Inactivation and Reactivation of Manganese Catalase: Oxidation-State Assignments Using X-ray Absorption Spectroscopy†

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ABSTRACT: The oxidation states of the Mn atoms in three derivatives of Mn catalase have been characterized using a combination of X-ray absorption near-edge structure (XANES) and EPR spectroscopies. The as-isolated enzyme has an average oxidation state of Mn(III) and contains a Mn(III) form, together with a reduced Mn(II) form and a variable amount (10–25%) of a Mn(III)/Mn(IV) mixed-valence derivative. Treatment with NH_2OH rapidly reduces the majority of the enzyme to a Mn(II) derivative with no loss of activity. Inactivation by treatment with $NH_2OH + H_2O_2$ converts all of the enzyme to a mixed-valence Mn(III)/Mn(IV) form. The inactive, mixed-valence derivative can be completely reactivated by long-term (>1 h) anaerobic incubation with NH_2OH, giving a reduced Mn(II)/Mn(II) derivative. These data suggest a catalytic model in which the enzyme cycles between a reduced Mn(II)/Mn(II) state and an oxidized Mn(III)/Mn(III) state.

The catalytic disproportionation of H_2O_2 to dioxygen and water (the catalase reaction) is important in protecting aerobic cells from oxidative stress. Most catalases contain the protoporphyrin IX prosthetic group; however, some bacteria have been found to contain non-heme catalases (Delwiche, 1961; Johnston & Delwiche, 1962, 1965; Jones et al., 1964). Recently, Fridovich and co-workers (Kono & Fridovich, 1983; Beyer & Fridovich, 1985) showed that the non-heme catalase from *Lactobacillus plantarum* ATCC 14431 contains manganese with an apparent stoichiometry of 2 Mn/subunit. Mn catalases have also been isolated from *Thermus thermophilus* HB8 (Barynin & Grebenko, 1986; Khangulov et al., 1986; Barynin et al., 1986) and *Thermoleophilum album* NM (Algood & Perry, 1986).

Low-temperature electron paramagnetic resonance (EPR)[1] spectra for both the *T. thermophilus* and the *L. plantarum* enzymes (Khangulov et al., 1986, 1987; Fronko et al., 1988) show a 16-line signal which has been attributed to a Mn(III)/Mn(IV) mixed-valence binuclear structure. This, together with a low-resolution crystal structure (Barynin et al., 1987), suggests that the enzyme has a binuclear Mn active site. In addition to the Mn(III)/Mn(IV) derivative, several other oxidation states have been identified for Mn catalase. Additional EPR signals for the *T. thermophilus* enzyme have been attributed to Mn(II)/Mn(III) and Mn(II)/Mn(II) derivatives (Khangulov et al., 1986, 1987). Finally, the optical spectrum for Mn catalase (Kono & Fridovich, 1983a) has been interpreted as arising from an oxo, bis(carboxylato)-bridged Mn(III)/Mn(III) cluster (Wieghardt et al., 1986; Sheats et al., 1987; Wieghardt, 1989).

Given this range of species, the identity of the *active* oxidation states in Mn catalase is unclear. Hydrogen peroxide disproportionation is a two-electron process; thus, potential catalytic cycles most likely involve one of eq 1–3 (assuming

