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The Lysozyme of Bacteriophage λ

II. AMINO ACID AND END GROUP ANALYSIS*

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SUMMARY

The frequencies of the amino acid residues in λ-lysozyme have been determined. A molecular weight of 17.9 × 10^3 was computed from these frequencies, in agreement with the value determined previously from the sedimentation behavior of this enzyme. Analyses of the amino-terminal residues by the cyanate method and of the carboxyl-terminal residues by hydrazinolysis and by exposure to carboxypeptidase A indicate that λ-lysozyme consists of a single polypeptide chain containing 159 residues bounded by methionine at the amino terminus and valine at the carboxyl terminus. It contains no disulfide bonds. The λ-lysozyme exhibits similarities with the lysozymes of coliphages T2 and T4 in regard to total number of residues, their distribution among different groups of amino acids, the lack of disulfide bonds, and the nature of the amino terminus. In addition, a weak immunochemical cross-reaction between the λ- and T4-lysozymes was shown.

In the preceding article (1) we described efficient purification procedures that yield monodisperse preparations of λ-lysozyme, and, by analysis of the sedimentation behavior of such preparations, determined that this protein exhibits a molecular weight of 17.9 × 10^3. In this paper we begin an analysis of the primary structure of λ-lysozyme by determining its amino acid composition and terminal residues.

It is shown here that the minimum molecular weight calculated from the residue frequencies of the constituent amino acids is 17.9 × 10^3, giving independent confirmation of the value obtained by sedimentation analysis. The values for the residue frequencies eliminate repeated identical polypeptides and disulfide bonds as elements of the λ-lysozyme structure. Furthermore, the results presented here show that this protein contains only 1 amino-terminal residue (methionine) and only 1 carboxyl-terminal residue (valine). Hence λ-lysozyme consists of one polypeptide chain in which the 159 residues are covalently linked solely by peptide bonds.

In regard to size, amino acid composition, and lack of disulfide bonds, the λ-lysozyme is similar to the lysozymes of coliphages T2 and T4 (2) and dissimilar to egg white lysozyme (3). The similarity between the phage lysozymes is also indicated by a weak immunochemical cross-reaction described here.

EXPERIMENTAL PROCEDURE

Materials

Dowex AG50W-X2, 100 to 200 mesh, was a product of Calbiochem, and was washed in turn with 1 M NaOH, H_2O, 6 M HCl, and finally H_2O. Hydrazine was anhydrous hydrazine obtained from the International Chemical and Nuclear Corporation and was used without further treatment. Potassium cyanate, purchased from the J. T. Baker Chemical Company and recrystallized from ethanol, was a gift of G. R. Stark (Stanford University). The hippuryl-L-phenylalanine and hippuryl-L-arginine used in the assays of carboxypeptidase A and B were obtained from Cyclo Chemicals. 5,5′-Dithiobis-(2-nitrobenzoic acid) was purchased from Aldrich.

The λ-lysozyme was prepared according to the procedure described in the preceding paper (1). Carboxypeptidase A (Batch Co A-DFP613) was obtained from Worthington. When assayed for carboxypeptidase B (4), the ratio of B to A activities was less than 1 × 10^{-3}.

Methods

Amino Acid Analyses—Protein samples were hydrolyzed in 6 N HCl in sealed, evacuated tubes at 110° according to the methods described by Moore and Stein (5). The hydrolysates were analyzed in the Beckman-Spinco model 120B amino acid analyzer either at the unmodified sensitivity of the instrument or after increasing the sensitivity with a longer light path and more sensitive recorder, the protein samples being 1 to 2 mg and 0.08 mg, respectively.

Two independently purified preparations of λ-lysozyme were used. Preparation 1 was hydrolyzed for 20, 48, and 75 hours and the resulting hydrolysates were analyzed at the lower sensitivity of the analyzer. Preparation 2 was hydrolyzed for 20 and 48 hours and analyzed at the higher sensitivity.

Serine, threonine, and tyrosine were determined by extrapo-

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lation of the logarithms of values found after the various times of hydrolysis to zero time. A small crystal of phenol was added before hydrolysis to protect against oxidation of tyrosine, which exhibited a loss of only 0.07% per hour of hydrolysis. The rate of loss of threonine and serine was 0.2 and 0.6% per hour, respectively.

