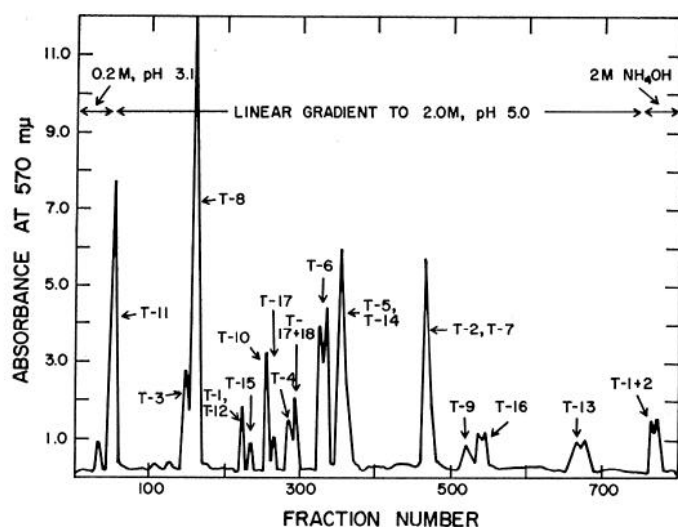


FIG. 1. Elution pattern for tryptic fragments from RCm-turkey egg white lysozyme as separated on a Dowex AG 50W-X2 column (2×150 cm). Plotted on the ordinate are the absorbances of aliquots of the fractions after alkaline hydrolysis and reaction with ninhydrin. Canfield's numbering system for the tryptic fragments from RCm-chicken egg white lysozyme (8) has been used here and throughout the remainder of the paper.

of the reaction by dialyzing the mixture in the dark against water, with the use of a dialysis sac prepared from $1\frac{1}{2}$ -inch cellulose tubing (Union Carbide Corporation, Food Products Division; this grade of cellulose tubing retains polypeptides having molecular weights greater than about 3600 (6)). The precipitate of RCm-lysozyme that formed during dialysis was washed three times with water and freeze-dried.

Tryptic Hydrolysis of RCm-Turkey Egg White Lysozyme and Separation of Tryptic Fragments—The RCm-lysozyme was digested as a 1% suspension in water at pH 8.0 and at 23° for 3 hours with 2% of its weight of trypsin (Worthington, treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone as described by Kostka and Carpenter (7)).

The tryptic digest from 600 mg of the RCm-lysozyme was chromatographed on a column (2×150 cm) of Dowex AG 50W-X2, 200 to 325 mesh. The column was eluted first at 40° with 500 ml of 0.2 M, pH 3.1 pyridine-acetic acid buffer followed by a linear gradient made up from 3.5 liters of the 0.2 M, pH 3.1 pyridine-acetic acid buffer and 3.5 liters of 2.0 M, pH 5.0 pyridine-acetic acid buffer. The final stage of the elution was carried out at 50° with 500 ml of 2 M NH_4OH . The flow rate during all stages was 60 ml per hour. The volume of the fractions was 10 ml. The elution pattern (Fig. 1) was determined by alkaline hydrolysis (9) followed by reaction with ninhydrin (10).

Peptides T-4, T-7, T-10, T-13, T-16, T-17, and T-17 + 18 were purified by chromatographing the appropriate Dowex AG 50W-X2 fractions on Whatman No. 1 paper with 1-butanol-acetic acid-water (4:1:5). Peptides T-1, T-5, T-12, and T-14 were purified by eluting them from Dowex AG 1-X8, 200 to 400 mesh, with the *N*-ethylmorpholine- α -picoline-pyridine-acetic acid system described by Schroeder (11). The mixture of peptides T-3 and T-8, obtained in the Dowex AG 50W-X2 separation procedure, was resolved by high voltage electrophoresis on Whatman No. 3MM paper at pH 3.5 (pyridine-acetic acid-water buffer, 1:10:289). Peptides were eluted from paper with 50% pyridine-water mixture.

