Crystallographic Studies of Protein Denaturation and Renaturation.

2. Sodium Dodecyl Sulfate Induced Structural Changes in Triclinic Lysozyme†

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ABSTRACT: Cross-linked triclinic lysozyme was denatured with sodium dodecyl sulfate. Removal of the denaturant resulted in a refolding of the protein to a conformation similar to but not identical with the native one. Three-dimensional x-ray diffraction data out to 3.2-Å resolution were collected for two states in the refolding pathway, and appropriately weighted electron density difference maps were constructed. An analysis of these maps reveals that a sodium dodecyl sulfate molecule is trapped in the interior of the protein, and results in a separation of regions of the polypeptide chain. Our results are discussed in terms of current models for protein folding.

A great deal of information about the three-dimensional structure of globular proteins has become available from x-ray crystallography. However, while crystal studies provide an essentially static structural picture it has become clear that proteins have a considerable degree of conformational flexibility (see, for example, Lakowicz and Weber, 1973), which may play an important functional role. Flexibility has been probed by a variety of techniques, most of them spectroscopic, but essentially no structural information as to the type of conformational fluctuation proteins undergo is available. Similarly, both protein folding and denaturation have been characterized kinetically (Baldwin, 1975), but it has not yet been possible to identify a specific structural change along either pathway.

In this work we have used x-ray crystallography as a probe of conformational changes accessible to a native protein. In particular, a crystal-structure determination at various points along the denaturation-renaturation cycle (Yonath et al., 1977a) of cross-linked triclinic lysozyme has been carried out. The availability of isomorphous cross-linked crystals makes the use of x-ray crystallography possible and thus allows a detailed description of a conformational deformation induced by a denaturant molecule.

This study pertains to the specific case of sodium dodecyl sulfate denaturation. We have previously described (Yonath et al., 1977b) the effects of a number of other denaturants on triclinic lysozyme and find that they can be divided into two main classes, hydrophilic and detergent-like. Sodium dodecyl sulfate belongs to the latter group whose predominant site of attack is the core rather than the surface of the protein. This type of interaction affects the entire protein and therefore is likely to produce global conformational changes. We have been able to obtain a detailed three-dimensional picture of these changes and find that large fragments of lysozyme maintain their conformational integrity despite the presence of a sodium dodecyl sulfate molecule in the interior of the protein. Our results suggest that lysozyme contains independent structural domains and thus support current thinking on the mechanism of protein folding.

Evidence has accumulated which indicates that proteins fold through the piecemeal formation of tertiary structure from smaller structural fragments, although the detailed mechanism is far from clear. Anfinsen (1972) has suggested, for example, that certain regions may flicker in and out of their final conformation and stabilize one another in the native protein. Creighton's (1975) studies of the folding of pancreatic trypsin inhibitor show that certain conformational states must be reached before folding can proceed correctly. Protein crystallographers have also identified structural subunits that seem to have formed independently. Observations of this type have been summarized by Wetlauffer (1973) who emphasized the existence of large domains and suggested that their mutual assembly might be a late stage in a folding pathway. These domains appear to be thermodynamically unstable in all cases that have been studied with the exception of the constant and variable regions of antibodies (Davies et al., 1975). Smaller distinct structural fragments such as helices are usually assumed to play a role, such as a nucleation site, along a folding pathway.

In a recent publication (Honig et al., 1976) it has been suggested that rigid units connected by flexible hinges may be used to represent many conformational changes in proteins. Using this description and assuming that β bends correspond to flexible chain segments, it was shown that only certain conformational deformations were accessible to a native protein. Moreover, it was possible to separate large fragments of the protein through the correlated rotation of only a small number of backbone angles, thus suggesting that structural subunits could maintain their integrity during early stages of an unfolding pathway or during conformational fluctuations such as breathing vibrations. In this study we use the model of rigid fragments connected by flexible hinges to fit the differences between native and renatured lysozyme. Our results are consistent with the assumptions of this model.

