A Model for Oxidative Modification of Glutamine Synthetase, Based on Crystal Structures of Mutant H269N and the Oxidized Enzyme

Shwu-Huey Liaw,[‡] Joseph J. Villafranca,[§,⊥] and David Eisenberg*[,‡]

Molecular Biology Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024, and Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Received February 17, 1993; Revised Manuscript Received April 28, 1993

ABSTRACT: Proteolytic degradation of glutamine synthetase (GS) in *Escherichia coli* is known to follow "marking" by oxidative modification. At an early stage of the degradative pathway, oxidation of His 269 and Arg 344 abolishes GS enzymatic activity. We propose a mechanism for the early stage of oxidative inactivation of GS on the basis of the crystal structure of H269N and tryptophan fluorescence spectra of H269N and H269NR344Q: (1) Oxidation of Arg 344, adjacent to the n_2 metal ion site, decreases ATP binding. (2) Oxidation of His 269 to Asn destroys the n_2 site, consistent with the function of His 269 as a ligand for the n_2 metal. (3) Loss of Mn^{2+} at the n_2 site destroys the integrity of the ATP binding site. (4) Destruction of the ATP site results in the observed low enzymatic activity of H269N and H269NR344Q. During later stages of oxidative modification, the n_1 metal ion site is destroyed and the active site of the enzyme becomes flexible as suggested by X-ray data collected from an oxidized crystal of GS. Thus, studies of mutant and oxidized enzymes confirm that there are at least two stages of oxidative modification of GS. These studies suggest that the early modification occurs at the n_2 metal ion site, eliminating enzyme activity, and the later modification occurs at the n_1 metal ion site, relaxing the GS structure, perhaps enabling proteolytic degradation. These studies also illuminate the differing roles of the two bound metal ions: the tightly bound n_1 ion enhances the stability of the catalytically active conformation, and the less tightly bound n_2 ion participates in ATP binding.

Levels of intracellular proteins, reflecting a balance between the rate of synthesis and the rate of degradation, are regulated by the nutritional state of organisms. A mechanism for regulation of these levels has been proposed by Stadtman, Levine, and co-workers (Levine, 1985; Stadtman & Oliver, 1991; Stadtman, 1992). First, proteins are "marked" by oxidative modification which abolishes activity and also renders proteins susceptible to proteolytic attack. Second, proteases of strict specificity degrade the marked protein but not the native form. When degradation systems fail, as in aging (Oliver et al., 1987a; Starke et al., 1987) and some pathological processes (Garland et al., 1986; Oliver et al., 1987b; Chapman et al., 1989), oxidized proteins accumulate.

Past studies of GS[1] oxidation have suggested the following: (1) At least two residues, His 269 and Arg 344, are oxidized in the presence of both oxygen and Fe^{2+} to Asn and γ-glutamyl semialdehyde, respectively, during initial oxidation. This destroys catalytic activity (Farber & Levine, 1986; Climent et al., 1989). (2) Oxidation of other residues during long-term exposure, including one more His residue (Rivett & Levine, 1990), is required to increase the susceptibility of GS to specific proteases which have been isolated from mammalian tissues (Rivett, 1985) and from *Escherichia coli* (Roseman & Levine, 1987). Here we propose mechanisms for the oxidative destruction of GS activity and for the oxidative induction of conformational relaxation leading to proteolysis. These proposals are based on studies of the engineered *E. coli* GS mutants H269N and R344Q, the double mutant H269NR344Q, and the oxidized GS of *Salmonella typhimurium* by X-ray crystallographic and fluorometric methods.

MATERIALS AND METHODS

Purification of Mutant GS. Mutant *E. coli* cells, strain YMC21E, were prepared by Mr. J. Yanchunas at the Pennsylvania State University. Mutant proteins were purified as native GS by ammonium sulfate precipitation and a Cibracon Blue affinity column (Liaw, 1992). Mutants are fully unadenylylated due to the lack of adenylyltransferase in this strain. H269N displays a biosynthetic activity of 4.1 units/mg, 1/30-fold the native enzyme activity, whereas the double mutant H269NR344Q in which His 269 is replaced by Asn, and Arg 344 by Gln, displays a biosynthetic activity of 0.12 unit/mg.

Tryptophan Fluorescence Spectroscopy. Tryptophan fluorescence spectra were recorded using a Perkin-Elmer luminescence spectrometer LS50 equipped with an Epson recorder. All fluorescence spectra were measured at room temperature. The buffer solution was 15 mM imidazole–HCl (pH 7.0) and 2.2 mM $MnCl_2$. Fluorescence emission spectra were recorded from 310 to 450 nm by excitation at 300 nm.