$$\text{Mn(II)/Mn(II)} \leftrightarrow \text{Mn(III)/Mn(III)} \quad (1)$$

$$\text{Mn(II)/Mn(III)} \leftrightarrow \text{Mn(III)/Mn(IV)} \quad (2)$$

$$\text{Mn(III)/Mn(III)} \leftrightarrow \text{Mn(IV)/Mn(IV)} \quad (3)$$

that only the Mn atoms are redox-active). The mechanism in eq 1 is consistent with the catalase chemistry observed for a binuclear Mn(II) model system (Mathur et al., 1987). The proposal in eq 2 would be consistent with our earlier report (Fronko et al., 1988) that the active, as-isolated catalase shows an Mn(III)/Mn(IV) EPR signal and that the intensity of this signal correlates with activity. The mechanism in eq 3 was first proposed by Beyer and Fridovich (1988). Although it invokes an as yet unidentified Mn(IV)/Mn(IV) derivative, this mechanism is consistent with the known coordination chemistry of Mn (Pecoraro, 1988; Larson & Pecoraro, 1991a,b). An attactive feature of eq 3 is that it invokes higher oxidation states of Mn, similar to those believed to be present for the Mn sites in the photosynthetic oxygen-evolving complex (Pecoraro, 1988; Vincent & Christou, 1989; Wieghardt, 1989; Brudvig & Crabtree, 1989). The present study was undertaken in order to identify the active Mn oxidation states in Mn catalase.

Mn catalase is inactivated when treated with a mixture of NH_2OH and H_2O_2 (Kono & Fridovich, 1983b; Kono, 1984, 1986). This observation is surprising, since NH_2OH alone has no effect on activity and H_2O_2 is the substrate. Although the mechanism of inactivation is at present unknown, the existence of the inactive enzyme nevertheless provides a valuable chemical perturbation of the Mn active site. The inactivation is accompanied by a bleaching in the optical spectrum (Kono & Fridovich, 1983b). This was interpreted as indicating that Mn is oxidized from Mn(III) to Mn(IV), with the latter oxidation state being inactive. More recently it has been reported (Beyer & Fridovich, 1989) that the $NH_2OH + H_2O_2$ inactivated enzyme shows the same 16-line EPR signal as is observed in the native enzyme (Fronko et al., 1988). This would be consistent with the mechanism in eq 2 if the NH_2OH +

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[1] Abbreviations: EPR, electron paramagnetic resonance; XANES, X-ray absorption near-edge structure; XAS, X-ray absorption spectroscopy.

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H_2O_2 treatment resulted in a covalent modification of the protein that blocked the Mn(III)/Mn(IV) → Mn(II)/Mn(III) half of the catalytic cycle. Alternatively, the Mn(III)/Mn(IV) derivative may represent an inactive oxidation state (Beyer & Fridovich, 1988; Kono & Fridovich, 1983). The latter seems more likely in view of the fact that the inactive enzyme can be partially reactivated by dithionite (Kono & Fridovich, 1983b). Additional evidence against covalent modification is found in the fact that a completely different protocol, oxidation with KIO_4, has been used to inactivate the *T. thermophilus* catalase (Khangulov et al., 1987) and that this procedure once again gives a Mn(III)/Mn(IV) derivative. We have used NH_2OH and H_2O_2 treatments to prepare different forms of Mn catalase and have used EPR and X-ray absorption spectroscopies, together with reactivity measurements, to characterize these derivatives.

Experimental Procedures

Mn catalase was isolated using a modification of published procedures (Kono & Fridovich, 1983; Beyer & Fridovich, 1985), in which batchwise DE-52 extraction followed by DE-52 chromatography was replaced by a single fast-flow Sepharose column.[2] Enzyme activity was assayed using a Clark-type oxygen electrode (YSI 5331). Protein concentrations were determined either spectroscopically (Warburg & Christian, 1941) or using a bicinchoninic acid coupled assay (Smith et al., 1985). Typical specific activities were ca. 3500 AU/mg. Values ranged from 3000 to 3800 AU/mg (1 activity unit = decomposition of 1 μmol of H_2O_2/min with $[H_2O_2]$ = 0.02 M). All enzyme samples were prepared and assayed in buffer A (50 mM phosphate, pH 7.0, and 0.1 mM EDTA).

EPR spectra were measured using a Bruker ER-200E-SRC spectrometer equipped with a TM(110) cavity operating at ca. 9.3 GHz. Typically, measurements were made at 77 K using 20-mW microwave power, 5-G peak-to-peak modulation, and 100 kHz modulation frequency. Under these conditions, saturation is not a problem.