Experimental Section

Data Collection. Denaturation-renaturation experiments were carried out with cross-linked triclinic lysozyme crystals...
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The calculated phases used are probably very similar to those of the protein display and manipulation program described elsewhere (Katz and Levinthal, 1972; Honig et al., 1973, 1976). All manipulations were controlled by typing commands at the terminal with the program operating under the TSO time sharing system. The details of the graphics system will be described in a future publication (A. Podjarny et al., manuscript in preparation). A Labquip molecular model of lysozyme which we constructed provided a description of the entire protein and aided in the interpretation of the local features displayed on the scope.

To simplify the display, we “boxed” the triclinic lysozyme molecule in an orthogonal coordinate system by rotation about the three principal axes (Moult et al., 1976). As a result, the triclinic conformation is expressed in Cartesian coordinates which correspond to the tetragonal crystal form. We shall refer to this representation as the “orthogonal” one. The difference maps are sectioned perpendicular to the “orthogonal” Z axis.

Results

Map Interpretation. Two sodium dodecyl sulfate crystals were studied at different stages of renaturation. During the data collection, crystal no. 1 was in contact with a solution of 0.35 M sodium dodecyl sulfate and no. 2 was in contact with 0.0 M sodium dodecyl sulfate. Details of the data collection, cell constants, and a comparison with cross-linked triclinic lysozyme crystals are given in Table I. The electron density difference maps between the renatured and cross-linked crystals were calculated as described in the previous section and intercorrelated. The correlation factor between the difference maps for 0.35 and 0.0 M sodium dodecyl sulfate was 0.8, indicating a great similarity between the two renatured states. Furthermore, the agreement factors between the observed structure factors of the 0.35 and 0.0 M sodium dodecyl sulfate renatured and the native cross-linked crystals are 19 and 17%, respectively. However, the agreement factor between the calculated native and observed renatured structure factors is 39%. Moreover, the minimum spacing observed in x-ray patterns of the refolded protein is 2.9 Å as compared to less than 1.1 Å for the native and cross-linked cases. The differences in the overall temperature factor that were obtained from Wilson plots are of the order of 60. Thus, it appears that the refolding results in somewhat disordered crystals of a protein whose conformation is similar but not identical with that of native lysozyme.

Most of the features in the difference map were readily interpretable in terms of structural changes, and the average noise level was remarkably low. In regions far from the protein the local standard deviation was lower than 0.7 of the overall...
There are at least three elongated peaks in both difference maps that on the basis of their size and location cannot be interpreted as parts of the protein. We assigned a sodium dodecyl sulfate molecule to each of these peaks, and for at least one of them we could clearly distinguish between the hydrocarbon chain and the SO$_3^{2-}$ head. A description of their locations, the type of contacts they make with the protein, and their influence on the protein conformation is given below.

Neither renatured crystal contains a single population of molecules. Certain regions of the difference maps appear to have particularly strong features and these correspond to portions of the protein whose conformations are identical in each molecule, but different from that of native lysozyme. Other portions are different from the native structure in only a fraction of the molecules so that in the difference map they appear to have a lower occupancy. Finally, there are several regions in the difference map that are clean, and these correspond to parts of the protein that are in their native conformation throughout the crystal.

Similar considerations indicate that the occupancies of the sodium dodecyl sulfate sites are less than 100%. Moreover, from the relative intensities of interior holes and peaks it seems that the crystals contain at least two molecular forms in different relative populations: one is close to the original native conformation and the other has a sodium dodecyl sulfate molecule in the interior of the protein which induces displacements of a large number of residues. Many of the strong features in the difference maps correspond to the intermolecular packing regions. This suggests that the intermolecular contacts of all the molecules in the renatured crystal are similar, but different from those of native lysozyme. Thus, it appears that crystal forces, symmetry requirements, and, to some extent, cross-linking, lead to a unique packing arrangement between molecules whose overall conformations in the core region differ slightly from one another.