Crystal Growth. Crystallization conditions of H269N from *E. coli* are similar to those of native GS from *S. typhimurium* (Liaw et al., 1993). Crystals are grown by the hanging drop method of vapor diffusion. Drops are prepared by combining 10 μL of 30 mg/mL protein in 15 mM imidazole–HCl (pH 7.0), 2.2 mM $MnCl_2$, and 600 μM NaATP, with 10 μL of precipitant solution, containing 2 mM 2-methyl-2,4-pentanediol (MPD) and 1.4 mM spermine tetrahydrochloride.

* To whom correspondence should be addressed.
‡ University of California.
§ The Pennsylvania State University.
⊥ Present address: Bristol Meyers Squibb, P.O. Box 4000, Princeton, NJ 08543-4000.

[1] Abbreviations: GS, glutamine synthetase; MPD, 2-methyl-2,4-pentanediol; MetSox, L-methionine *S*-sulfoximine.

0006-2960/93/0432-7999$04.00/0

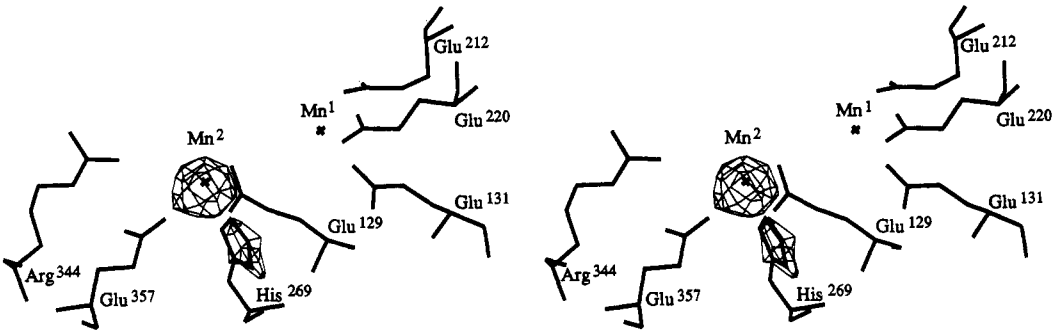

FIGURE 1: The 12-fold averaged Fourier difference map $F_{o,H269N} - F_{o,nat}$ at the 2σ contour level. A single significant negative peak is observed at the 3σ contour level, showing the absence of Mn^{2+} at the n_2 site. The peak next to the strongest one shows that half of the imidazole ring of His 269 is missing because the peak represents Asn minus His. This map suggests that oxidation of His 269 to Asn destroys the n_2 site.

The reservoir is a 2% (v/v) solution of MPD. Crystals measuring 0.7 mm × 0.5 mm × 0.2 mm grow within 1 month at 21 °C and are stable for at least 3 months.

Iron-Catalyzed Oxidation of Native GS Crystals. Fully unadenylylated crystals of GS from *S. typhimurium* (native GS) were grown. Ascorbate (pH 7.0) at a concentration of 15 mM and $FeCl_3$ at a concentration of 6 mM were dissolved in a synthetic mother liquor containing 15 mM imidazole–HCl (pH 7.0), 3 mM $MnCl_2$, 3 mM spermine tetrahydrochloride, and 10% MPD. Since sudden replacement of all mother liquor with the synthetic mother liquor impairs the diffraction quality of GS crystals, one-third of the real mother liquor containing $MnCl_2$ was replaced in five steps by this synthetic mother liquor containing $FeCl_3$. Thus, crystallization drops contain relatively large amounts of $FeCl_3$ and little of $MnCl_2$. Oxidized GS crystals turned yellow overnight, and X-ray data from these crystals were collected 30 h later. Both oxidized and native GS crystals were dissolved in the protein buffer containing 15 mM imidazole (pH 7.0) and 2.2 mM $MnCl_2$, and their glutamyltransferase activities were measured. The transferase activity of oxidized crystals is less than 0.1 unit/mg, whereas that of native GS crystals is 100 units/mg.

