Role of an Intrachain Disulfide Bond in the Conformation and Stability of Ovalbumin

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Ovalbumin, which contains one intrachain disulfide bond and four cysteine sulfhydryls, was reduced with dithiothreitol under non-denaturing conditions, and its conformation and stability were compared with those of the disulfide-bonded form. The CD spectrum in the far-UV region revealed that the overall conformation of the reduced form is similar to that of the disulfide-bonded one. Likewise, the inaccessibility to trypsin and the non-reactivity of the four cysteine sulfhydryls, exhibited by the native disulfide-bonded ovalbumin, were still retained in the disulfide-reduced form. Thus, the reduced ovalbumin appeared to substantially take the native-like conformation. However, the near-UV CD spectrum slightly differed between the native and disulfide-reduced forms. Protein alkylation with a fluorescent dye and subsequent sequence analysis showed that the two sulfhydryls (Cys73 and Cys120) originating from the disulfide bond are highly reactive in the reduced form. Furthermore, upon proteolysis with subtilisin, the N-terminal side of Cys73 was cleaved in the reduced form, but not in the disulfide-bonded one. Upon heat denaturation, the transition temperature of the reduced form was lower, by 6.8°C, than that of the disulfide-bonded one. Thus, we concluded that ovalbumin has a native-like conformation in its disulfide-reduced form, but that the local conformation of the reduced form fluctuates more than that of the disulfide-bonded one. Such local destabilization may be related to the decreased stability against heat denaturation.

The "serpins" are a superfamily of homologous proteins that are believed to have developed from a common ancestral protein (1). Most of the proteins retain the original serine proteinase inhibitor activities (α-antitrypsin, α,-antichymotrypsin, antithrombin III, etc.), but the rest have lost the proteinase inhibitor functions (ovalbumin, angiotensinogen, and thyroxine binding protein). Although the homology among the superfamily is about 30%, all members have been hypothetically deduced to have a similar conformation to α-antitrypsin, a typical serine proteinase inhibitor, this protein in its nicked form, being the only example in which the molecular structure has been constructed based on the results of X-ray crystallographic analysis (2). Very recently, however, the three-dimensional structures of both the native (3) and nicked forms of ovalbumin have been resolved (4), thereby the structural similarity to α-antitrypsin being confirmed. Since ovalbumin has no inhibitor activity, the egg white protein, along with α-antitrypsin, is a useful model protein for the study of the relationship between the structures and activities of the serine proteinase inhibitors.

Ovalbumin has an intramolecular disulfide bond, which is not a common feature of the serpins; α-antitrypsin contains no intramolecular disulfide (1). The role of the disulfide bond has not been established in relation to the stability and conformation of ovalbumin. Generally, small disulfide proteins consisting of a single domain take a fully unfolded or native-like conformation in their disulfide-reduced forms. Bovine pancreatic trypsin inhibitor and RNase A unfold upon the reduction of their intramolecular disulfides (5, 6); in contrast, the disulfide-reduced forms of the Fc fragment of immunoglobulin and human growth hormone take conformations indistinguishable from their disulfide-bonded forms (7, 8). With regard to a multi-domain protein, we have shown that the fully reduced form of ovotransferrin takes an intermediate conformation between the native and unfolded ones (9, 10).

In the present study, we examined the effect of disulfide reduction on the conformation and stability of ovalbumin. The overall conformation of the reduced form of ovalbumin was evaluated as to the CD spectrum, resistance to protease digestion, and reactivity of cysteine sulfhydryls toward alkylation reagents. Here we report that the disulfide-reduced form of ovalbumin takes a native-like conformation, except that the local conformation around the two sulfhydryl groups originally involved in the disulfide bond is slightly loosened in the reduced form.

MATERIALS AND METHODS

Materials—Ovalbumin was purified from fresh egg white by crystallization in an ammonium sulfate solution (11) and recrystallized three times. Dephosphorylated ovalbumin was prepared by acid phosphatase treatment and ion-exchange chromatography as described elsewhere (12). Trypsin and subtilisin-BPN' were purchased from Sigma. The reduced form of ovalbumin was prepared under...
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non-denaturing conditions by incubating the native protein at 1.0 mg/ml with 15 mM dithiothreitol (DTT) at 37°C for 2 h in Buffer A (50 mM Tris-HCl, pH 8.2/1 mM Na2EDTA).

