ULTRAVIOLET LIGHT-INDUCED CHANGES IN RIBONUCLEASE CONFORMATION

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Received June 28, 1971

Summary: Ultraviolet light-induced inactivation of RNase A is accompanied by a decrease in the circular dichroism (CD) at the extrema observed at 239 nm and 275 nm. As inactivation progresses a new CD band centered near 325 nm also develops. For RNase maintaining up to one half of its original activity an isoelliptic point may be noted near 257 nm. These findings are consistent with initial normalization of a "buried" tyrosyl residue followed by chemical modification of the protein. Further chemical change and extensive conformational reorganization of the protein appear to accompany advanced inactivation.

Of the six tyrosine residues present in RNase A three lie on the outer surface of the protein; the remaining are buried in the hydrophobic interior of the polypeptide chain (1,2). Exposure of the protein to denaturing solvents such as urea (3), guanidine HCl-urea (4) or ethylene-glycol (5) normalizes the properties of "buried" tyrosines. Thermal denaturation also produces normalization of tyrosyl residues (6).

In view of the changes in the tyrosine environment which accompany the thermal or the solvent induced denaturation of ribonuclease, it is of interest to examine whether similar changes occur as a result of UV-light irradiation. Irradiation has been known to disrupt hydrogen and disulfide bonds present in the native protein conformation (7,8).

Materials and Methods

Lyophilized Ribonuclease A (Sigma Chem. Co. Lot #49B-8043) was dissolved (1.3×10^{-4} M) in 0.1 M sodium phosphate buffer at pH 7.0 and 2 ml aliquots of the solution were placed in quartz tubes fitted with Teflon valves (Fisher and Porter, Warminster, Pa.). After degassing and flushing with Argon the solutions were

irradiated in a Rayonet Chamber (Southern New England Ultraviolet Co., Middletown, Conn.) at 3°C. The tubes were rotated past a fixed, essentially monochromatic, UV source (1.65×10^{12} photons/sec/cm^3 at 2537 Å) at a distance of 2 cm. Intermittent but frequent stirring was achieved as the tubes moved past a magnetic stirrer. Enzyme activity was measured from the rate of hydrolysis of cytidine 2',3'-cyclic phosphate (9) using a Cary 15 spectrophotometer.

Circular dichroism measurements were performed at 3°C immediately after irradiation on a Durrum-Jasco ORD/CD/UV-5 spectropolarimeter modified to a maximum sensitivity of 2×10^{-3} deg/cm. Molar ellipticities, $[\theta]$, in deg. cm^2/decimole, were computed on a mean residue weight (MRW) of 110, as:

$$[\theta]_{MRW} = \frac{100 \times \theta}{l \times c}$$

θ is the observed ellipticity in degrees, l is the cell path length in centimeters, and c is the molar concentration.

Results

The circular dichroism of irradiated RNase A maintaining various degrees of activity are shown in Fig. 1. The native protein exhibits a negative extremum near 275 nm and a positive extremum near 239 nm. Upon irradiation the intensity of both extrema is decreased and a new band appears with a maximum near 325 nm. For RNase maintaining up to approximately one half of the activity of the native enzyme an isoelliptic point is present near 257 nm.

The 275 nm band in native RNase is reported to contain contributions originating from disulfide bonds and tyrosyl residues both "buried" and exposed (10,11). "Buried" tyrosines apparently contribute to the circular dichroism associated with the 239 nm

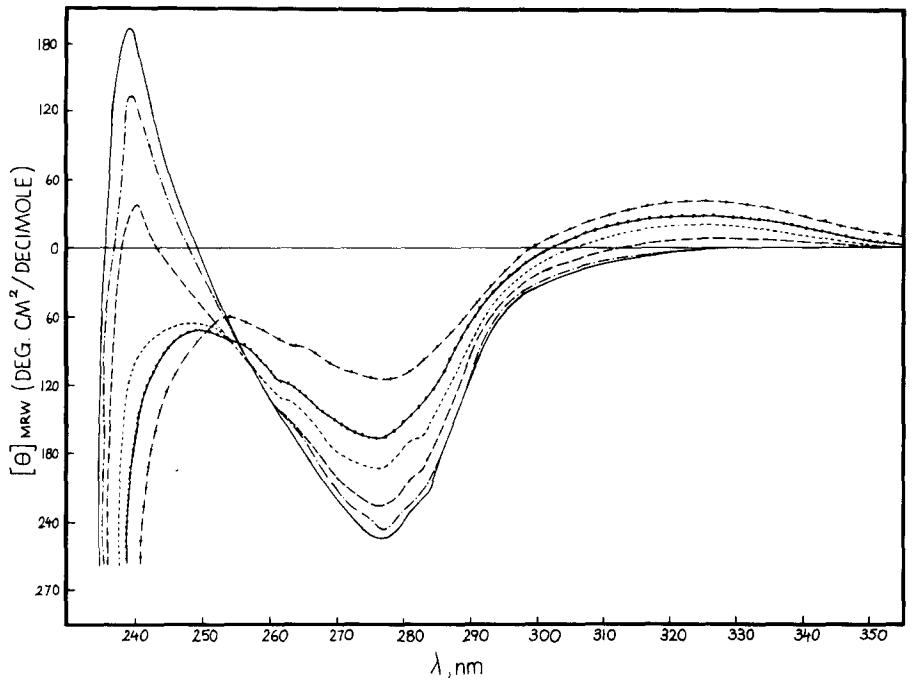

Figure 1. Circular dichroism of UV-irradiated RNase A (1.3×10^{-4} M) obtained in a 5 mm thermostated cell at 3°C. The remaining enzymic activity is: 90%, 73%, 54%, 42%, 21%. Unirradiated RNase is indicated by a solid line.

band as well (12). The decrease in ellipticity and the red shift noted for the 239 nm band upon irradiation may be attributed to the normalization of a tyrosyl residue. Since exposed tyrosines may be associated with a positive circular dichroism band near 227 nm (13), which is normally overshadowed by strong negative polypeptide transitions, any blue shift in the positive component of the 239 nm maximum would be expected to result in a shift of the 239 nm maximum to the opposite direction. Such decreases in ellipticity and shift in the 239 nm maximum have also been noted to accompany the thermal denaturation of RNase (6).