Data Collection and Electron Density Map Calculation. H269N and oxidized GS crystals are isomorphous with respect to the native enzyme, belonging to space group *C*2 with cell dimensions a = 235.5 Å, b = 134.5 Å, and c = 200.1 Å and β = 102.8° (Janson et al., 1984). X-ray data were collected from one crystal of each with a RAXIS-II (Rigaku), image plate detector at UCLA: 77 216 unique reflections ($F_o > 1\sigma$) were measured to 2.8-Å resolution with an R merge of 5.44%, representing 60% of the complete data for H269N, and 54 133 unique reflections ($F_o > 1\sigma$) were measured to 3.5-Å resolution with an R merge of 9.1%, representing 70% of the complete data for the oxidized crystal. We computed 12-fold averaged Fourier difference maps $F_{o,H269N} - F_{o,nat}$ at 2.8-Å resolution and $F_{o,ox} - F_{o,nat}$ at 3.5 Å using CCP4 programs (a suite of programs for protein crystallography, SERC, Daresburg Laboratory, Warrington, England) implemented on a DEC VAX4000. Phases of a 2.8-Å native GS model (Liaw, 1992) omitting metal ions were used in computing Fourier difference maps.

RESULTS

Inactivation of GS by Mutation. Genetic engineering studies showed that His 269 but not Arg 344 is crucial for GS activity (Abell et al., 1989, and unpublished observations). However, the near lack of enzymatic activity in H269NR344Q suggested the importance of Arg 344 for activity once His 269 is oxidized to Asn.

Detection of Metal Ions on GS. Divalent metal ions bind at two sites on each GS monomer. The more tightly binding site was called n_1 by Denton and Ginsburg (1969), and the less tightly binding site was called n_2. Since Mn^{2+} is the heaviest atom of GS, and since its affinity to GS is high, electron density maps of the types $F_o - F_o$, F_o, and $2F_o - F_c$ are sensitive indicators of the level of Mn^{2+} occupancy.

There is only one negative peak appearing at the 3σ contour level in the Fourier difference map $F_{o,H269N} - F_{o,nat}$, and it is at the n_2 site. This shows that the n_2 site is empty in H269N. This is consistent with our earlier finding (Yamashita et al., 1989) that His 269 is a ligand for the n_2 ion. A second negative peak appears nearby at the 2σ contour level, showing the difference of the imidazole ring of the His 269 residue and of the smaller Asn in H269N (Figure 1).

The strongest negative peak in the Fourier difference map $F_{o,ox} - F_{o,nat}$ at the 2σ contour level appears at the n_1 site. A weaker negative density peak appears at the 1.3σ contour level at the n_2 site. This difference map shows that no metal ions, neither Mn^{2+} nor Fe^{2+} nor Fe^{3+}, are bound to the n_1 or n_2 site (Figure 2). Thus, vacancies of metal ions in H269N and oxidized GS can be inferred from F_o and $2F_o - F_c$ maps in which electron density peaks are not observed at the n_2 site in H269N and at neither the n_1 nor n_2 site in oxidized GS. Moreover, $2F_o - F_c$ maps from oxidized crystals show no electron densities are observed for residues around the n_1 and n_2 sites, for example, the six protein ligands and three arginine residues, Arg 344, Arg 339, and Arg 359. This disorder of the active site in oxidized GS may be due to loss of the n_1 ion.

Tryptophan Fluorescence Measurements. Previous studies by Atkins and Villafranca (1992) on *E. coli* GS mutants suggested that ATP binding results in a conformational change at the Trp 57 region. We have used fluorescence enhancement (Timmons et al., 1974; Fisher & Stadtman, 1992) to monitor ATP binding.

Addition of ATP to H269N enhances fluorescence by 20% (Figure 3). This fluorescence enhancement is only about one-fifth that displayed by the native form. This result suggests that loss of the n_2 ion results in some deficiency of the ATP binding site. One surprising result is that the addition of both ADP and spermine to H269N induces a 60% fluorescence enhancement, whereas the addition of ADP alone to H269N exhibits a 20% fluorescence increase (Figure 3). However, fluorescence enhancements induced by addition of ATP alone to H269N and by the addition of both ATP and spermine to H269N are similar (data not shown). Moreover, no fluorescence increase is induced by addition of ATP to H269-NR344Q, suggesting that the ATP binding site in H269-NR344Q is nearly destroyed.