Determination of the Number of Reactive Cysteine Sulfhydryl Groups by Polyacrylamide Gel Electrophoresis—The numbers of disulfide bonds and reactive sulfhydryl groups in dephosphorylated ovalbumin were determined by selective two step alkylation and subsequent PAGE, as described before (13). Briefly, as the first step, dephosphorylated ovalbumin was alkylated by incubation with 50 mM iodoacetamide (IAA) or 15 mM 5-[2-[[iodoacetyl]-amino]ethyl]aminonaphthalene-1-sulfonic acid (IAEDANS) at 37°C for 10 min in the presence and absence of 8 M urea. Ovalbumin was co-precipitated with a carrier protein (alkylated l-lysylase) in cold acetone/1 N HCl (98:2), washed three times with acetone/1 N HCl/H2O (98:2:10), dissolved in Buffer B (8.0 M urea/50 mM Tris-HCl, pH 8.2/1 mM Na2EDTA), and then fully reduced by incubation with 3.5 mM DTT at 37°C for 30 min. As the second step, the fully reduced sample was alkylated by incubation with 10 mM iodoacetamide (IAM) at 37°C for 10 min. After the second step alkylation, the samples were resolved by urea-denaturing PAGE.

Identification of Reactive Sulfhydryl Groups—The locations of reactive sulfhydryls in the reduced form of ovalbumin were determined by sequence analysis after protein alkylation with IAEDANS and fragmentation with trypsin, followed by gas-phase sequencing. The reduced form of dephosphorylated ovalbumin (0.5 mg) was alkylated with 15 mM IAEDANS at 37°C for 10 min in 1 ml of Buffer A containing 5 mM DTT. Excess IAEDANS was trapped by incubation with 18 mM DTT at 37°C for 5 min. The alkylated ovalbumin was mixed with 10 volumes of cold acetone/1 N HCl/H2O (98:2:10), kept at −20°C for 1 h, precipitated by centrifugation (3,000 x g, 10 min), and then washed three times with cold acetone/1 N HCl/H2O (98:2:10). The alkylated protein was dissolved in 120 μl of 0.1 M Tris-HCl buffer (pH 8.2) containing 6 M urea and then incubated at 50°C for 10 min. After the urea concentration had been decreased to 2 M by the addition of 240 μl of 0.1 M Tris-HCl buffer (pH 8.2), digestion was immediately performed at 30°C for 3 h with trypsin at an ovalbumin to protease ratio of 100:1 (w/w). The mixture (120 μl) was applied to a reverse phase HPLC column (YMC AP-302) and peptides were eluted with an acetonitrile linear gradient (0-80%) in 0.1% trifluoroacetic acid (pH 2.0). Peptide peaks exhibiting IAEDANS fluorescence (excitation, 340 nm; emission, 510 nm) were collected and further purified by the same reverse phase HPLC with an acetonitrile linear gradient (20-80%) in 0.5 M triethylamine/acetic acid buffer (pH 5.0). The purified peptides were subjected to amino acid composition analysis with an amino acid analyzer (Hitachi, model 835-30) and primary sequence analysis with a gas-phase protein sequencer (Applied Biosystems, model 477A/120A). CD Spectral Measurement—CD spectra were recorded with a J-500C spectropolarimeter (JASCO). The far-UV CD spectra of the native and reduced ovalbumin were measured at 1 mg/ml in 20 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM Na2EDTA, with a 0.1-mm cell, in the wavelength range of 190 to 250 nm. DTT in the reduced ovalbumin solution was removed on a gel filtration column (PD-10, Pharmacia-LKB-Biotechnology) prior to CD measurements. The near-UV CD spectra (250 to 340 nm) of the native and reduced ovalbumin were measured at 1.0 mg/ml in 50 mM Buffer A, with a 10 mm cell. The far-UV CD spectral data were expressed in mean residue ellipticity (degree-cm²/decimol) and the near-UV CD spectral ones in molar ellipticity.