The half-cystine values given in Table I were determined as cysteic acid after performic acid oxidation of the protein for 3 hours at 0° followed by acid hydrolysis for 18 hours at 110° (6). Free sulfhydryl groups were determined by the method of Shramm (7) modified so that guanidine hydrochloride replaced the combination of sodium dodecyl sulfate and urea. After a slow initial reaction of the enzyme with 5,5′-dithiobis-(2-nitrobenzoic acid), guanidine hydrochloride was added to a final concentration of 4.5 M to bring the reaction to completion.

Tryptophan was determined from the absorption spectrum of the protein in 0.01 M potassium phosphate buffer, pH 7, and in 0.1 M NaOH (8).

Amino-terminal Residues—The cyanate method of Stark and Smyth (9) was used to determine the amino-terminal residues. The second Dowex 50 column was used to remove spurious glutamic acid arising from pyrrolidone-carboxylic acid formation (9). We note here certain variations in the method as applied to two separate samples of λ-lysozyme (Experiment I and II, Table IIa).

The carbamylation solution included 4 M guanidine hydrochloride and this denaturing solvent was removed either by dialysis (Experiment I) or by precipitation with acetone (Experiment II). Acetone was used in the second case because of the considerable loss (about 50%) observed in Experiment I during dialysis from the concentrated salt solution.

The second experiment differed from the first in two other regards. Serotonin creatinine sulfate was added during cyclization in Experiment II to protect possible tryptophan end groups (9). The medium column (57 cm) of the Beckman-Spinco amino acid analyzer was used in Experiment I for analysis of neutral and acidic amino acids. The long column (150 cm) was used in Experiment II in order to allow the detection of any aspartic acid that might have been obscured by the methionine sulfoxides observed in Experiment I. Use of the long column revealed the two stereoisomeric methionine sulfoxides (10) as clearly resolved peaks and no aspartic acid.

The amount of carbamylated protein subject to the end group analysis was determined in each case by hydrolyzing an aliquot of this protein in 6 N HCl and assaying for the constituent amino acids.

Carboxyl-terminal Residues by Hydrazinolysis—The method of Akabori *et al.* (11) for the hydrazinolysis of proteins was applied to λ-lysozyme in the following modified form. The protein (0.575 mg) was dried over P_2O_5 and 1.5 ml of anhydrous hydrazine were added to it, this operation taking place in a dry box containing P_2O_5. The tube containing the mixture was placed in a bath of methanol and Dry Ice to freeze the hydrazine and then evacuated and sealed.

After heating at 100° for 5 hours (see Table IIb), the hydrazine was evaporated over P_2O_5 and the residue was taken up in 1 ml of H_2O. After adding 0.3 ml of benzaldehyde to precipitate the hydrazides and shaking the tube, it was kept at 0° for 30 min and then centrifuged at 3000 × *g* for 10 min. The aqueous phase was combined with that resulting from washing the precipitate and organic phase with 0.5 ml of H_2O and again 0.3 ml of benzaldehyde was added. After 20 min at 0°, the mixture was centrifuged as before and the aqueous phase was decanted. Two more 0.3-ml aliquots of benzaldehyde were added and removed by this procedure, the final aqueous phase having a volume of 1.35 ml. After adding an equal volume of pH 2.2 citrate buffer (12), one half of the solution was analyzed for basic amino acid and the other half for neutral and acidic amino acids.

Two other samples of λ-lysozyme were treated with hydrazine as above for 10.5 and 16.7 hours (Table IIb). The benzaldehyde extraction was omitted in these cases, and the dried amino acids plus hydrazides, dissolved directly in the pH 2.2 citrate buffer, were applied only to the column used for analysis of neutral and acidic amino acids and from which the hydrazides fail to elute.