Function of Arg 344. Participation of Arg 344 in ATP binding was suggested by protein engineering studies and the

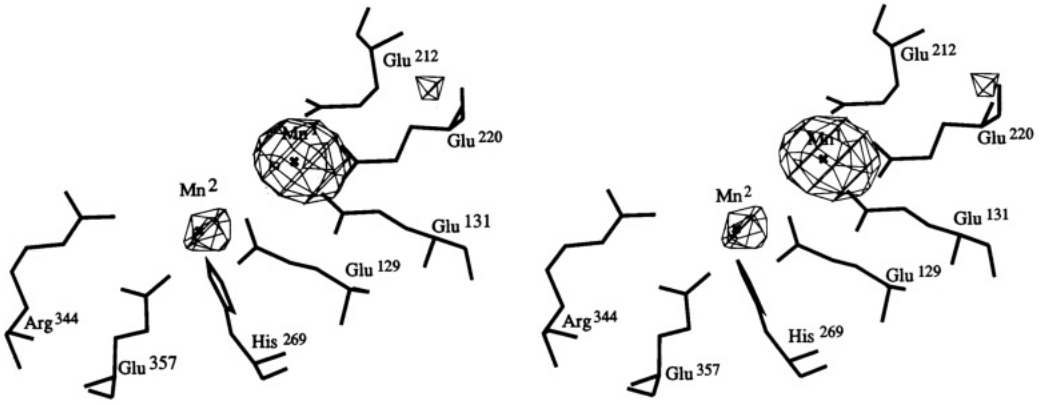

FIGURE 2: The 12-fold averaged Fourier difference map of oxidized GS ($F_{o,ox} - F_{o,nat}$) at the 1.3σ contour level. Notice a single negative peak at the 2σ contour level at the n_1 site; another negative peak is observed at the n_2 site at the 1.3σ level. This map suggests decreased binding of the n_1 and n_2 ions after oxidative modification.

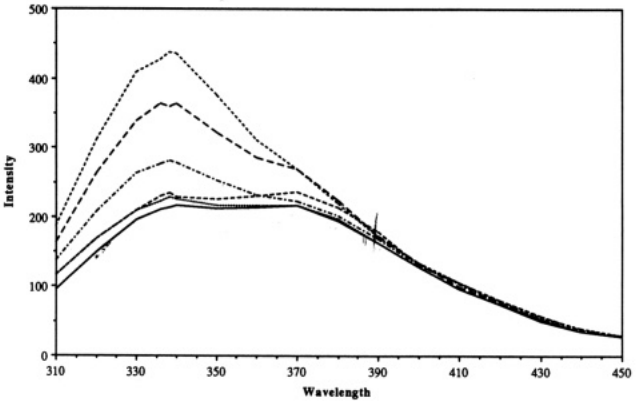

FIGURE 3: Tryptophan fluorescence spectra and enhancement of fluorescence emission induced by the addition of 2.5 mM ATP to native and mutant GS: from the bottom to the top, curve 1, fluorescence intensity of 0.7 μM GS–Mn; curve 2, fluorescence intensity of 0.7 μM H269N–Mn; curve 3, fluorescence intensity of 0.7 μM H269NR344Q–Mn + 2.5 mM ATP; curve 4, like curve 2, + 2.5 mM ATP; curve 5, like curve 2, + 2.5 mM ADP and 17 mM spermine tetrahydrochloride; curve 6, like curve 1, + 2.5 mM ATP. The interpretation of these fluorescence changes is given in the text.

GS structures. K_m values for ATP increase, from 1.4 to 240, 91.2, and 36.9 μM, respectively, as Arg 344 is replaced by His, Gln, and Lys (J. J. Villafranca, unpublished data). The smaller increase for K_m of R344K implies the importance of the positive charge at this residue. Comparison of fluorescence spectra of H269N and H269NR344Q also implies the involvement of Arg 344 in ATP binding (Figure 3). Direct evidence on the role of Arg 344 is provided by crystal structures of GS-AMP-PNP (an ATP analog, 5′-adenylylimide diphosphate) and GS-ADP (data not shown). These structures show interactions of positive charges at the n_2 site, the n_2 ion, and Arg 344 with β-phosphate groups of ADP and ATP. Thus, Arg 344 could compensate for loss of the n_2 ion to recover some activity in H269N. Also, interactions of Arg 344 with Glu 357 (Figure 5a) may be important for the stability of the active site since there are seven Glu residues and one Asp residue of the adjacent subunit within 10 Å of the n_2 site, in addition to the negatively charged substrates. Of the seven Glu residues, five serve as ligands for the two metal ions. Therefore, Arg 344 can neutralize in part negative charges of Glu 357 and ATP. The absence of both the n_2 ion and Arg 344 may somewhat destabilize the enzyme structure, perhaps increasing susceptibility to proteases (~25%) (Rivett, 1985; Rivett & Levine, 1990), and the loss of all of the n_1 and n_2 metals and Arg 344 may result in a less stable GS, increasing protease susceptibility (100%) (Rivett & Revine, 1990).