Susceptibility to Protease Digestion—Since the native, disulfide-bonded form of ovalbumin is highly resistant to trypsin, we used its susceptibility to the protease as a probe for evaluation of the conformation of the disulfide reduced form. Ovalbumin, in the disulfide-bonded or disulfide-reduced form, was incubated at 0.2 mg/ml with various concentrations (0-0.5 mg/ml) of trypsin at 25°C for 1 min. The digestion was stopped by incubation at 0°C for 5 min with soybean trypsin inhibitor, the concentration of which was twice that of the protease. The proteins in SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 7.0/1% SDS/10% glycerol/20 mM 2-mercaptoethanol) were pretreated in a boiling water bath for 2 min, electrophoresed on a SDS polyacrylamide gel (10% polyacrylamide monomer/0.27% N,N'-methylenebisacrylamide) according to the standard method of Laemmli (14), and then stained with Coomassie Brilliant Blue R250. The amount of trypsin-resistant ovalbumin was determined from the band intensity, which was measured with a densitometer (Shimadzu CS-910). The susceptibility of the reduced ovalbumin was also examined using subtilisin. The ovalbumin solution (1.0 mg/ml) was incubated in Buffer A with 0.125 μg/ml of subtilisin (subtilisin-BPN' type V) at 25°C, in a total volume of 20 μl. After various times, proteolysis was stopped by the addition of an equal volume of 1.0% trifluoroacetic acid solution. The mixture was dried in vacuo, dissolved in 100 μl of the SDS-PAGE sample buffer, and then pretreated in a boiling water bath for 2 min. A part (0.1 volume) of the mixture was analyzed by SDS-PAGE in the same way, except that 15% polyacrylamide/0.4% N,N'-methylenebisacrylamide gel was used.

N-Terminal Analysis of Proteolytic Fragments—The disulfide-reduced ovalbumin (1.0 mg/ml) was incubated in Buffer A with 0.5 μg/ml of subtilisin at 25°C for 3 h, in a total volume of 250 μl, and then mixed with an equal volume of a 1% trifluoroacetic acid solution. The sample was dried in vacuo, dissolved in 250 μl of the SDS-PAGE sample buffer, pretreated in a boiling water bath, and then electrophoresed on a 10% polyacrylamide gel (13.5 × 13.5 × 0.1 cm).

The protein was transferred to a polyvinylidene difluoride membrane (Millipore, Immobilon) with a semi-dry transfer unit (Sartoblot II S) as described (15). The proteins blotted onto the membrane were stained with 0.1% Ponceau solution. After washing with a 1% acetic acid solution and distilled water, the membrane was dried. The protein bands were cut and subjected to sequence analysis with the protein sequencer.

Differential Scanning Calorimetry—The ovalbumin solution (1.0 mg/50 μl) in 20 mM potassium phosphate buffer, pH 7.0, was placed in a silver pan, and DSC thermograms were recorded with a differential scanning calorimeter attached to a thermal analysis station (Seiko Instruments, DSC100). The rate of heating was 1°C/min.
RESULTS

Effect of Disulfide Reduction on the Reactivity of Sulfhydryl Groups—Native ovalbumin was incubated with DTT under non-denaturing conditions, and the extent of disulfide reduction was evaluated by a method comprising two-step alkylation and PAGE, which enables the estimation of protein species with different numbers of free sulfhydryls (13). As shown in Fig. 1 (lane f), when ovalbumin had been pretreated with 15 mM DTT at 37°C for 2 h, all the six half-cystines were detected as reactive sulfhydryl groups in the presence of 8 M urea in the first step of alkylation. The disulfide-intact ovalbumin showed four reactive sulfhydryl groups (Fig. 1, lane c). These numbers demonstrate that the disulfide bond in ovalbumin is completely cleaved under the non-denaturing pretreatment conditions.

Under non-denaturing conditions the four cysteine sulfhydryls in disulfide-bonded ovalbumin are all non-reactive to alkylation reagents, such as IAA (13). The reactivity, under non-denaturing conditions, of the six sulfhydryls of the reduced ovalbumin was analyzed by the same PAGE method. When ovalbumin was incubated in the first alkylation step with IAA, only one of the six sulfhydryl groups of the disulfide-reduced protein was modified by the anionic alkylation reagent, but no reactive sulfhydryl was detected in the disulfide-intact protein (Fig. 1, lanes e and b). When the reduced form of ovalbumin was incubated with another anionic alkylation reagent, IAEDANS, a protein species with two introduced IAEDANS molecules as well as one with no introduced IAEDANS molecule was detected (Fig. 1, lane h). Although the reason why the introduced number of molecules differs between the two anionic alkylation reagents is not clear, the incubation of the reduced form with IAA and IAEDANS in the first-step alkylation resulted in the introduction of two alkylation reagent molecules; no protein species with three introduced alkylation reagent molecules was detected (Fig. 1, lane j). Thus, it can be concluded that two cysteine sulfhydryls are reactive in the reduced form of ovalbumin.