X-ray absorption spectroscopy (XAS) measurements were made at the National Synchrotron Light Source beam lines X9A and X19A and at the Stanford Synchrotron Radiation Laboratory beam line 7-3 with either Si[111] (at NSLS) or Si[220] (at SSRL) monochromator crystals. The protein was concentrated to ca. 3 mM Mn using Centricon 30 microconcentrators, placed in Lucite cuvettes with 6-μm polypropylene windows, and rapidly frozen in liquid nitrogen. Samples were maintained at 77 K, and XAS spectra were measured at either 10 K or 77 K. The inactivated enzyme was prepared by dialysis for 4 h against buffer A containing 20 mM NH_2OH + 50 mM H_2O_2 followed by dialysis against two changes of buffer A containing 20 mM H_2O_2 (4 h each) and finally dialysis for 12 h against buffer A. The concentration of $S = {}^1/_2$ centers in the inactive catalase was determined by double-integration relative to a $CuSO_4$ (aqueous) standard. Total Mn was determined from the intensity of the 6-line $Mn^{2+}(H_2O)_6$ signal for the acid-denatured protein (pH = 1, HCl at 80 °C for 15 min). Long-term-incubated NH_2OH-treated samples were prepared either by dialysis against 10 mM NH_2OH (2 h) followed by anaerobic dialysis against buffer A or by injection of NH_2OH to a final concentration of 10 mM followed by incubation (2 h). The two protocols gave samples having indistinguishable XANES spectra. Short-term-incubated NH_2OH-treated samples were prepared by adding NH_2OH to a final concentration of 10 mM followed immediately by flash-freezing in liquid nitrogen.

[2] A 2-L bed volume of DEAE Fast-Flow Sepharose was equilibrated with 50 mM potassium phosphate buffer, pH 7.0. A flow rate of 2 mL/min was used to load 1.5 L of cell extract. A 50–350 mM linear gradient in buffer ionic strength was used to elute the enzyme. Total gradient volume = 4.0 L. The most active fractions (typically eluting between 3.0 and 3.5 L) were pooled, concentrated, and used as previously described.

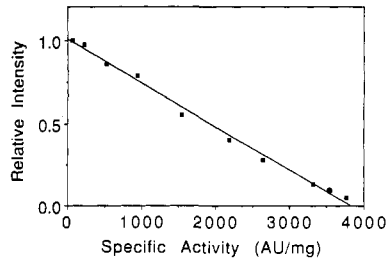

FIGURE 1: Correlation between enzyme activity and the intensity of the 16-line EPR signal. The relative EPR intensity was taken as the average size (peak minus trough) for the central transition (ninth line from the low-field side) normalized to the average size for the inactivated protein. Data shown here were measured during NH_2OH reactivation of the NH_2OH + H_2O_2 inactivated protein. An equivalent correlation is observed during inactivation (data not shown). The as-isolated enzyme is indicated by a circle (specific activity ca. 3500 AU/mg).

XAS spectra were measured as fluorescence excitation spectra using either a large solid-angle ion chamber (Stern et al., 1982) with a Cr filter (Stern & Heald, 1979) or a 13-element solid-state detector array (Cramer et al., 1988). XAS data were reduced following standard procedures (Penner-Hahn et al., 1990) and were calibrated by assigning the pre-edge peak in a simultaneously measured $KMnO_4$ standard at 6543.3 eV.