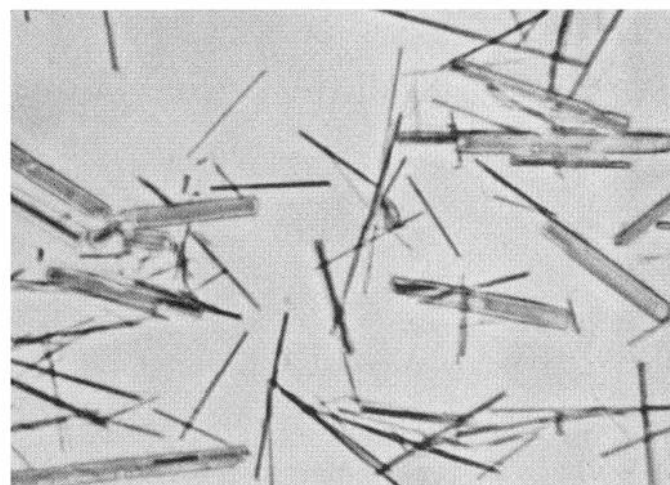


FIG. 2. Crystalline turkey egg white lysozyme at a magnification of 4600.

TABLE I

Amino acid composition of RCm-turkey egg white lysozyme

Except for the tryptophan value, which was obtained with unmodified turkey egg white lysozyme, the turkey lysozyme data are averages computed from duplicate runs on samples hydrolyzed for 24, 48, and 72 hours. The threonine and serine values have been calculated by extrapolating to zero hydrolysis time. Values for RCm-chicken egg white lysozyme are given for comparison.

Amino acid	RCm-turkey lysozyme		RCm-chicken lysozyme
	Found	Integral value	
	<i>residues/mole</i>		
Lysine.....	7.00	7	6
Histidine.....	1.90	2	1
Arginine.....	10.02	10	11
S-Carboxymethylcysteine.....	8.36	8	8
Aspartic acid.....	19.52	20	21
Threonine.....	7.00	7	7
Serine.....	10.10	10	10
Glutamic acid.....	2.98	3	5
Proline.....	1.94	2	2
Glycine.....	12.88	13	12
Alanine.....	13.00	13	12
Valine.....	4.92	5	6
Methionine.....	1.88	2	2
Isoleucine.....	5.80	6	6
Leucine.....	8.98	9	8
Tyrosine.....	3.78	4	3
Phenylalanine.....	1.92	2	3
Tryptophan.....	6.5	6-7	6

Chymotryptic Hydrolysis of Peptides T-7 and T-16 and Separation of Chymotryptic Peptides—Reaction of 3.8 μ moles of peptide T-7 with 2 mg of chymotrypsin (salt-free, Calbiochem A grade) in 1.45 ml of 0.1 M NH_4HCO_3 at 37° for 10 hours, followed by separation of the peptides on a column (0.5 $\times$ 50 cm) of Dowex AG 1-X2, 200 to 400 mesh, by elution with *N*-ethylmorpholine- α -picoline-pyridine-acetic acid buffer (11) gave Peptides T-7-CHT-2 and T-7-CHT-3 as the principal products and only a small amount of Peptide T-7-CHT-1 (the relationship of these chymo-

TABLE II

Amino acid analysis of tryptic peptides from RCm-turkey egg white lysozyme

For each of these peptides the number of amino acid residues assumed (given in parentheses) is the same as that of a peptide from RCm-chicken egg white lysozyme.

Amino acid	Peptides								
	T-3	T-6	T-8	T-9	T-11	T-14	T-15	T-17	T-17 + 18
Lysine.....	0.94 (1)	0.97 (1)			1.00 (1)		0.95 (1)		
Ammonia.....		1.54 (1)		1.51 (1)	3.48 (3)	0.82 (1)			
Arginine.....			0.87 (1)	1.04 (1)		1.00 (1)		1.03 (1)	1.00 (1)
S-Carboxymethylcysteine.....	0.91 (1)	1.00 (1)		1.05 (1)	3.09 (3)		1.05 (1)	0.90 (1)	0.99 (1)
Aspartic acid.....		0.74 (1)	3.90 (4)	1.91 (2)	3.92 (4)	1.00 (1)			
Threonine.....			1.82 (2)		0.99 (1)				
Serine.....		0.98 (1)	1.83 (2)		3.90 (4)				
Glutamic acid.....	0.98 (1)		0.90 (1)						
Proline.....					1.03 (1)				
Glycine.....		2.03 (2)	2.10 (2)	1.01 (1)				1.07 (1)	1.00 (1)
Alanine.....	3.10 (3)	2.06 (2)			3.14 (3)				
Valine.....		0.94 (1)			1.03 (1)				
Methionine.....	1.03 (1)								
Isoleucine.....			2.15 (2)		2.18 (2)				
Leucine.....	1.05 (1)	1.03 (1)	1.09 (1)		3.08 (3)				1.00 (1)
Tyrosine.....		0.99 (1)	0.85 (1)						
Tryptophan.....		1.24 (1)		1.90 (2)					