Preparation of Antibodies to λ-Lysozyme—Five milligrams of λ-lysozyme in 0.5 ml of water and 0.5 ml of Freund's adjuvant containing 5 mg of heated and dried *Micrococcus butyricum* were thoroughly homogenized and then injected into the toepads and neck of a rabbit. Twenty-four hours later 1 mg of alum-precipitated enzyme in 1 ml of suspension (13) was injected. After 5 weeks the animal was again injected with 1 mg of the alum-precipitated protein in 1 ml of suspension. One week after this injection, a relatively strong antigen-antibody precipitin line was observed on agar plates (see "Results"), and 20 ml of antiserum were collected.

Other Procedures—The assay of λ-lysozyme has been described (1); T2-lysozyme was assayed by the same method. Carboxypeptidase A was assayed by the method of Folk and Schirmer (14). The Cary model 14 recording spectrophotometer was used in the measurement of the carboxypeptidase activities. Other optical densities were obtained with a Zeiss PMQII spectrophotometer. Unless otherwise stated, protein concentrations were determined by the method of Lowry *et al.* (15), with bovine serum albumin as a standard. The use of this method and standard results in correct values for purified λ-lysozyme (1).

RESULTS

Amino Acid Composition and Molecular Weight

The frequencies of amino acid residues in λ-lysozymes are ordered in Table I according to increasing values. A minimum molecular weight for this enzyme can be computed from these frequencies in several ways. The two methods used here yield values consistent with each other and with the value of 17.9 × 10^3 found independently by sedimentation analysis (1).

The first method is the common one for determining minimum residue numbers and is given in Table I. It yields a total of 159 residues per molecule and a consequent molecular weight of 17,873.

The second method used here is adapted from that proposed by Nyman and Lindskog (16). A set of molecular weight values to be tested is chosen. The residue frequencies are used to calculate residue numbers for each molecular weight in the set. The difference between the calculated residue number and the nearest integer is taken as a measure of the fit of the frequencies to the molecular weight. However, the individual differences must be weighted to account for the fact that the smaller the residue number the more significant is its deviation from an integral value. Further, it would be useful to express the result in terms that allow easy comparison to that obtained if random

errors in the measurement of the frequencies become dominant. To accomplish both aims the following procedure was adopted.

The difference between the calculated residue number and the nearest integer was divided by the integer and the sum of such weighted differences computed for each molecular weight. The maximum value that such a sum can take is 0.5 times the sum of the reciprocals of the integers. The ratio of the experimental to this maximum sum is the measure of fit used here and forms the ordinates of the two plots in Fig. 1. Values of this ratio near 0.5 are expected if random errors in the frequencies dominate the result. A plot of this ratio against molecular weight should yield the smallest value at the minimum molecular weight for the enzyme. Molecular weights that represent multiples of the minimum value will tend to exhibit ratios closer to 0.5 since the effect of errors will be proportionately higher.

In the range of molecular weights from 12,000 to 39,000, the deepest minimum has a value of about 0.2 at 18,000 (*upper right curve* in Fig. 1). The expanded plot for molecular weights near 18,000 indicates the minimum to be at a molecular weight of 17,900 when 100 is the size of the interval between nearest values. The closest integers to the calculated residue numbers for a molecular weight of 17,900 are those listed as minimum residue numbers in Table I.

The finding of only 1 cysteic acid residue per molecule of oxidized protein (Table I) indicates that there are 1 cysteine residue and no disulfide bonds in λ-lysozyme. This is confirmed by the observation that the reaction between 5,5′-dithiobis-(2-nitrobenzoic acid) and enzyme (see "Methods") indicates the presence of 0.84 mole of free sulfhydryl groups per mole of λ-lysozyme.

TABLE I

Frequencies and minimum residue numbers for amino acids in λ-lysozyme

Type of residue, i	Residue frequency,[a] F_i	Minimum residue no.[b]	Standard deviation of F_i
Half-cystine[c]	0.0068	1	0.0006
Histidine[d]	0.0184	3	0.0002
Methionine[e]	0.0184	3	0.0005
Tryptophan[f]	0.0196	3	
Tyrosine[g]	0.0308	5	0.0008
Phenylalanine	0.0308	5	0.0004
Proline	0.0325	5	0.0026
Threonine[g]	0.0392	6	0.0002
Valine[h]	0.0447	7	0.0002
Isoleucine[h]	0.0556	9	0.0007
Serine[g]	0.0627	10	0.0019
Lysine[d]	0.0743	12	0.0014
Arginine[d]	0.0746	12	0.0020
Alanine	0.0824	13	0.0014
Leucine[h]	0.0876	14	0.0016
Glycine	0.0956	15	0.0040
Glutamate	0.1072	17	0.0029
Aspartate	0.1186	19	0.0016
$\sum$		159	

[a] Except as indicated, the residue frequencies represent the average of the determinations for both preparations of the enzyme (see "Methods").