Results

Inactivation on Treatment with NH_2OH + H_2O_2. Consistent with previous reports (Kono & Fridovich, 1983; Kono, 1984, 1986), we see no loss of activity when Mn catalase is incubated with NH_2OH alone. This is true even for long-term (several hour) incubations at high (25 mM) NH_2OH concentrations. If NH_2OH is added to Mn catalase and the solution is frozen promptly (short-term treatment), there is also no change in the 16-line EPR signal. However, if H_2O_2 is added to the Mn catalase + NH_2OH solution, we observe a progressive loss of activity and an increase in the intensity of the 16-line EPR signal. The intensity of the 16-line EPR signal is negatively correlated with enzyme activity, as shown by the data in Figure 1. Aside from the increase in intensity, there is no detectable change in the 16-line EPR signal on inactivation, thus suggesting that the same species gives rise to this signal in the as-isolated enzyme and in the inactivated enzyme. EPR spin quantitation indicates that >90% of the Mn in the inactive catalase contributes to the 16-line EPR signal.

XAS of Mn Catalase. X-ray absorption near-edge structure (XANES) spectra refer to the structured absorption in the near vicinity (±25 eV) of a core electron excitation threshold (Bart, 1986). Mn K-edge XANES spectra have proven useful for characterizing the oxidation state of Mn in the oxygen-evolving complex (Kirby et al., 1981; Goodin et al., 1984; Cole et al., 1987; Penner-Hahn et al., 1990). An important aspect of XANES is that it is sensitive to all of the Mn in a sample, thus providing a useful complement to EPR, which may detect only minor species.

Detailed interpretation of XANES spectra in terms of molecular structure is extremely difficult. For the present purposes, it is important only that Mn(II) XANES spectra are readily distinguished from Mn(III) and Mn(IV) spectra,

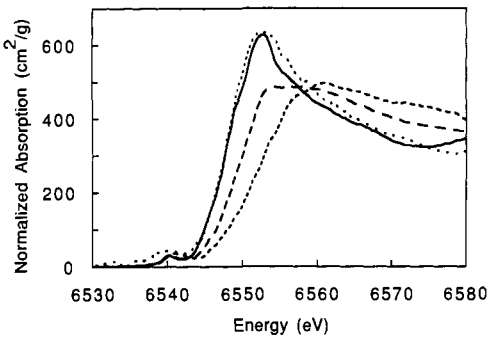

FIGURE 2: Normalized XANES spectra for Mn catalase. Solid line = long-term NH_2OH-reduced; dotted line = short-term NH_2OH-reduced; long dash = as-isolated; short dash = $NH_2OH + H_2O_2$ inactivated. Spectra normalized to an Mn K-edge step of 415.1 cm^2/g. Normalization of the spectrum for the short-term NH_2OH-reduced sample is less precise due to the higher noise level of these data at high k.

both by a shift to higher energy and by a change from sharp to broad-edge structure. These differences allow ready identification and quantitation of Mn(II) and, to a lesser extent, allow us to distinguish between Mn(III) and Mn(IV) (Penner-Hahn et al., 1990). The XANES spectra for the as-isolated Mn catalase, for NH_2OH short-term and long-term NH_2OH-treated Mn catalase, and for $NH_2OH + H_2O_2$ inactivated Mn catalase are shown in Figure 2.

From Figure 2, it is clear that NH_2OH treatment *does* affect the Mn in Mn catalase, even though it has no effect on either the activity or the EPR spectrum. In quantitative XANES fits using model XAS spectra (Penner-Hahn et al., 1990), the long-term NH_2OH-treated sample can be fit using only Mn(II), with no need to include higher oxidation-state models. The short-term NH_2OH-treated enzyme is best fit with ca. 85% Mn(II) with the remaining 15% being either Mn(III) or Mn(IV). The as-isolated enzyme is at an intermediate oxidation state, most consistent with an average composition of Mn(III), and the $NH_2OH + H_2O_2$ inactivated sample is more highly oxidized.[3] The latter is consistent with a mixture of Mn(III) and Mn(IV).