TABLE III

Amino acid analysis of tryptic peptides from RCm-turkey egg white lysozyme

The amino acid analysis of each of these peptides differs from that of an analogue from RCm-chicken egg white lysozyme, although the total number of residues is in each instance the same. The numbers of residues assumed for the turkey lysozyme peptides are given in parentheses. Values for corresponding chicken lysozyme peptides are shown in **boldface**.

Amino acid	Peptides					
	T-1 + 2	T-5	T-7	T-10	T-13	T-16
Lysine.....	0.93 (1) 1			1.01 (1)		
Histidine.....			1.00 (1)			0.85 (1)
Ammonia.....		1.47 (1) 1	3.76 (3) 4			1.86 (0) 1
Arginine.....	1.01 (1) 1	1.00 (1) 1	1.05 (1) 1	1	1.00 (1) 1	0.89 (1) 1
Aspartic acid.....		2.07 (2) 2	3.00 (3) 3		2.04 (2) 3	1.16 (1) 1
Threonine.....			1.79 (2) 2	0.87 (1) 1		1.06 (1) 1
Serine.....			0.94 (1) 1	1.00 (1) 1	0.90 (1) 1	
Glutamic acid.....			1.08 (1) 2			1
Proline.....				0.88 (1) 1		
Glycine.....	1.17 (1) 1	1.01 (1) 1		1.09 (1) 1	2.97 (3) 2	1.22 (1) 1
Alanine.....			1.08 (1) 1		2.96 (3) 2	1.19 (1) 1
Valine.....	1.01 (1) 1				1.02 (1) 2	0.81 (1) 1
Methionine.....					1.02 (1) 1	
Isoleucine.....					1.03 (1) 1	0.81 (1) 1
Leucine.....		1.94 (2) 1				
Tyrosine.....	0.87 (1)	0.99 (1) 1				
Phenylalanine.....	1		1.80 (2) 2			
Tryptophan.....					2.00 (2) 2	1.16 (1) 1

tryptic peptides to peptide T-7 is shown in Fig. 3). In order to obtain Peptide T-7-CHT-1 in reasonable yield, Peptide T-7 was hydrolyzed under more vigorous conditions (5.0 μ moles of peptide and 5 mg of chymotrypsin in 2.25 ml of 0.1 M NH_4HCO_3 ; reaction carried out at 40° for 12.75 hours), and the mixture of peptides was chromatographed on a column (0.5 $\times$ 40 cm) of Dowex AG 50W-X2, 200 to 325 mesh, as described by Schroeder (12). The latter hydrolysis conditions and separation procedure

were used for hydrolyzing Peptide T-16 and isolating Peptide T-16-CHT-1.

COOH-terminal Analysis with Carboxypeptidases A and B—The conditions used were virtually the same as those described by Doppeide, Moore, and Stein (13).

Cyanogen Bromide Cleavage of Peptide T-13 and Separation of Cyanogen Bromide Peptides—Taniuchi and Anfinsen's procedure (14) was employed for the cleavage. The cyanogen bromide

T-1 + 2		Lys - Val - Tyr - Gly - Arg					T-5		Leu - Gly - Leu - Asp - Asn - Tyr - Arg						
		→	→	→					→	→	→	→	←	←	
	Edman								Edman						
	1	0.09	0.91	0.94	1.10	1.07			1	1.05	0.99		2.04	0.98	n.d.
	2	0.08	0.11	0.86	1.06	1.09			2	1.01	0.17		2.01	0.97	0.84
	3	0.05	0.11	0.15	0.98	1.02			3	0.34	0.13		2.04	0.96	0.87
									4	0.31	0.15		1.55	1.00	0.86
									Carboxypeptidases A + B (6.75 hours)					0.95	0.94
T-7		Phe - Glu - Ser - Asn - Phe - Asn - Thr - His - Ala - Thr - Asn - Arg													
		→	→	→											
	Edman														
	1	0.97	0.98	0.90	2.98		1.90	0.98	0.98		1.10				
	2	1.04	0.21	0.91	3.15		2.01	0.86	1.02		1.00				
	3	1.00	0.20	0.35	3.00		1.97	1.01	1.00		1.00				
T-7-Cht-1		Phe - Glu - Ser - Asn - Phe													
						←	←								
Composition		1.79	1.01	1.02	1.17										
Carboxypeptidase A	(2.5 hours)				0.73	0.81									
T-7-Cht-2		Phe - Glu - Ser - Asn - Phe - Asn - Thr - His													
						←	←	←							
Composition		1.84	0.96	0.96	2.16		1.12	0.99							
Carboxypeptidase A	(40 min.)					0.20	0.29	0.55							