The most striking aspect of the difference map is the radial displacement of large fragments of the protein away from the sodium dodecyl sulfate located in its hydrophobic core. The direction of this motion and the locations of the three sodium dodecyl sulfate molecules are shown in Figure 1. The overall pattern corresponds to the separation of the two wings of lysozyme that have been identified from observations of the crystal structure (Imoto et al., 1972; Wetlauffer, 1973). The first wing consists of residues 1-38 and 103-129 and the second of residues 43-100.

It is of interest to determine the extent to which the differences between the native and renatured proteins involves rigid-body displacements of the two wings. This should be describable in terms of torsional motions localized at the segments of the main chain that separates the wings. Practically, this means that rotations about the $\phi$ and $\psi$ angles in this segment, which may be regarded as a flexible joint, should move large parts of the native protein from regions of negative electron density toward positive peaks in the difference map.

A procedure to test this possibility was developed and is described in the next section.

The Wing Separation Procedure. We assigned the chain segment around residue 42 as a flexible joint since it separates the two wings and since residues on either side of it are displaced in opposite directions. Torsional motion in the joint was obtained by defining an attractive "potential" between points (atoms and residues) of the native protein which are in holes in the difference electron density maps and the corresponding locations of the peaks which could be clearly assigned to them in the difference map. Then minimizing this potential with respect to the variable angles in the joint moves the residues which have been kept fixed together to their new positions. A consistent rigid body displacement is obtained only if at least two points can be moved simultaneously to their assigned peaks. For the first wing the side chain of His-15 and the main chain of Ala-9 and Ala-10 were successfully moved together by rotation about the $\phi$ and $\psi$ angles of residues 39-42 (which comprise the $\beta$ turn separating the two wings). For His-15 this involves a movement of 1.6 Å and for Ala-9 and Ala-10 a movement of about 2.7 Å. This result implies that at least these points move together in a correlated manner. The number of other residues that move from holes to peaks as a result of this displacement define the extent to which the entire wing moves as a single unit.

The second wing was treated in a similar fashion. Here the guide points were the main chain of Ile-88 (movement of 4.2 Å) and the S-S bond from Cys-76 to Cys-94 (shift of 3.2 Å). The rotating bonds are those of residues 43-47, which are apparently disordered by an external sodium dodecyl sulfate (no. 3, see below). The net result of this treatment was the creation of a "new" molecule, that we refer to as the "opened" protein. In Figure 2 we show some of the regions that are most affected by the wing separation. Figure 2a shows the superposition of the native triclinic lysozyme structure on the electron density difference map while Figure 2b shows the superposition of the "opened molecule" on the difference map. Note that the residues that were in holes in Figure 2a are now in or near peaks. However, if both wings really move apart as rigid bodies, the entire "opened" protein should move from holes to peaks in the difference map.

The Opened Molecule. The structure of native lysozyme has been described in detail by Imoto et al. (1972). The first wing consists largely of three helices: residues 4-15, 23-36, and 108-116. Most of the first and second helices seem to move together as a rigid body and account for many features of the difference map for this wing. The other features can be inter-
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FIGURE 2: Regions of the native molecule (a) and the "open" one (b) that are most affected by the wing separation. These are superimposed on the 0.35 M electron density difference maps, contoured at 2σ. Full lines represent positive regions and dashed lines represent negative regions.

FIGURE 3: The internally trapped sodium dodecyl sulfate interaction site. The long hydrophobic chain is represented as peaks A, B, and C and the salt bridge SO₄ to e-amino of Lys-1 is peak D. The superimposed model is of the native molecule.