[b] The minimum residue numbers, n_i are calculated by (*a*) assuming that the value for the amino acid with lowest frequency is 1, (*b*) dividing the next larger frequency by successive integers and selecting n_i as that integer yielding a quotient closest to the lowest frequency, and (*c*) continuing the process in order of increasing frequencies, always matching the quotients for selection of a given n_i with the mean of all previously determined values of F_i/n_i. The mean of all F_i/n_i is 0.0063 and the range (excluding the half-cystine) is 0.0061 to 0.0065, indicating a deviation from the integral n_i of no more than 3%.

[c] Two independent determinations of half-cystine as cysteic acid were performed with Preparation 1 only.

[d] Basic amino acids were determined only in the two analyses of Preparation 2.

[e] Methionine determined after performic acid oxidation was 2.8 residues per molecule as compared to 2.93 residues obtained from the above data on the unoxidized protein.

[f] Single determination, Preparation 1.

[g] Determined by extrapolation for each preparation ("Methods"); the residue frequency is the mean of the two extrapolated values and the standard deviation is derived from them.

[h] The 20-hour hydrolysates are not included.

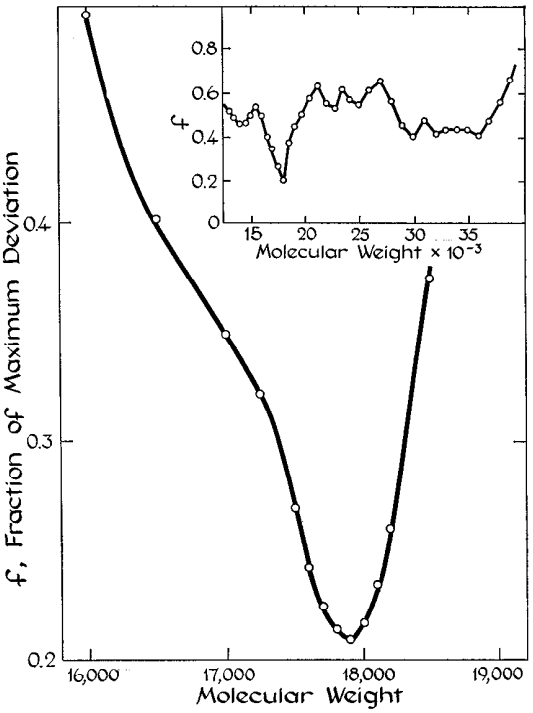

FIG. 1. The fit of molecular weight to the amino acid frequencies in λ-lysozyme. The *ordinate* for both curves is the fraction of the maximum deviation, f, defined as

$$f = \frac{\sum(\Delta_i/I_i)}{0.5\sum(1/I_i)}$$

where Δ_i is the numerical difference between the number (n_i) of residues of amino acid, i, and the nearest integer, I_i. n_i is a function of molecular weight (M), the amino acid residue frequencies (F_i of Table I), and the residue molecular weight (R_i), such that

$$n_i = F_i \cdot [(M - 18)/\sum F_i \cdot R_i]$$

All sums are taken over 18 different types of residues, the glutamate-glutamine and aspartate-asparagine pairs each being considered as single types.

Terminal Residues

Amino Terminus—The results obtained from the cyanate method for the determination of amino-terminal residues (see "Methods") are presented in Table IIa. No significant amounts of neutral and acidic amino acids other than methionine and its sulfoxides were detected. The three basic amino acids were obtained in very low amounts in each of the two experiments (about 0.06 mole each of lysine, histidine, and arginine per mole of protein). In sum, these results indicate that the λ-lysozyme molecule contains only 1 amino-terminal residue and that it is methionine.