FIG. 3. Edman and carboxypeptidase degradations of peptides from RCm-turkey lysozyme. The primary structure of RCm-chicken egg white lysozyme is shown in parentheses where the amino acid sequence was not determined.

peptides were separated by the method used for isolating Peptide T-7-Cht-1.

Edman Degradations—The procedure of Taniuchi and Anfinsen (14) was followed except that in degrading Peptide T-13-CNBr-1 the peptide was purified after each cyclization step by Konigsberg and Hill's method (15).

Amino Acid Analysis of Peptides and RCm-Turkey Egg White

Lysozyme—The tryptic peptides that gave a positive test with Ehrlich's reagent were hydrolyzed for tryptophan analysis by digesting with papain and leucine aminopeptidase according to the procedure of Smyth, Stein, and Moore (16). For determining the other amino acids, hydrolysis was carried out in constant boiling HCl at 105° in evacuated, nitrogen-flushed tubes. A model 120 Beckman-Spinco amino acid analyzer was used for

all of the analyses. The unmodified protein was analyzed for tryptophan by the method of Spies and Chambers (17).

Comparison of Chicken and Turkey Egg White Lysozyme Lytic Activity toward *Micrococcus luteus*² Cells—A Gilford model 2000 recording spectrometer adjusted for 0.5 absorbance unit at full scale was used for the absorbance measurements. The suspension of *M. luteus* cells was prepared by mixing 13 to 15 mg of the dried cells (Miles Laboratories) with 90 ml of 0.67 M, pH 6.8 sodium phosphate buffer and 10 ml of 1% NaCl. The absorbance of this suspension at 550 m μ was 0.4 to 0.5. The lysozyme solutions contained various amounts of the enzyme (either the twice crystallized turkey egg white lysozyme or Worthington thrice crystallized chicken egg white lysozyme) dissolved in this same phosphate buffer-NaCl mixture. For the assay, 0.29 ml of the cell suspension was mixed with 10 μ l of enzyme solution at ambient temperature (approximately 23.5°), and the change in absorbance was recorded over a period of 2 to 3 min. A plot of the initial slopes was linear over the range of from 0 to 3.3 μ g of protein per ml.

RESULTS

Purification and General Properties of Turkey Egg White Lysozyme—Turkey egg white lysozyme crystallized readily with approximately 30% loss of activity to the mother liquor during each crystallization. In spite of the losses that occur when the mother liquors are not reworked, crystallization has been the method of choice for purifying the enzyme on account of the ease of carrying out this procedure and the degree of purification that it yields: two crystallizations removed minor protein peaks that appeared when the crude enzyme was chromatographed on carboxymethylcellulose columns, and the twice crystallized product gave a single band on disc electrophoresis at either normal loads or overloads of 200 μ g. The appearance of crystals obtained by this method is shown in Fig. 2.

On comparing the activities of the turkey and chicken lysozymes toward *M. luteus* cells, the twice crystallized turkey lysozyme was almost exactly 50% more active than was three times crystallized, salt-free Worthington chicken egg white lysozyme. In a further comparison these two lysozymes gave virtually identical optical rotatory dispersion curves in the region of from 200 to 300 m μ .

Amino Acid Analysis of RCm-Turkey Egg White Lysozyme and of Tryptic Fragments—The results from amino acid analysis of RCm-turkey egg white lysozyme are shown in Table I; the basic amino acids have been referred to lysine and the neutral and acidic amino acids to alanine in normalizing the molar ratios. The integral values show a difference of either 6 or 7 residues between the chicken and turkey egg white lysozymes, depending on whether the number of tryptophan residues is taken as 6 or 7 from the analysis for tryptophan carried out on the unmodified protein. Except for the number of lysine residues, this composition for the turkey enzyme agrees reasonably well with that reported by Imanishi (18) (Imanishi gave the number of lysine residues as 6).