The portion of the map corresponding to residues 16–20 is clean. These residues are part of an extended chain which as a result of the opening should have a small movement which is not expected to produce strong features in the difference map. Moreover, residues 21–22 are involved in packing interactions with residues 65–68 of a neighboring molecule and therefore resist the opening motion. Thus, the extended chain 16–20 is pushed at one end but anchored at the other. It is reasonable that these forces are accommodated by rotations about glycines 16 and 22 which are both in hairpin turns and may serve as flexible joints. Particular attention should be paid to the loop 20–23, since the maps of the two renatured crystals show some different holes and peaks in this region. This is probably a result of different relative populations of the "opened" and "native" protein in the two stages of renaturation. In the 0.35 M crystal the "opened" protein determines the conformation of the loop. In the 0.0 M crystal the "native" protein is predominant, and the packing arrangement of this loop is similar but not identical with the one in the native structure of lysozyme.

The residues 23–29 are displaced along their helix axis by the wing separation, and move toward nearby peaks. These residues follow the rigid body motion of the first wing and maintain their contacts with residues 9–15 which are directly influenced by the sodium dodecyl sulfate. This helix extends as far as Asn-37, but is broken at Cys-30, even in the native structure, possibly by the 30–117 S–S bond. There is evidence that residues 30–37 follow the general direction of the rigid body movement although this region appears to be quite disordered in the 0.35 M crystal. The existence of a peak outside the protein in both maps suggests the presence of a sodium dodecyl sulfate (no. 2) molecule which is bound to the surface of the protein and displaces the side chains of Phe-38, Trp-123, and Lys-33, thus forming a hydrophobic "pocket". Figure 4 shows this region for the 0.35 M sodium dodecyl sulfate treated...
molecule. This position is also occupied when bromoethanol is used as the denaturing agent (Yonath et al., 1977a) and the apparent affinity of this site for the detergent may explain the disorder.

The main structural features of the second wing are an antiparallel \( \beta \) sheet consisting of residues 42–60, a long outer loop 64–74, a small helical segment 79–84, and a large helical region 88–99 which is partially disordered at Cys-94. Residues 43–47 which were used as a flexible joint appear to be partially disordered. This may be due in part to a surface sodium dodecyl sulfate molecule which attacks residues 42–47. This sodium dodecyl sulfate (no. 1) is located at the same position that is believed to be occupied by an acetate molecule in the native form (Moult et al., 1977).

Residues 42–47, which are in the anti-parallel \( \beta \) sheet, although partially disordered, still maintain contacts with residues 49–60 which appear in a clear region in both difference maps. Thus, the anti-parallel \( \beta \) sheet at residues 42–60 does not undergo any significant displacements. The only exceptions are the side chain of Ser-50, Gly-54, and Gln-57 which are in contact with Thr-47 and moves together with it as a result of interactions with the surface sodium dodecyl sulfate molecule which attacks residues 42–47. This sodium dodecyl sulfate (no. 3) is located at the same position that is believed to be occupied by an acetate molecule in the native form (Moult et al., 1977).

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Figure 5 shows residues 61–64. The pattern of holes and peaks in this region can be explained by the rigid body radial movement used to define the "opened" structure. Trp-62 and Trp-63 have been displaced, so as to occupy peaks in the difference map. Residues Asn-65 and Pro-70 are involved in intermolecular contacts and, as a result, large peaks and holes characterize the entire region. Arg-68 probably moves along with Tyr-23 in order to maintain intermolecular hydrogen bonds and solvent bridges. The outer loop 70–74 appears in a relatively clean region of the difference map, though there is some doubt about the accuracy of the native structure determination for this region (Moult et al., 1976).

In both maps residues 75 to 78 seem to follow the radial opening motion of this wing. In particular, the displacement of S–S 76–94 was used to define the wing separation. However, residues 77 and 78 are disordered by a surface sodium dodecyl sulfate which is bound to 46–48 of the neighboring molecule. Residues 79 to 87 are essentially in the native conformation, with the exception of the side chain of Leu-83 and the main chain of Leu-84 which are displaced by indirect contacts with the sodium dodecyl sulfate.