Identification of the Reactive Sulfhydryl—To determine the locations of the reactive sulfhydryl groups in the ovalbumin sequence, we introduced the fluorescent probe, IAEDANS, into the reduced ovalbumin under non-denaturing conditions. The modified protein was digested with trypsin and peptides were purified by reverse phase HPLC. As shown in Fig. 2, four peaks showed IAEDANS fluorescence. Each of the four fluorescent peak materials was further purified by rechromatography by the same reverse phase HPLC. The four peaks were first characterized by amino acid analysis. Peak 1 was found to be a non-peptide substance, since no amino acid was detected in the peak. The complete sequences of the other three peak materials were determined, from their N-terminals to C-terminals, to be as follows:

Peak 2: FDKLPGFGDSIEAQXGTSVNVHSSLR
Peak 3: YPILPEYLQXVK
Peak 4: LYAEERYPILPEYLQXVK

In the light of the established primary structure (16), peak 2 corresponds to the fragment, Phe59-Arg84. In this fragment, Cys73 is included. Therefore, the X in peak 2 can be taken to be Cys73. Peaks 3 and 4 correspond to Tyr11-Lys122 and Leu105-Lys122, respectively. Thus, the Xs in peaks 3 and 4 should be Cys120. Cys73 and Cys120 are both involved in the
The CD spectrum of the disulfide-intact (solid line) and disulfide-reduced (dotted line) ovalbumin were measured in the far-UV region (185-250 nm; panel A) and in the near-UV region (250-340 nm; panel B).

Resistance to Trypsin Digestion—Although ovalbumin contains twenty lysine and fifteen arginine residues, it is highly resistant to trypsin in its native disulfide-bonded state (18). Heat-denatured ovalbumin, however, is quite sensitive to trypsin digestion (19). Thus, trypsin resistance can be regarded as a probe for the compactness of an ovalbumin molecule. To evaluate the overall protein conformation, we examined the resistance to trypsin digestion between the native and reduced ovalbumin. In contrast, in the near-UV region, the CD spectrum of the disulfide-reduced ovalbumin showed slightly reduced ellipticity at 260, 265, and 275 nm, but the wavelengths of the extrema remained unchanged in comparison to in the case of the disulfide-bonded form (Fig. 3B). Both the positive peak at 290 nm and the negative peak at 305 nm were almost exactly the same for the two forms of ovalbumin.

Fig. 4. Trypsin digestion of ovalbumin. The native (lanes a, b, c, and d) and the reduced (lanes e, f, g, and h) ovalbumin were incubated at 0.2 mg/ml in Buffer A (pH 8.2) with trypsin at 25°C for 1 min. The ratios (w/w) of trypsin to ovalbumin were 0 for lanes a and e, 0.25 for lanes b and f, 0.5 for lanes c and g, and 2.5 for lanes d and h. The reaction was stopped by incubation with soybean trypsin inhibitor. The samples were resolved by SDS-PAGE and stained with Coomasie Brilliant Blue. The open arrow indicates the limit of migration; the closed arrows, OVA and TI complex, indicate the positions of intact ovalbumin, and the complex of trypsin and trypsin inhibitor, respectively.

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Fig. 5. Susceptibility of ovalbumin to subtilisin. The disulfide-intact (panel A) and the disulfide-reduced (panel B) ovalbumin were incubated at 1.0 mg/ml with 0.125 μg/ml of subtilisin-BPN' for 0 (lane a), 1 (lane b), 5 (lane c), 30 (lane d), 60 (lane e), 90 (lane f), 120 (lane g), 150 (lane h), or 180 min (lane i). The reaction was terminated by the addition of an equal volume of 1% TFA. The proteins were electrophoresed on a 15% polyacrylamide gel and then stained with Coomasie Brilliant Blue. The molecular masses of the fragments were estimated from their migration positions using molecular weight markers (glutamate dehydrogenase, 55.4 kDa; lactate dehydrogenase, 36.5 kDa; soybean trypsin inhibitor, 20.1 kDa; cytochrome c, 12.5 kDa; bovine pancreatic trypsin inhibitor, 6.5 kDa).
of the disulfide-reduced form of ovalbumin. As shown in Fig. 4 (lanes b–d), the disulfide-intact ovalbumin appeared completely resistant to proteolysis with 2.5-fold trypsin (w/w). Surprisingly, the reduced ovalbumin was also resistant to the same high concentration of trypsin (Fig. 4, lanes f–h). These findings were confirmed by densitometric analysis: the original amounts of both the native and the reduced ovalbumin were completely retained during the trypsin digestion. Thus, in terms of trypsin resistance, the conformation of the reduced ovalbumin was indistinguishable from that of the disulfide-bonded ovalbumin.