Molar ratios obtained for the amino acids of the tryptic peptides, as calculated by the method of Hirs, Moore, and Stein (19) (the exceptions are the tryptophan values), are presented in Tables II and III. A comparison of these data with the composition of the tryptic peptides from RCm-chicken egg white lyso-

zyme, indicated in Table III, corroborates a difference of 6 amino acid residues between the chicken and turkey lysozymes, and it indicates that this difference arises from an amino acid sequence difference of 7 residues distributed over six of the tryptic peptides.

Two tryptic fragments from the RCm-turkey lysozyme, T-4 and T-12, which were isolated as free arginine and lysine, respectively, are not included in Table II.

Amino Acid Sequence Determinations for Peptides from RCm-Turkey Egg White Lysozyme—Results from Edman and carboxypeptidase degradations are presented in Fig. 3. These data and the specificity of trypsin establish the amino acid sequences given here, except that they do not decide whether the residue next to the carboxy terminus in Peptide T-7 is asparaginyl or aspartyl; this residue was identified as asparaginyl by observing that Peptide T-7-Cht-3 moves toward the negative electrode during high voltage paper electrophoresis at pH 6.4. It will be noted that Peptides T-13 and T-13-CNBr-1 were both degraded by the Edman method, with much better conversion of the latter at each stage. This improvement over the data obtained with Peptide T-13 was considered necessary and was achieved by applying Konigsberg and Hill's modification (15), which gives better results with Peptide T-13-CNBr-1 than it does with Peptide T-13 because Peptide T-13-CNBr-1 contains no aromatic amino acid residues.

DISCUSSION

The method given here affords a simple means of preparing crystalline turkey egg white lysozyme in good yield. According to the criteria used the twice crystallized enzyme is homogeneous.

Although the complete primary structure of the turkey lysozyme has not yet been established, the present investigation has shown that this lysozyme has the same number of amino acid residues as has the lysozyme from chicken egg white and that differences in these two proteins occur at least at Positions 3, 15, 41, 73, 99, 101, and 121. The work having to do with determining the amino acid sequence may be summarized as follows. Chromatography of tryptic hydrolysates from RCm-turkey egg white lysozyme has yielded arginine, lysine, and 15 peptides. Nine of the peptides are identical in composition with tryptic peptides from RCm-chicken egg white lysozyme; 6 have the same number of residues as do their chicken lysozyme analogues but differ by one or more amino acid substitutions. Finally, the positions of substitutions in the latter group of peptides have been ascertained. The tentative primary structure shown in Fig. 4 is based on this information and the assumption that the order of the tryptic fragments in the intact proteins is the same for the RCm-chicken and RCm-turkey lysozymes.

Although the effects of amino acid sequence differences on conformation and the related phenomena of binding and catalytic activity cannot yet be predicted accurately for even similar enzymes, it is interesting to note that where the sequences of the turkey and chicken lysozymes differ, the side chains, except that of Residue 99, occupy surface positions in the Phillips structure for crystalline chicken lysozyme (2), remote from the Glu₃₅ and Asp₅₂ carboxyl groups which have been presumed to function in the catalytic mechanism (2). It may be significant also that, although 3 of the residues that are different in the two lysozymes lie within conformationally regular regions of the crystalline chicken lysozyme, 2 of these, Residues 15 and 41, are located—at the end of the helix extending from Residue 5 through Residue 15 and at the beginning of the region of β -structure embracing

² Formerly *Micrococcus lysodeikticus*.