Residues 88 and 89 are in direct contact with sodium dodecyl sulfate (no. 1) (Figure 3). In fact, the displacement of He-88 was used to define the movement of the second wing. As a result of this motion the helical fragment 88–94 moves as a rigid body. The difference map in the region of the remaining residues of the helix 95–99 is rather complex and less clearly interpretable. Thus, it appears that the disulfide bridge formed by Cys-94 divides the helix into two fragments, one which follows the wing opening, and one which might not. A similar pattern was observed for the helical segment 24–36.

Residues 100–104 are very flexible and partially disordered in all native crystal forms. This fragment connects the two wings and we expect it to be stretched as a result of their separation. Due to its disordered nature we cannot detect changes in its conformation. Residues 105 and 129 which encircle the first wing are involved in many interactions with neighboring molecules. The corresponding portions of the map are mostly clean with the exceptions of side chains of Met-105, Trp-108, and Trp-123 and the main chain at Ala-124 and Arg-125. The side chains of Met-105 and Trp-108 follow the movement of Tyr-23 and Glu-35. Trp-123 is involved in interactions with the sodium dodecyl sulfate (no. 2) molecule that binds to Phe-38 and Lys-33 (Figure 4). Ala-124 and Arg-125 make intermolecular contacts with the packing loop 65–68 and the flexible loop 101–103, and undergo a displacement as a consequence of these packing interactions.

Native triclinic lysozyme contains several residues which are not well defined so that the comparison between the "opened" and "native" molecule is subject to uncertainties. These residues include Arg-21, Arg-45, Trp-62, Arg-68, Arg-73, Asp-101, Asp-103, and Glu-121.

Discussion

The System. Before attempting to characterize the conformational changes that accompany sodium dodecyl sulfate binding to lysozyme, it is important to recall the inherent constraints of the system. First, the molecule in the crystal must be cross-linked in order to prevent the crystal from dissolving in the presence of denaturant. However, there are only a few cross-links and these are highly flexible (Yonath et al., 1977b) so that the structural restrictions they impose are not expected to be severe. Second, the crystallinity itself may limit access of denaturant to some parts of the molecule as well as freedom of the molecule to unfold. These restrictions may operate up to the point where the x-ray pattern is lost. Unfortunately, the effects that crystalline forces may have on the properties of the denatured protein and its refolding pathway are extremely difficult to assess, though the large volume changes observed in the crystal at high denaturant concentrations suggest that the degree of unfolding is extensive (Yonath et al., 1977b).

It should also be pointed out that the disulfide cross-links are still intact in our denatured crystals. While it is likely that such a denatured state may be regarded as a random coil in that all single bonds are free to rotate (Tanford, 1968), it is
clear that the possible configurations of the chain are somewhat restricted.

The high correlation between the two sodium dodecyl sulfate maps shows that the two renatured crystals are very similar. The differences are at least partially due to different occupancies of sodium dodecyl sulfate molecules that are bound to the protein in the 0.35 and 0.0 M crystals, as well as to the different level of disorder induced by them.

Although it appears reasonable to identify this particular renatured lysozyme as a state along a folding or unfolding pathway, it cannot be demonstrated to be a true intermediate in either direction. Unfortunately, stages in sodium dodecyl sulfate denaturation have been impossible to isolate crystallographically since the diffraction pattern is lost in a sudden highly cooperative transition (Yonath et al., 1977b). On the other hand, refolding in the crystal is not complete (the sodium dodecyl sulfate can never be totally removed) so that we are not dealing with a true renaturation.

Despite these reservations we believe that our results may be used to describe conformational transitions that are really possible for lysozyme. At the least they represent an identification of a major structural deformation induced by a denaturant and as such provide important information as to the internal degrees of freedom available to a native protein. It is likely, moreover, that the renatured protein is in fact a close approximation to a late stage along a folding pathway and would form the native structure without first unfolding if somehow the internal sodium dodecyl sulfate could be removed. Furthermore, it seems reasonable to assume that sodium dodecyl sulfate induced denaturation proceeds along a similar pathway in the reverse direction and that the change from the native to the renatured conformation may resemble an early unfolding transition. A possible objection to these conclusions is that the presence of the disulfide bonds in the denatured state may influence the refolding pathway. However, although it is clear that S-S bonds stabilize protein structures, there is considerable evidence that they play only a minor role in defining a pathway of folding (Creighton, 1975; Wetlaufer and Ristow, 1973).