Susceptibility to Proteolysis by Subtilisin—The disulfide-bonded ovalbumin is hydrolyzed by a serine proteinase, subtilisin, at specific sites to generate a nicked form, which is called plakalbumin (18). The cleavage sites are the peptide bonds on the C-terminal sides of Glu346, Asp350, and Ala352, accordingly, plakalbumin is a nicked form consisting of AcGly1–Glu44 (42.0 kDa) and Ser352–Pro365 (3.7 kDa), lacking the hexapeptide (Ala352–Ala365). The time course of the proteolysis of ovalbumin was monitored by SDS-PAGE. Under the present conditions, we confirmed that the disulfide-bonded ovalbumin was hydrolyzed into two large fragments of 42.0 and 3.7 kDa (Fig. 5, panel A). These two fragments were stable on prolonged incubation for 3 h. Furthermore, on sequence analysis of a sample that had been digested for 3 h and then passed through a Sephadex G-25 column to remove a low molecular weight fragment, only the N-terminal sequence of Ser-Val-Ser-Glu-Glu-Phe-Arg-, which corresponds to the N-terminal sequence of the 3.7 kDa fragment, was detected, suggesting that the N-terminal amino acid of the 42.0 kDa fragment is an acetylated glycine.

As to the disulfide-reduced ovalbumin, subtilisin digestion proceeded in such a way that the temporal appearance of a 42.0 kDa fragment was followed by the accumulation of a 32.5 kDa fragment (Fig. 5, panel B). In addition to the same 3.7 kDa fragment, a fragment of 5.8 kDa was also generated. The data were consistent with the idea that the 42.0 kDa fragment is further digested into 32.5 and 5.8 kDa fragments in the reduced form of ovalbumin. To address this possibility, we analyzed the N-terminal sequence of the newly generated 32.5 kDa fragment, after the peptide had been transferred from the SDS-PAGE gel onto a polyvinylidene difluoride membrane. The N-terminal sequence of this fragment was determined to be X-Gly-Thr-Ser-Val-, which can be found in the known sequence at Cys73 to Val77. Thus, we conclude that in addition to the known cleavage sites in the disulfide-bonded ovalbumin, in the disulfide-reduced form, the N-terminal side of Cys73, which is involved in the disulfide bond in the native ovalbumin, is also susceptible to subtilisin.

**Thermal Stability**—The preceding data indicate that the conformational change induced by the disulfide reduction may be locally limited to the region close to the original disulfide bond. We examined, by differential caloriometry, whether or not such local destabilization affects the overall stability against heat denaturation. Figure 6 shows that the endothermograms of the disulfide-bonded and reduced ovalbumin were quite different from each other. The denaturation temperature of the disulfide-bonded form was 78.9°C, which was very close to the previous datum (20). The reduced ovalbumin was found to be less stable, by 6.8°C, than the disulfide-intact form.