The 16-line EPR signal has been interpreted as arising from an $S = {}^1/_2$ Mn(III)/Mn(IV) dimer (Khangulov et al., 1986, 1987; Fronko et al., 1988). However, there is some question as to whether Mn(III)/Mn(IV) and Mn(II)/Mn(III) dimers can be distinguished using EPR spectroscopy alone (Mabad, 1985). In the case of inactivated Mn catalase, the XANES spectra clearly rule out the latter possibility and confirm that the 16-line EPR signal arises from a Mn(III)/Mn(IV) cluster.

Reactivation of Mn Catalase. Fridovich et al. (1983) reported that the $NH_2OH + H_2O_2$ inactivated Mn catalase could be partially reactivated by treatment either with superoxide or with dithionite but that oxidants and other reductants were ineffective. We find, in contrast, that long-term anaerobic incubation with NH_2OH will completely reactivate Mn catalase. This reactivation is accompanied by the complete disappearance of the Mn(III)/Mn(IV) EPR signal. If the as-isolated protein is incubated with NH_2OH, there is a small

[3] XANES fits for the as-isolated enzyme used in this study indicate the presence of ca. 40% Mn(II); however, this fraction is variable from preparation to preparation (G. S. Waldo and J. E. Penner-Hahn, unpublished results). There is no evidence for Mn(II) in the fits for the inactivated enzyme. The precise ratio of Mn(III) to Mn(IV) cannot be reliably determined using XANES. It is clear that the inactivated enzyme is more highly oxidized than the as-isolated enzyme; however, the extent of oxidation is difficult to determine given the likelihood that ligation changes may complicate the oxidation-state-dependent XANES changes.

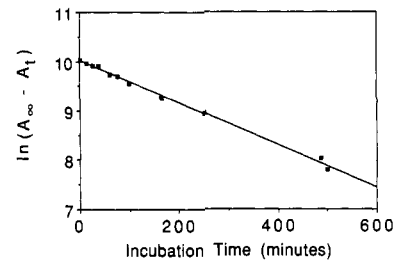

FIGURE 3: Reactivation kinetics for $NH_2OH + H_2O_2$ inactivated Mn catalase. Inactive protein was incubated anaerobically with 1.5 mM NH_2OH, and samples were withdrawn periodically and assayed for activity. A_t = activity (arbitrary units); A_∞ = activity after 24-h incubation. Solid line is linear least-squares fit to a straight line; first order rate constant = 0.39 h^{-1}.

(typically 10–20%) increase in catalytic activity.

The reactivation shows pseudo-first-order kinetics (Figure 3). The apparent first-order rate constant is 0.39 h^{-1} when $[NH_2OH]$ = 1.5 mM. This very slow reactivation rate probably accounts for earlier failures to completely reactivate Mn catalase. Earlier work utilized aerobic NH_2OH incubation. Since NH_2OH is slowly autoxidized with the consequent reduction of O_2 to H_2O_2, the reactivated catalase will be continually re-inactivated.

Discussion

The XANES data demonstrate that the majority of the Mn in the as-isolated Mn catalase is rapidly reduced by NH_2OH, with no loss of activity. This indicates that the Mn(II) derivative must either be active or be readily convertible to the active protein. Khangulov et al. (1987) have previously shown that NH_2OH treatment of the *T. thermophilus* catalase results in formation of an EPR-active species assigned as Mn(II)/Mn(II). The EPR detection is complicated, however, by the need to add anionic inhibitors in order to convert the protein into an EPR-active form (Khangulov et al., 1987, 1990a). Quantitation of the EPR spectra relies on the assumption (Khangulov et al., 1987, 1990a) that all of the Mn sites except for the Mn(III)/Mn(III) derivatives are EPR-active. In contrast, XANES spectra are sensitive to all of the Mn in the sample, regardless of spin state, and XANES spectra can be measured under conditions where there is no inhibition of enzymatic activity.