Tryptic fragment	Amino acid sequence
T-1	Lys 1
T-2	(Phe) Val-Tyr-Gly-Arg 5
T-3	[Cys(Cm), Glu, Leu, Ala, Ala, Ala, Met]Lys 10
T-4	Arg
T-5	(His) Leu-Gly-Leu-Asp-Asn-Tyr-Arg 15 20
T-6	[Gly, Tyr, Ser, Leu, Gly, Asn, Trp, Val, Cys(Cm), Ala, 25 30 Ala]Lys (Gln)
T-7	Phe-Glu-Ser-Asn-Phe-Asn-Thr-His-Ala-Thr-Asn-Arg 35 40 45
T-8	[Asn, Thr, Asp, Gly, Ser, Thr, Asp, Tyr, Gly, Ile, Leu, Gln 50 55 Ile, Asn, Ser]Arg 60
T-9	[Trp, Trp, Cys(Cm), Asn, Asp, Gly]Arg 65
T-10	(Arg) [Thr, Pro, Gly, Ser]Lys 70
T-11	[Asn, Leu, Cys(Cm), Asn, Ile, Pro, Cys(Cm), Ser, Ala, 75 80 Leu, Leu, Ser, Ser, Asp, Ile, Thr, Ala, Ser, Val, 85 90 Asn, Cys(Cm), Ala]Lys 95
T-12	Lys
T-13	(Val) (Asp) Ile-Ala-Ser-Gly-Gly-Asp-Gly-Met-Asx-Ala[Trp, 100 105 Val, Ala, Trp]Arg 110
T-14	Asn-Arg
T-15	Cys(Cm)-Lys 115
T-16	(Gln) [Gly, Thr, Asp]Val-His[Ala, Trp, Ile]Arg 120 125
T-17	[Gly, Cys(Cm)]Arg
T-18	Leu

FIG. 4. Tentative primary structure for RCm-turkey egg white lysozyme arranged as tryptic fragments by analogy with RCm-chicken egg white lysozyme. Positions at which differences have been found between the turkey and chicken lysozymes are indicated by showing the corresponding amino acid residue of the chicken lysozyme in parentheses above the line. Where the turkey lysozyme amino acid sequence was not determined, the pri-

Residues 41 through 45 and 50 through 54—such that an appreciable conformational change may not occur when Residue 15 becomes leucyl and Residue 41 becomes histidyl.

The most striking of the apparent amino acid sequence differences between the turkey and chicken lysozymes, that at Residue 101, relates to substrate binding for the reason that the x-ray crystallographic examination of chicken lysozyme complexes with certain competitive inhibitors has implicated the Asp₁₀₁ carboxyl group in these binding phenomena (2). Substrate binding by the turkey lysozyme has yet to be examined thoroughly. Nevertheless, this enzyme is a good catalyst for the hydrolysis of *M. luteus* cell walls and, thus, must bind some substrates efficiently. The effect on substrate binding by replacing Asp₁₀₁ by glycyl, then, may be viewed in either of two ways: (a) the Asp₁₀₁ carboxyl group is involved in binding larger substrates such as the *M. luteus* cell wall hexasaccharide to the chicken lysozyme, and the turkey lysozyme binds these substrates somewhat differently; or (b) the Asp₁₀₁ carboxyl group is not important for binding these substrates to the chicken lysozyme. Lin and Koshland's recent observation (20) that chitin oligomers did not protect the Asp₁₀₁ carboxyl group against conversion into $-\text{CO}-\text{NHCH}_2\text{SO}_3^-$ but *did* prevent this transformation for the Asp₃₂ carboxyl group is an indication that the latter interpretation is correct. In fairness it must be mentioned, however, that it is not certain whether the Asp₁₀₁ side chain $-\text{CO}-$ can or cannot form a hydrogen bond with substrates when the carboxyl group has been transformed into $-\text{CO}-\text{NHCH}_2\text{SO}_3^-$; therefore Lin and Koshland's report that over 50% of the original lytic activity remained after modifying all of the chicken lysozyme carboxyl groups in this way, except those of Glu₃₈ and Asp₃₂, cannot substantiate any argument against a requirement for substrate binding to the Asp₁₀₁ carboxyl group of chicken lysozyme.

There are as yet available only limited data for other avian egg lysozyme primary structures, so that few comparisons with the turkey and chicken lysozymes can be made at this time. It is noteworthy, however, that a preliminary report concerning the amino acid sequences of duck lysozymes II and III from the amino terminus through Residue 53 (21) has indicated that the amino acid sequences of these proteins differ at six positions from that of the chicken lysozyme and that 2 of the residues that are different (at Positions 3 and 15) are as observed in the turkey lysozyme. It will be interesting to note whether there are any other similarities between the turkey and duck lysozymes when the complete primary structures of the duck lysozymes become known.

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