**Sodium Dodecyl Sulfate-Protein Interactions.** It is of interest to consider the nature of sodium dodecyl sulfate attack as reflected by its binding sites to lysozyme. Sodium dodecyl sulfate molecules were found in three different locations, each representing a different type of interaction (Figure 1). All of them have one feature in common which is probably characteristic of sodium dodecyl sulfate binding: the hydrocarbon chain makes hydrophobic contacts with the protein while the SO$_4^{2-}$ tail is salt bridged to charged amino acids. This is consistent with the description of sodium dodecyl sulfate attack suggested by Burkhard and Stolzenberg (1972). The sites of attack differ in the extent to which the local disorder is propagated throughout the protein. One sodium dodecyl sulfate (no. 3) is bound to the surfaces of two molecules and causes only small local disorder in its vicinity (near residues 47 and 77-78 of a neighboring molecule). The second (no. 2) penetrates the protein between two aromatic side chains (Phe-38 and Trp-123), displaces them, and induces some local disorder. The third sodium dodecyl sulfate molecule (no. 1) penetrates deeply into the hydrophobic core of the protein, between Ala-9, Ala-10, His-15, Ile-88, and Thr-89 and its sulfate forms a salt bridge with the epsilon group of Lys-1. The presence of this sodium dodecyl sulfate leads to a separation of the two wings of lysozyme, and thus the perturbation is propagated throughout the protein with residues as far as 20 A away being clearly affected. This global dislocation is likely to be a component of the sharp unfolding transition observed in sodium dodecyl sulfate denaturation (Yonath et al., 1977a). It is likely that the existence of sodium dodecyl sulfate binding sites in the interior of the protein is responsible for the ability of sodium dodecyl sulfate to induce large conformational changes at low concentrations (Tanford, 1968).

**Implications for Folding.** The most striking features of the renatured crystals are the separation of large sections of the protein and the presence of a sodium dodecyl sulfate molecule with its hydrophobic part buried in the hydrophobic core. In order to interpret these results it is important to understand why the interior sodium dodecyl sulfate (no. 1) we observe is the only denaturant molecule that remains bound inside the protein, even after the crystal has been soaked in denaturant free mother liquor for more than 30 days. It seems highly likely that many sodium dodecyl sulfate molecules are associated with the protein in its denatured state and that most of these must be removed before the protein can refold. That only one internal sodium dodecyl sulfate remains implies that its presence does not inhibit the formation of tertiary structure. It seems most likely that the two domains separated by the sodium dodecyl sulfate refold independently, trapping the sodium dodecyl sulfate in their interface during a late stage in folding. An alternative hypothesis that both wings fold around the hydrophobic core can be maintained only if one makes the unlikely assumption that the sodium dodecyl sulfate denaturant actually helps nucleate protein folding.

Our results suggest then that the two wings of lysozyme are distinct structural entities which represent late intermediates in the refolding process. The presence of a trapped sodium dodecyl sulfate molecule allows us to isolate these intermediates and investigate their conformation.

It should be emphasized that the positions of large numbers of residues in both wings were not affected by the internal sodium dodecyl sulfate. This is probably due to the constraints imposed by residues involved in intermolecular contacts. The fact that parts of each wing moved by a few angstroms while others remained fixed implies a certain amount of local rearrangement within each wing. A detailed description of these changes will be available from a structure refinement now in progress. At this stage it appears that even within each wing individual residues behave as if they belong to small structural units. The difference map consists of a series of segments of 5-10 residues that are either in clean regions, that moved from holes toward peaks as a unit, or are disordered. The borders between them are quite sharp suggesting that the segments themselves may be treated as individual entities.