Carboxyl Terminus—The carboxyl-terminal residue of λ-lysozyme was determined by hydrazinolysis and by digestion with carboxypeptidase A.

The results of the hydrazinolysis experiments are given in Table IIb and indicate that valine is the carboxyl-terminal residue of the enzyme. The results from the 5-hour hydrazinolysis differ from those obtained after 10.5 and 16.7 hours not only in the smaller yield obtained but also in the appearance of alanine (0.38 mole per mole of enzyme), not found at the later times. We ascribe this aberrant alanine to contamination acquired during the 5-hour experiment. The yield of other amino acids was negligible (<0.08 mole per mole of enzyme) in each of the experiments. However, it should be noted that cysteine, asparagine, and glutamine would escape detection. Cysteine is unstable in hydrazine, while carboxyl-terminal asparagine and glutamine would form the β- and γ-hydrazides, respectively, thus escaping detection (11).

To allow for the detection of such carboxyl-terminal residues and to confirm the carboxyl-terminal valine found by hydrazinolysis, λ-lysozyme was treated with carboxypeptidase A. No valine and negligible amounts of other amino acids were released from λ-lysozyme when the mole ratio of carboxypeptidase A to λ-lysozyme was 0.01 under two solvent conditions. In the first experiment the hydrolysis of 39 mμmoles of native λ-lysozyme was attempted in 0.1 M ammonium carbonate, pH 8.3, for 24 hours at 37°. In the second experiment, denaturing conditions essentially the same as used by Halsey and Neurath (17) were used. Thus, 20 mμmoles of λ-lysozyme were reacted with carboxypeptidase A in 0.075 M sodium barbital, pH 8, 0.075 M NaCl, 6 M urea for 4 hours at 25°. In both cases the residual protein was removed by precipitation in 5% trichloracetic acid prior to assaying for amino acids with the analyzer.

The stability of the carboxypeptidase A in 4 M and 6 M urea was then tested. It was found that after 4 hours at 25° in 4 M urea 16% of the initial carboxypeptidase activity remained, whereas only 0.4% of this activity remained in 6 M urea. As a consequence of this information, the carboxypeptidase A was increased to a mole ratio of 0.1 and the denaturing solvent was changed to 4 M urea in 0.05 M sodium barbital, pH 8, 0.05 M KCl. Incubation of 20 mμmoles of λ-lysozyme in this solvent for 16 hours at 25° yielded 12 mμmoles of valine or 0.6 mole per mole of λ-lysozyme, and negligible amounts of other amino acids.

TABLE II

Terminal residues of λ-lysozyme

a. Amino-terminal residues

Experiment	λ-Lysozyme	Methionine + methionine sulfoxides	NH_2-terminal methionines per molecule
	mμmoles	*mμmoles*	
I	165	122	0.74
II	90	72	0.80

b. Carboxyl-terminal residues determined by hydrazinolysis

Experiment	Time of hydrazinolysis	Valine/λ-lysozyme mole ratio
	hrs	
I	5	0.45
II	10.5	0.78
III	16.7	0.77