The XANES results are consistent with the catalytic cycle shown in eq 1. In contrast, if one assumes that the Mn(III)/Mn(IV) derivative is active (eq 2), it is difficult to account for the XANES data indicating the presence of a large pool of short-term NH_2OH-reducible Mn sites in the as-isolated enzyme. The Mn(II) thus formed cannot come from reduction of Mn(III)/Mn(IV) centers to Mn(II)/Mn(III), since short-term NH_2OH treatment does not alter the EPR signal. The NH_2OH reduction cannot interchange active and inactive pools of enzyme, since short-term NH_2OH incubation has no effect on activity. The catalytic cycle in eq 2 would thus require that the short-term NH_2OH-reducible Mn (i.e., the majority of the Mn in the sample) contribute nothing to the observed activity. This, however, is inconsistent with the data in Figure 1. Equation 2 is thus ruled out as the catalytic cycle.

Finally, the catalytic cycle shown in eq 3 could be correct; however, in this case, it would be necessary that H_2O_2 rapidly oxidize both the Mn(II)/Mn(II) and the Mn(III)/Mn(III) derivatives, since the Mn(II)/Mn(II) protein shows no loss of activity. Recently, Khangulov et al. (1990a) have shown that treatment of the oxidized *T. thermophilus* catalase with H_2O_2 in the presence of chloride results in the production of

an EPR-detectable Mn(II)/Mn(II) species. Since chloride is a potent inhibitor of the *T. thermophilus* catalase, it is unclear whether this observation is relevant to the catalase activity (i.e., chloride may inhibit the enzyme by trapping it in an inactive oxidation state). *If* H_2O_2 also reduces Mn catalase to the Mn(II)/Mn(II) form in the absence of inhibitor, the catalytic scheme in eq 3 cannot be correct. However, both eq 1 and eq 3 are consistent with the data available at present.

Regardless of which scheme is correct, the 16-line EPR signal seen for the as-isolated protein must arise from inactive protein, probably formed by autoxidation of the active enzyme (Khangulov et al., 1990). We previously estimated that the Mn(III)/Mn(IV) derivative comprised ca. 60% of the Mn (Fronko et al., 1988); however, the present data (Figure 1) demonstrate that our current isolation procedure gives much smaller amounts of the Mn(III)/Mn(IV) derivative. Measurements analogous to those in Figure 1 indicate that the as-isolated Mn catalase has 10–25% of its Mn in the Mn(III)/Mn(IV) oxidation state. This new estimate of Mn(III)/Mn(IV) content is consistent with the increase in specific activity over our earlier preparations (3500 vs 2000 units/mg). We originally reported that the intensity of the Mn(III)/Mn(IV) EPR signal was correlated with enzyme activity (Fronko et al., 1988). The present results demonstrate that this apparent correlation was in fact due to the presence of an approximately constant percentage of inactive enzyme in the different preparations.

The observation that the Mn(III)/Mn(III) catalase is rapidly reduced by NH_2OH while the Mn(III)/Mn(IV) catalase is only slowly reduced under equivalent conditions is intriguing. This shows that the Mn(III)/Mn(III) derivative is a *better* oxidant than the Mn(III)/Mn(IV) enzyme, probably as a result of differences in the Mn ligation. The observation that the Mn(III)/Mn(IV) derivative is a poor oxidant provides an explanation for its lack of activity: It is too poor of an oxidant to oxidize H_2O_2 to O_2.