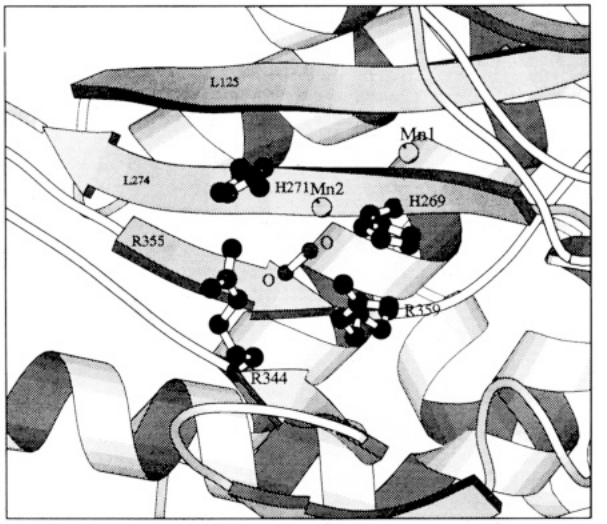

FIGURE 4: n_2 pocket of GS, the location of early oxidative modification, drawn by the Molscript program (Kraulis, 1991). His and Arg residues around the n_2 site: His 269, His 271, Arg 344, and Arg 359 are shown. To date only His and Arg residues have been found to be altered during oxidative modification of GS. Peroxide O–O is represented by a pair of spheres separated by 1.48 Å (labeled O), and is speculatively positioned between His 269 and Arg 344 which are the modified residues.

DISCUSSION

A Mechanism of Oxidative Inactivation of GS. Stadtman and co-workers have proposed a general mechanism for oxidative marking of proteins for proteolytic clearance (Stadtman & Oliver, 1991). Our structural studies of GS permit us to propose a more detailed model (Figure 5) for metal-catalyzed oxidation of His 269 to Asn and of Arg 344 to γ-glutamyl semialdehyde at the n_2 pocket. First Fe^{2+} replaces Mn^{2+} at the n_2 site. Then H_2O_2 interacts with the Fe^{2+}–GS complex. The adduct could extend across the n_2 pocket to Arg 344, whose guanidino group in our model is 3.5 Å from the n_2 site (Figures 4 and 5). The Fe^{2+}–peroxide complex could then dissociate into two reactive species, one being a $^{\bullet}HO$ radical and the other a Fe–O (ferryl ion). Both are extremely reactive, and because of the proximity to Arg 344 and His 269 (His 269 is 2.4 Å from the n_2 site), oxidation at these sites would occur rapidly. Multiple oxidations are required to cleave both the guanidino group of Arg and the imidazole group of His, and activated oxygen species such as those described above would be required to achieve this result (Figure 5c). After oxidative modification of His 269, the number of protein ligands to Fe^{2+} will have decreased by one, and Fe^{2+} can dissociate from the n_2 site (Figure 5d). The iron