A comparison of native and renatured lysozyme reveals that large fragments of the protein assume the same conformation, independent of the history of folding and of the presence of a bound sodium dodecyl sulfate. The changes that are observed seem to involve the reorientation of fixed segments with respect to one another. These segments may correspond to a large domain or to a few amino acids. It is possible that this type of dislocation reflects the internal flexibility of many proteins (Honig et al., 1976) and derives from a largely independent formation of structural regions in the molecule, which are later adjusted to an overall minimum energy conformation. In fact, McCammon et al. (1976) have recently calculated a force constant for the displacement of the two wings of lysozyme that are identified in this work.

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Selective Covalent Binding of Methionyl-Containing Peptides and Proteins to Water Insoluble Polymeric Reagent and Their Regeneration†

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ABSTRACT: A polymeric reagent of the type $\mathcal{P} \sim$ NHCOC$_2$H$_5$Cl (where $\mathcal{P}$ is Bio-Gel P-100) was prepared. This polymer covalently bound peptides and proteins specifically at methionine residues, under acidic conditions in the presence of a small amount of sodium iodide. Treatment of the polymer-peptide conjugate with 2-mercaptoethanol resulted in essentially complete removal of the peptide with regeneration of intact methionyl residues. In an alternative way, the polymer was suspended for 2 h in boiling water. This treatment resulted in the conversion of the bound methionyl residues to homoserine residues and cleavage and liberation of the bound peptides. The polymeric reagent was successfully applied to the separation of methionyl peptides from peptide mixtures and for specific covalent binding of enzymes and biologically active proteins via their exposed methionyl residues, with the retention of their biological activity.

Gundlach et al. (1959) and Stark and Stein (1964) showed that alkylating agents react with methionyl residues in peptides and proteins over a wide pH range and may be considered as specific agents toward methionine at a low pH where other reactive groups are protonated. The products formed, methionylsulfinium salts, are generally stable to performic acid oxidation (Neumann et al., 1962) and cyanogen bromide cleavage (Spande et al., 1970) but decompose on acid hydrolysis to give a variety of products (Gundlach et al., 1959; Goren et al., 1968). S-Carboxamidomethyl (CM)1-methionylsulfonium peptides undergo quantitative cleavage with formation of homoserine lactone on heating in a sealed tube for 2 h (Tang and Hartley, 1967). Naider and Bobak (1972) have shown that reaction of methionine sulfonium derivatives with several nucleophiles, particularly with sulfur nucleophiles, results in the regeneration of intact methionyl residues.

† Abbreviations used: 8CM-lysozyme, lysozyme derivative in which all four disulfide bonds were reduced and carboxymethylated; CM, S-carboxamidomethyl; Z, benzyloxycarbonyl; BAEE, N-acetyl-L-arginine ethyl ester; ATEE, N-acetyl-L-tyrosine ethyl ester; Chloramine-T, N-chloro-p-toluenesulphonamide; GSH, glutathione; $\mathcal{P}$-NHCOC$_2$H$_5$Cl, Bio-Gel P-100 derivative in which 2-chloroacetic acid was covalently attached; N-MeOt-Aba, a-MeOt-a-lactalbumin, a-lactalbumin derivative in which the single methionyl residue was oxidized to methionine sulfoxide; 2-MeOt-Kutzyn-trypsin inhibitor, Kutzyn trypsin inhibitor derivative in which both methionyl residues were oxidized to methionine sulfoxide; 1-MeOt-ribonuclease, ribonuclease derivative in which Met-29 was oxidized to methionine sulfoxide; $\mathcal{P}$-Met, Met, covalently linked through its methionyl residue to $\mathcal{P}$-NHCOC$_2$H$_5$Cl (the same kind of abbreviation is used for free methionine or other methionyl peptides, e.g., $\mathcal{P}$-Met); Hse, homoserine; RT, room temperature; DEAE, diethylaminoethyl.