It is instructive to compare the Mn site in the Mn catalase with that found in the photosynthetic oxygen-evolving complex (OEC). The latter contains four Mn atoms per complex and has been shown to have Mn-dependent catalase activity (Frasch & Mei, 1987; Mano et al., 1987). The Mn ions in the OEC are generally believed to be present as Mn(III) or Mn(IV) (Pecoraro, 1988; Vincent & Christou, 1989; Wieghart, 1989; Brudvig & Crabtree, 1989), although it is not clear which oxidation states are responsible for the catalase activity. Treatment of the OEC with very low (micromolar) concentrations of NH_2OH results in a two-flash delay in oxygen evolution. This has been interpreted as arising from rapid reduction of the OEC to a state formulated as S_{-1} (Beck & Brudvig, 1987, 1988; Sivaraja & Dismukes, 1988) although recent XANES studies suggest that reduction only occurs following illumination (Guiles et al., 1990). Treatment with higher (millimolar) concentrations of NH_2OH results in slow, irreversible loss of Mn and consequent inactivation of the OEC (Cheniae & Martin, 1971; Yocum et al., 1981). Even the latter, slower, reaction is ca. 100-fold faster than the reduction shown in Figure 3. In contrast to the OEC, Mn catalase appears to function in the *lower* oxidation states of Mn and is *stable* to NH_2OH treatment. When oxidized to the oxidation states believed to be present in the OEC, the Mn catalase is inactive. These differences in reactivity are probably due to differences in the Mn ligation in the Mn catalase and in the OEC. Experiments to characterize these differences are in progress.

Conclusions

We find that the majority of the Mn in Mn catalase is reduced by NH_2OH. The combination of EPR, XANES, and kinetic measurements has allowed us to distinguish between a rapidly reducible Mn(III)/Mn(III) derivative and an inactive Mn(III)/Mn(IV) derivative that is reduced only slowly. These results are most consistent with a Mn(II)/Mn(II) $\leftrightarrow$ Mn(III)/Mn(III) catalytic cycle, although a Mn(III)/Mn(III) $\leftrightarrow$ Mn(IV)/Mn(IV) catalytic cycle cannot be ruled out at this time. In order to be correct, the latter would require that both the Mn(II)/Mn(II) and the Mn(III)/Mn(III) derivatives be oxidized rapidly by H_2O_2.

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Estrogen-Induced Ribonuclease Activity in *Xenopus* Liver†

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ABSTRACT: Estrogen administration to male *Xenopus* causes the cytoplasmic destabilization of the hepatic serum protein coding mRNAs, most notably, albumin, yet has little effect on mRNAs encoding intracellular proteins such as ferritin. This report describes an estrogen-inducible ribonuclease activity found in liver polysomes that degrades albumin mRNA 4 times faster in vitro than it degrades ferritin mRNA. This differential rate of degradation was observed upon incubation of polysome extract with free liver RNA, isolated liver mRNPs, or transcripts from plasmid vectors. A cleavage fragment consisting of a doublet of approximately 194 nucleotides in length was consistently observed upon digestion of transcripts for the full length or 5′ half of albumin mRNA. The generation of this cleavage fragment was used as an assay to study properties of the polysome nuclease activity. The 194 doublet is produced by the action of a Mg^{2+}-independent endonuclease. This distinguishes the *Xenopus* liver enzyme from the enzymes that degrade histone or c-*myc* mRNA in vitro. It is inactivated by 400 mM NaCl or heating at 90 °C, but not by placental ribonuclease inhibitor or *N*-ethylmaleimide. Finally, the polysomal nuclease activity does not degrade double-stranded RNA. We believe the estrogen-induced nuclease activity contains an enzyme(s) that may mediate hormone-regulated changes in mRNA stability in this tissue.

The stabilization or destabilization of mRNAs within a cell effect significant changes in gene expression [reviewed in Brawerman (1988)]. Altering mRNA stability is the major mechanism regulating such diverse mRNAs as those encoding oncogenes [c-*myc* (Brewer & Ross, 1988; Pei & Calame, 1988), c-*fos* (Wilson & Treisman, 1988; Shyu et al., 1989)], translation elongation factors [eEf-TU (Rao & Slobin, 1988)], histones (Ross et al., 1986), tubulin (Pachter et al., 1987; Yen et al., 1988), and homeobox proteins (Brown & Harland, 1990), to name a few. The determinants of stability (or instability) for a number of RNAs have been identified and in many cases consist of a discrete nucleic acid sequence (e.g.,

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