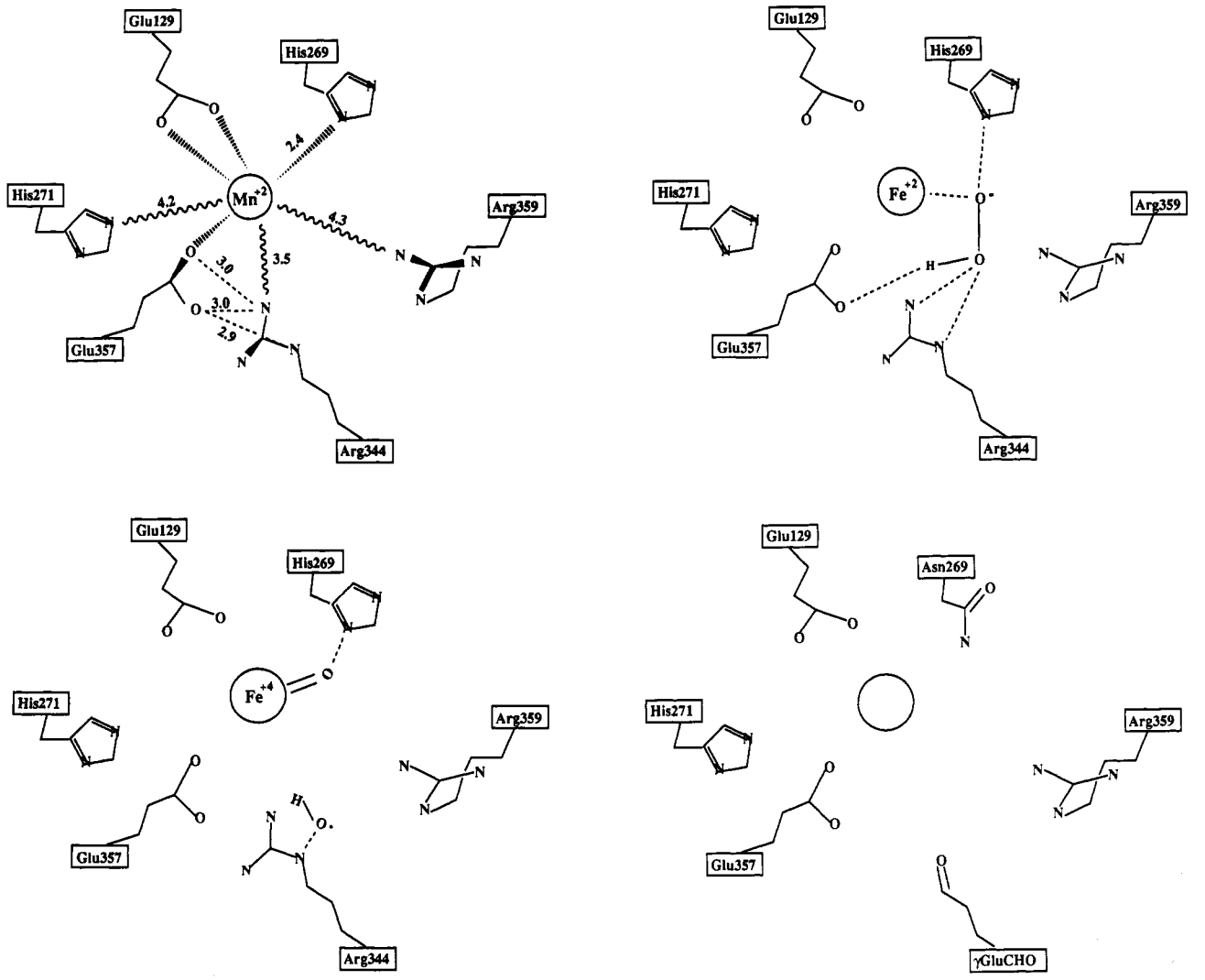

FIGURE 5: A model for metal-catalyzed oxidation of both His 269 and Arg 344. (a, top left) Mn^{2+} at the n_2 site and some surrounding amino acid residues are shown. Distances between oxygen atoms of Glu 357 and nitrogen atoms of Arg 344, displayed by dotted lines, are ~3 Å. (b, top right) Mn^{2+} is replaced by Fe^{2+}, and a model peroxide anion O–OH^- ligand is speculatively positioned between His 269 and Arg 344. (c, bottom left) An activated species, OH• radical, is proposed to dissociate and to interact with Arg 344. The superreactive Fe–O (ferryl ion) is proposed to attack His 269. (d, bottom right) His 269 and Arg 344 are oxidized into Asn and γ-glutamyl semialdehyde. Fe^{2+} is released from the protein (see text).

ion can then bind to another native GS molecule for another cycle of oxidation.

Release of Fe^{2+} from the n_2 site is supported by the absence of n_2 metals in H269N and in the oxidized GS crystal. The reason, of course, is that Asn is not as strong a metal ligand as His. This ion release is also supported by the known coordination chemistry of iron. Asn is not known to be a ligand for either Fe^{2+} or Fe^{3+} in any protein, but His is a common ligand for iron [see review by Howard and Rees (1991)].

Is There Oxidative Modification at the n_1 Site? The binding of MetSox (L-methionine *S*-sulfoximine) to the n_1 site has been observed to induce a fluorescence enhancement (Timmons et al., 1974). Identical fluorescence increases are induced by addition of MetSox to H269N and to native GS (data not shown). The crystal structure of H269N also shows that one Mn^{2+} ion still binds to the n_1 site and no significant conformational change is observed there. Moreover, H269N still possesses some activity although no metal ion is at the n_2 site. These results suggest that the n_1 site is still functional in H269N. Therefore, the n_1 site probably survives undamaged during the early stage of oxidative modification in which His 269 and Arg 344, both located at the n_2 pocket, are oxidized.