From the data shown in Fig. 1 and additional CD measurements the difference in ellipticities between native and irradiated RNase at 239 nm and 275 nm can be plotted as a function of

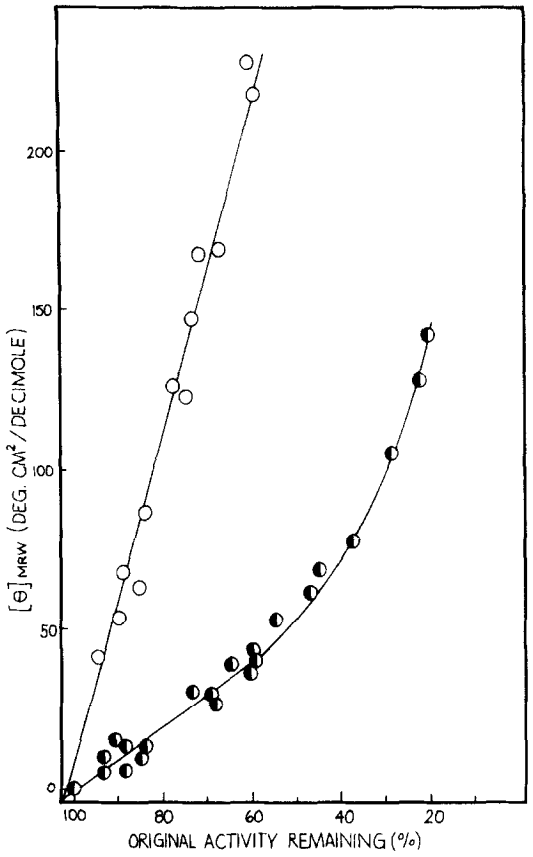

Figure 2. Differences in ellipticities between native and irradiated RNase (1.3×10^{-4} M) at ○ 239 nm and ◐ 275 nm plotted as a function of inactivation. Since the 239 nm band is a composite one and a positive extremum is not present in extensively inactivated RNase measurements at this wavelength are limited to enzyme with activity not less than 70%.

the inactivation of the enzyme (Fig. 2). Examination of Fig. 2 indicates that the relation between enzyme activity and ellipticity at 275 nm for up to approximately 50% inactivation remains linear. As inactivation exceeds this limit, however, the observed decrease in ellipticity is rapidly accelerated. A linear relationship is also observed for the ellipticity at 239 nm for RNase maintaining at least as much as 70% of the native activity.

The appearance of a new circular dichroism band in irradi-

ated RNase with a maximum near 325 nm is a definite indication that chemical modifications accompany the process of inactivation. Although the nature of these modifications is not clear, the wavelength at which the new circular dichroism band appears is consistent with structural changes in the aromatic residues. In this connection it may be noted that a modified aromatic ring system, namely bityrosine, has been detected among the products of hydrolysis of UV-irradiated poly-L-tyrosine (14).

Discussion

The loss of enzymic activity during the initial stages of RNase inactivation is accompanied by extensive changes in the environment of at least one "buried" tyrosyl residue as indicated by the dependence of ellipticity at 239 nm upon inactivation. This view is consistent with the results of spectrophotometric titrations which have indicated that normalization of one tyrosyl residue occurs during the early stages of γ-ray induced inactivation of RNase (15).

The decrease in circular dichroism noted at 275 nm is in agreement with the expected disruption of disulfide bonds. Such disruption is known to accompany the UV-light induced inactivation of the enzyme (7,8). Furthermore, additional contributions to the decrease at 275 nm may originate from a change in the proportion of the "buried" vs the exposed tyrosyl residues. Such residues may contribute differently to the circular dichroism in this region (10).

The isoelliptic point noted near 257 nm for RNase maintaining up to about half of the native activity indicates the presence of a mixture of two distinct types of molecules in the irradiated protein. At least one of these species is, obviously, partially or totally inactivated enzyme. The accelerated decrease in

ellipticities at 275 nm and the absence of the isoelliptic point for RNase with activities below 50% are evidence that at the later stages of inactivation major conformational changes take place. Thus, in highly inactivated RNase more than two, and probably several, types of macromolecules are present which may differ in conformation and/or chemical structure. Although chemical changes have been associated with the process of enzyme inactivation, the exact role of these changes has not as yet been clarified. One view has been that the disruption of specific disulfide bonds followed by conformational changes constitutes the only essential feature of the process of inactivation (16). Alternatively, the importance of the relation between the chemical modification or destruction of amino acid residues and inactivation has been emphasized (17,18).

Our results do not permit an assessment of the relative significance of these various factors in the process of inactivation. It is clear, however, that initial inactivation is associated with changes in the environment of a tyrosine and that this change occurs before any chemical modification of aromatic residues can be detected by CD measurements.

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