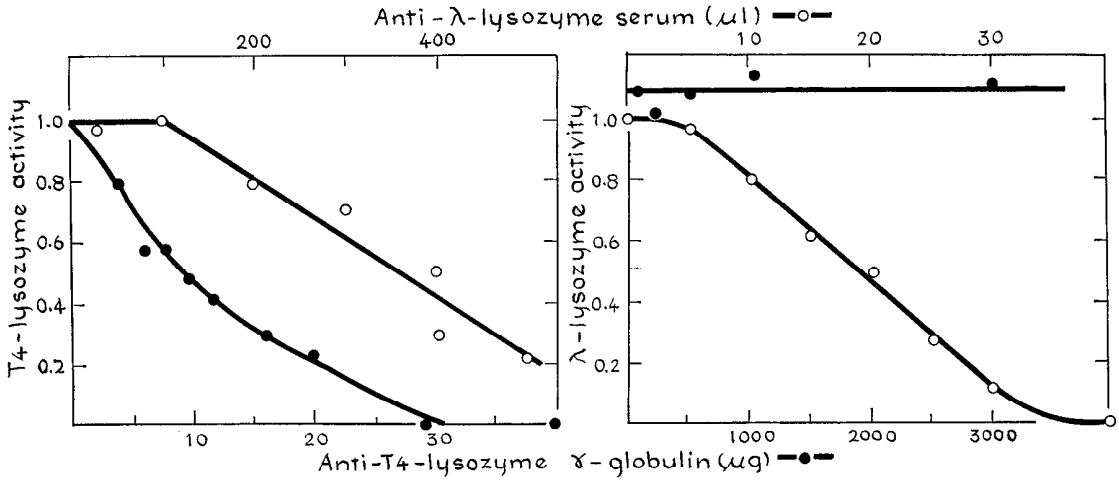

FIG. 2. Immunochemical cross-reaction between λ lysozyme and T4 lysozyme. Anti-λ-lysozyme serum or anti-T4-lysozyme γ-globulin was added to 1.0 μg of λ-lysozyme per ml of solvent (0.014% bovine serum albumin in 0.05 M potassium phosphate buffer, pH 7.0) and to 0.22 μg of T4-lysozyme per ml of solvent (0.020% bovine serum albumin in the above buffer). The mixtures were kept overnight at 2° and 0.05-ml aliquots were removed for assay of lysozyme activity before and after centrifugation for 20 min at 12,000 rpm. The values before and after centrifugation did not differ significantly. The *upper abscissa* of both graphs indicates the amount of anti-λ-lysozyme serum added, while the *lower abscissa* indicates the amount of anti-T4-lysozyme γ-globulin added in each case. ○, anti-λ-lysozyme serum; ●, anti-T4-lysozyme γ-globulin.

The removal of the carboxyl-terminal valine was again attempted under nondenaturing conditions of 0.075 M ammonium carbonate, pH 8.3, 25°, by further increasing the carboxypeptidase A to a mole ratio of 0.2. After 17 hours of incubation only 0.31 mole of valine was found per mole of λ-lysozyme.

These results confirm those obtained from the hydrazinolysis in indicating that the λ-lysozyme molecule contains only 1 carboxyl-terminal residue, valine. They also indicate that this protein has a native configuration that is resistant to attack by carboxypeptidase A; moreover, λ-lysozyme apparently has a penultimate amino acid residue which impedes further hydrolysis by this exopeptidase, even under denaturing conditions.

Immunochemical Cross-Reaction Between λ-Lysozyme and T4-Lysozyme

Having prepared an antiserum to λ-lysozyme for other purposes and having available both the T4-lysozyme and antibodies to it as gifts from G. Streisinger (University of Oregon), the similarities in their composition (see "Discussion") induced us to ask whether structural similarities between the enzymes could be observed at the level of antigen-antibody reactions.

Double diffusion of λ-lysozyme (1 mg per ml) and T4-lysozyme (1 mg per ml) against anti-λ-lysozyme serum and anti-T4-lysozyme γ-globulin (10 mg per ml) was carried out in agar in 4° (18). Homologous antigen-antibody precipitin lines were observed, but there was no evidence of heterologous interaction by this technique.

The effects of anti-λ-lysozyme serum and anti-T4-lysozyme γ-globulin on the enzyme activities were then measured, and the results are given in Fig. 2. Both enzyme activities are inhibited by the homologous antibodies and the T4-lysozyme activity could be inhibited by the anti-λ-lysozyme serum. However, this cross-reaction is very weak, the amount of antiserum required per enzyme molecule being about 100-fold more for the heterologous reaction. Serum from rabbits not exposed to either enzyme caused no inhibition of the T4-lysozyme when added in equal amounts. No inhibition of the λ-lysozyme activity was observed by anti-T4-lysozyme γ-globulin, even at levels about 20-fold greater than necessary for complete inhibition of an equivalent weight of T4-lysozyme.