Studies of GS sampled after various times of oxidative modification have demonstrated loss of a second His residue (Rivett & Levine, 1990). There are five His residues around the active site: His 269 is a ligand for the n_2 site; His 271 points toward the n_2 site, ~4 Å away; His 210 points toward the n_1 site, ~5 Å away, whereas His 209 and His 211 point away from the n_1 site, about 12 and 10 Å away. Either His 210 or His 209 was speculated by Rivett and Levine (1990) to be the second oxidized His. There are four reasons to support His 210 as a better candidate for being the second oxidized His during long-time oxidation. First, oxidation at His 269 would deplete the reagent of high oxidation potential at the n_2 site. Second, as we have just argued, Fe^{2+} does not bind to the n_2 site after oxidation of His 269. Thus, the late oxidative modification is not expected to occur at the n_2 site, and His 271 would not be oxidized. Third, slow oxidation at the n_1 site seems more likely, because for oxidation to occur at the n_1 site, Fe^{2+} must replace Mn^{2+} or Mg^{+2}, and the affinity of Mn^{2+} and Mg^{+2} to the n_1 site is much higher than to the n_2 site (Ginsburg, 1972). As replacement occurs at the n_1 site, His 210 would be slowly altered, and then a lag-enhanced proteolytic susceptibility would be produced, as was observed by Rivett and Levine (1990). Fourth, the oxidized GS crystal

reveals decreased ion binding at both the n_1 and n_2 sites after extended oxidation (Figure 2) as was also observed by Levine and co-workers (Cervera & Levine, 1988; Rivett & Levine, 1990). Furthermore, engineered replacement of His 210 with Asp abolishes enzyme activity perhaps due to some conformational change induced by the negative charge (J. J. Villafranca, unpublished observation). Therefore, the n_1 site is probably damaged in "late oxidative modification", and His 210 is the most likely target.

Proteolytic Susceptibility and Conformational Changes during Oxidation. The lack of conformational change observed in the structure of H269N implies that H269 is not a good substrate for proteolytic degradation, even though the n_2 ion is absent. However, the oxidized GS crystal is more disordered than native GS or H269N, especially in the active site, resulting in lower resolution X-ray diffraction, suggesting that some conformational change occurs at the late stage of oxidative modification, e.g., relaxation of the GS structure, which we have just argued may involve loss of the n_1 ion. This conformational relaxation is supported by a weakening of GS subunit interactions by oxidation (Fisher & Stadtman, 1992). Also, the binding studies of $^{54}Mn^{2+}$ to GS by equilibrium dialysis showed that the binding of the first 12 Mn^{2+} (presumably at the n_1 site) appeared to induce a significant overall conformational change for the conversion of the "relaxed" GS (metal ion free) into the "tightened" GS (Shapiro & Ginsburg, 1968; Denton & Ginsburg, 1969). Moreover, dissociation, denaturation, and relaxation enhance the enzyme susceptibility to proteases (Rivett, 1985). Therefore, the loss of the n_1 ion, but not the n_2 ion, may destabilize the GS structure, enhancing its susceptibility to proteolysis (Rivett, 1985).

Structural Stability of GS Contributed by Metal Ions. The two metal ions are located at the active site of GS which is at the interface of two subunits in the same hexagonal ring (Almassy et al., 1986; Yamashita et al., 1989). Ginsburg and co-workers demonstrated the functions of the metal ions in GS (Ginsburg, 1972; Hunt et al., 1975). Our H269N structure confirms that the metal ion at the n_2 site is associated in ATP binding. The lower resolution X-ray diffraction of the oxidized GS crystal is consistent with the importance of the n_1 ion for stabilization of the GS structure.

Structural enhancement by the metal ions may be mainly due to their positive charges (that is, to electrostatic forces) instead of direct metal–protein bonding because none of the protein ligands reside at the subunit contact sites and because there is no direct bonding of metal ions themselves with the adjacent subunit. Interactions of positively charged metal ions with the five negatively charged Glu ligands, and with the substrates Glu and ATP, probably strengthen the active conformation.

Once GS loses its metal ions, the charge balance is shifted, and it might be expected that the GS structure would become more flexible, and that the negatively charged substrates Glu and ATP can no longer bind to GS. Ginsburg and co-workers termed the catalytically active GS "taut" in contrast to the inactive relaxed GS from which metal ions have been removed by EDTA (Ginsburg, 1972). Consistent with this hypothesis of the importance of electrostatic forces to the stability of GS, addition of more than 2 mM ATP or ADP to crystallization drops dissolves crystals, perhaps due to the added negative charges (Liaw et al., 1993).