**DISCUSSION**

Both the data as to the far-UV CD spectrum (Fig. 3, panel A) and the resistance to trypsin digestion (Fig. 4) show that ovalbumin takes a compact and native-like conformation in its disulfide-reduced state. Upon subtilisin digestion, however, the disulfide-bonded and the disulfide-reduced ovalbumin do not behave in the same way. The disulfide-bonded ovalbumin is partially cleaved by subtilisin, which yields a nicked form known as "plakalbumin"; there is at least one additional cleavage site in the reduced protein, which corresponds to the N-terminal side of Cys73. This cysteine sulphydryl is originally disulfide-bonded with Cys120 in the native ovalbumin. The acquired susceptibility to subtilisin suggests local destabilization of the conformation of the reduced protein. Essentially the same conclusion can be drawn from the data as to the reactivity of the sulphydryl groups toward IAEDANS: only the two cysteine sulphydryls, Cys73 and Cys120, which are disulfide-bonded in the native protein, reacted with this alkylation reagent. These two cysteine groups are separated in the three-dimensional structure from the other four cysteines, each of which is involved in helix A, helix B, strand 4 in β-sheet B, and strand 5 in the same β-sheet, respectively (4). These secondary structural units, that are included in the internal space, may be retained in the disulfide-reduced ovalbumin, so that the four free sulfhydryls are not reactive with the alkylation reagent. At present, it is not clear why a local sequence around Cys120 is insusceptible to subtilisin. It may be related to that Cys120, but not Cys73, is included in an α-helix (helix E) (4). If the helical conformation is retained in the disulfide-reduced protein, subtilisin, which has a greater molecular size than IAEDANS, may not be able to gain access to cleavage sites. The complete resistance to trypsin digestion can be explained by the absence of basic amino acid residues around Cys73. Lys122, which is the basic amino acid residue nearest to Cys120, is also involved in helix E.

The local destabilization may be related to the slight difference in the near-UV CD spectrum between the
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reduced and native proteins (Fig. 3, panel B). The positive peaks at 260, 265, and 275 nm are slightly decreased, but both the positive shoulder peak at 290 nm and the negative peak at 305 nm are retained in the reduced form. Previously, the latter two peaks were assigned to the three tryptophan residues in ovalbumin, Trp148, Trp184, and Trp267 (21, 22). These three tryptophan residues are all separated from Cys135 and Cys186 in the three-dimensional structure (4). Trp148 is included in helix F, which is separated from both the half-cystines by ß-sheet A. Trp184 is located in the loop between strand 3 in ß-sheet A and helix F1, and this loop is located almost at the opposite pole to the two half-cystines. Trp267 included in helix H is separated from the two half-cystines by helix A. Thus, it is very likely that the circumstances of the three tryptophan residues, which are separated from the two half-cystines, are indistinguishable between the native and reduced proteins. Although the contribution of the disulfide bond in the near-UV CD spectrum has not been established for ovalbumin, the slight ellipticity difference observed at 260, 265, and 275 nm between the two forms of ovalbumin might be accounted for by local conformational destabilization around some other aromatic side chains. Four tyrosines, Tyr106, Tyr111, Tyr117, and Tyr125, which appear to be close to the disulfide bond in the three-dimensional structure (4), could be candidates for such aromatic residues.

An archetypical serpin, ß1-antitrypsin, undergoes the proteolytic cleavage of its reactive center (Met358-Ser359) during the expression of its inhibitory activity. This results in a large scale conformational change, including the incorporation of the newly generated C-terminal (Met358) into the major ß-sheet (2). Ovalbumin, which has no inhibitor activity, is also cleaved by subtilisin at a putative reactive center (Ala352-Ser353) (18). Ovalbumin, however, does not undergo such a large scale conformational change upon proteolytic cleavage (3, 4, 23, 24). The difference between these two proteins might be explained by the presence of the disulfide bond in ovalbumin, but not in ß1-antitrypsin, since this bond is located adjacent to the major ß-sheet (4). In the present study, however, the conformation of the nicked ovalbumin could not be compared between the disulfide-bonded and disulfide-reduced proteins, because an alternative truncated form was generated in the case of the disulfide-reduced ovalbumin (Fig. 5, panel B). The question of whether or not a large conformational change occurs in the disulfide-reduced ovalbumin will not be answered until the same nicked form is prepared for the disulfide-bonded and disulfide-reduced proteins.

In conclusion, the disulfide bond in ovalbumin is not essential for its overall conformation, but affects, to a limited extent, its local conformation around the two half-cystines that are involved in the disulfide bond in the native protein. Such local destabilization may be reflected in the decreased stability against heat denaturation, as revealed on differential scanning calorimetry, although the free energy change for denaturation could not be quantitatively determined because of the lack of reversible denaturation of this protein (25). The present locally destabilized conformation should be differentiated from the “Molten-globule”-like state that has been found for disulfide-bonded ovalbumin at acid pH (26), since at the extreme pH the near-UV CD is almost completely diminished (26). In addition, no difference in intrinsic viscosity was observed between the native and disulfide-reduced ovalbumin (unpublished data). We, therefore, believe that the disulfide-reduced form of ovalbumin, at neutral pH, takes a native-like compact conformation.

REFERENCES