TABLE III

Comparison of amino acid residues in λ- and T4-lysozyme

Amino acid	No. of residues in	
	λ-Lysozyme	T4-lysozyme[a]
Aliphatic		
Gly	15	11
Ala	13	15
Val	7	9
Ile	9	10
Leu	14	16
Σ	58	61
Hydroxyl		
Ser	10	6
Thr	6	11
Σ	16	17
Aromatic		
Phe	5	5
Tyr	5	6
Trp	3	3
Σ	13	14
Acidic (+ amides)		
Asx	19	22
Glx	17	13
Σ	36	35
Strongly basic		
Lys	12	13
Arg	12	13
Σ	24	26
His	3	1
Pro	5	3
Cys	1	2
Met	3	5
Total residues	159	164

[a] The amino acid sequence of T2-lysozyme differs from that of T4-lysozyme at only three positions.

DISCUSSION

Among the 10 amino acids appearing with lowest frequencies in λ-lysozyme, nine have residue numbers which are odd and include the values 1, 3, 5, 7, and 9, the lone even number being 6 (Table I). This is consistent with a model of the enzyme that involves only one polypeptide chain, but is inconsistent with models having multiple identical polypeptide chains.

Models with multiple and different polypeptides are excluded by the finding of one methionine and one valine and no other amino acids in the amino- and carboxyl-terminal positions, respectively. The exceptions to this exclusion must not only contain at least 1 blocked amino-terminal residue, but also contain at least 1 carboxyl-terminal residue which is both resistant to attack by carboxypeptidase A and does not yield an amino acid upon hydrazinolysis. We consider the likelihood of such exceptions for a protein molecule of this small size to be negligible, and conclude that λ-lysozyme consists of a single polypeptide chain of 159 amino acids residues bounded by methionine and valine.

There is only 1 cysteine residue among the 159 and its sulfur is in the reduced state. We have not determined how critical is this state to the catalytic activity. However, it has been reported that *p*-chloromercuribenzoate (19) and certain heavy metal ions (20) inhibit this activity, suggesting that the sulfhydryl group is involved in specifying the active configuration or is quite close to the active site.

The lack of disulfide bonds in λ-lysozyme is one of several characteristics which couples it to the lysozymes of T2 and T4 (2) to form a distinctly separate group from egg white lysozyme (3) and the lysozymes of higher organisms in general (21). Both the T2- and T4-lysozymes have 2 cysteine residues, each with a free sulfhydryl group, whereas in egg white lysozyme four disulfide bonds link the 8 cysteine residues. Indeed, such multiple disulfide bonds are a common characteristic of the lysozymes in higher organisms (21).

As is indicated in Table III, the distribution of the 159 residues

in λ-lysozyme among five functional groups is similar to that exhibited by the 164 residues in T2- or T4-lysozyme. Egg white lysozyme, on the other hand, contains only 129 residues which are distributed quite differently among these groups (3).

While the similarities between λ- and T4-lysozyme given in Table III are an inadequate base for an effective argument that the two polypeptide chains have similar configurations, they do induce such speculation, particularly in light of the immunochemical cross-reaction that was observed between them. The weakness of the cross-reaction between configurationally similar chains may be imagined to be due to different but functionally related side groups at various positions in the chain.

Two explanations for the structural differences between the lysozymes of higher organisms and the coliphage lysozymes come to mind. The first, more trivial speculation is that the structural differences correlate with unknown differences in catalytic specificity. It is not known which bonds are sensitive to attack by λ-lysozyme, although it is clear that one substrate of egg white lysozyme, penta-*N*-acetylglucosamine, is not hydrolyzed by λ-lysozyme (1). On the other hand, both T2- and T4-lysozyme appear to be muramidases (22, 23), as is egg white lysozyme.

Alternatively, the difference in structure may be imagined to derive from selective forces related to enzyme stability or rate of enzyme formation, or both. Rapid formation of lysozyme could be advantageous to phage reproduction in which lysis of the host cell occurs within 20 to 30 min, whereas a high degree of stability might not be relevant. In the case of higher organisms with their longer time scales, a reciprocal relation can be supposed in which high stability is the important factor and rapid formation the irrelevant one. We have observed that the coliphage lysozymes are significantly less stable to heat than is egg white lysozyme, and this difference may reasonably be attributed to the absence of disulfide bonds in the coliphage lysozymes and their multiple presence in egg white lysozyme. Similarly, the rate-limiting reaction in the formation of lysozymes with multiple disulfide bonds may be the construction of such bonds, and be a disadvantageous restriction in the formation of the coliphage ysozymes.

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