The n_1 metal ion binding is much tighter than the n_2 ion binding by 100–1000-fold because the n_1 ion interacts with three Glu residues (three negative charges) whereas the n_2 ion interacts with only two Glu residues (two negative charges), and because interactions of positively charged residues, His 269, Arg 344, and His 271, with two Glu residues at the n_2 site may weaken ligation of Glu 129 and Glu 357 to the n_2 ion. These positively charged residues may compensate the absence of the n_2 ion for charge balance and permit ATP binding in H269N. At the n_1 site, His 210 is the only positively charged residue interacting with one of three Glu ligands. Thus, the n_1 ion enjoys stronger interactions with its ligand than does the n_2 ion. Moreover, there are no other positively charged residues near the n_1 site which can substitute for the n_1 ion in its roles of structural stability and Glu binding. In short, the n_1 ion is crucial for the stability of the GS structure.

Conclusion. The present studies on H269N and oxidized GS confirm the roles of His 269, Arg 344, and metal ions in GS oxidation. A mechanism is proposed for the oxidative modification of GS: oxidative inactivation is caused by loss of Arg 344 and the n_2 ion, and then a conformational relaxation is caused by destruction of the n_1 site.

REFERENCES

Abell, L. M., Keck, P. J., & Villafranca, J. J. (1989) *Biochemistry 28*, 1940.

Almassy, R. J., Janson, C. A., Hamlin, R., Xuong, N.-H., & Eisenberg, D. (1986) *Nature 323*, 304–309.

Atkins, W. M., & Villafranca, J. J. (1992) *Protein Sci. 1*, 342–355.

Cervera, J., & Levine, R. L. (1988) *FASEB J. 2*, 2591–2595.

Chapman, M. L., Rubin, B. R., & Gracy, R. W. (1989) *J. Rheumatol. 16*, 15–18.

Climent, I., Tsai, L., & Levine, R. L. (1991) *Arch. Biochem. Biophys. 289*, 371–375.

Denton, M. D., & Ginsburg, A. (1969) *Biochemistry 8*, 1714–1725.

Farber, J. M., & Levine, R. L. (1986) *J. Biol. Chem. 261*, 4574–4778.

Fisher, M. T., & Stadtman, E. R. (1992) *J. Biol. Chem. 267*, 1872–1880.

Garland, D., Zigler, J. S., Jr., & Kinoshita, J. (1986) *Arch. Biochem. Biophys. 251*, 771–776.

Ginsburg, A. (1972) *Adv. Protein Chem. 26*, 1–79.

Howard, J. B., & Rees, D. C. (1991) *Adv. Protein Chem. 42*, 199–280.

Hunt, J. B., Smyrniotis, P. Z., Ginsburg, A., & Stadtman, E. R. (1975) *Arch. Biochem. Biophys. 166*, 102–124.

Janson, C. A., Almassy, R. J., Westbrook, E. M., & Eisenberg, D. (1984) *Arch. Biochem. Biophys. 228*, 512–518.

Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946–950.

Levine, R. L. (1985) *Curr. Top. Cell. Regul. 27*, 305–316.

Liaw, S. (1992) Ph.D. Thesis, University of California, Los Angeles.

Liaw, S., Jun, G., & Eisenberg, D. (1993) *Protein Sci. 2*, 470–471.

Oliver, C. N., Ahn, B. W., Moreman, E. J., Goldstein, S., & Stadtman, E. R. (1987a) *J. Biol. Chem. 262*, 5488–5491.

Oliver, C. N., Levine, R. L., & Stadtman, E. R. (1987b) *J. Am. Geriatr. Soc. 35*, 947–956.

Rivett, A. J. (1985) *J. Biol. Chem. 260*, 300–305.

Rivett, A. J., & Levine, R. L. (1990) *Arch. Biochem. Biophys. 278*, 26–34.

Roseman, J. E., & Levine, R. L. (1987) *J. Biol. Chem. 262*, 2101–2110.

Shapiro, B. M., & Ginsburg, A. (1968) *Biochemistry 7*, 2153–2167.

Stadtman, E. R., & Oliver, C. N. (1991) *J. Biol. Chem. 266*, 2005–2008.

Stadtman, E. R. (1992) *Science 257*, 1220–1224.

Starke, P. E., Oliver, C. N., & Stadtman, E. R. (1987) *FASEB J. 1*, 36–39.

Timmons, R. B., Rhee, S. G., Luterman, D. L., & Chock, P. B. (1974) *Biochemistry 13*, 4479–4485.

Yamashita, M. M., Almassy, R. J., Janson, C. A., Cascio, D., & Eisenberg, D. (1989) *J. Biol. Chem. 264*, 17681–17690.