EXTRINSIC PROPERTIES OF OPTICAL ACTIVITY IN ADRENAL IRON-SULFUR PROTEIN (ADRENODOXIN)

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Summary From studies on the protein denaturation of adrenal iron-sulfur protein (adrenodoxin) by urea and guanidine hydrochloride, it was revealed by optical absorption, optical rotatory dispersion and circular dichroism measurements that the iron-sulfur chromophore, per se, is optically inactive. The origin of multiple Cotton effects in the untreated protein at the visible region is due to asymmetric interaction of an ordered structure of polypeptide chain with the chromophore. The initial phase of the denaturation process is reversible upon the removal of the denaturants.

Adrenodoxin which is an oxidation-reduction intermediate in the adrenal mitochondrial steroid hydroxylation reactions (1,2) displays strong multiple Cotton effects in the visible region (3,4). Upon reduction, the ORD maximum as well as CD maximum changes dramatically, shifting the principal effect to shorter wavelength. The detailed structure of the chromophore is not yet known. However, our chemical determinations revealed that one iron atom (two iron atoms per mole of the protein) chelates with two cysteinyl sulfur and one labile sulfur atoms (5,6). In this communication, we wish to report that when adrenodoxin is treated with urea or guanidine-HCl in the presence of air, the original ORD and CD spectra are largely destroyed instantly with the appearence of new weak ORD and CD spectra, whereas the visible optical absorption spectrum remains with little change at certain period of

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The abbreviations used are as follows: ORD, optical rotatory dispersion; CD, circular dichroism.

time. Upon removal of the protein denaturant, the optical activity can be restored partially to the original level, and the regenerated protein shows the enzymatic activity towards NADPH-cytochrome c reduction in the presence of adrenodoxin reductase (a flavoprotein).

Methods Adrenodoxin prepared as described previously (6) had a ratio of A_{414} : A_{276} as 0.76 and contained 103 mμatoms of iron per mg protein. Protein was determined by a biuret reaction.

Results and Discussion The nature of aerobic denaturation profile in the presence of protein denaturants is shown in Fig. 1. Changes in absorbance at 414 mμ may reflect various steps involved in the denaturation process: the initial phase with a rate constant of k_1 (urea) = 3.8×10^{-3} min^{-1} and k_1 (guanidine-HCl) = 2.2×10^{-2} min^{-1}, and this process has activation energy of 15 kcal per mole. The second phase with a rate constant of k_2 (urea) = 1.47×10^{-2} min^{-1} and k_2 (guanidine-HCl) = 9.0×10^{-2} min^{-1} causes deterioration of the

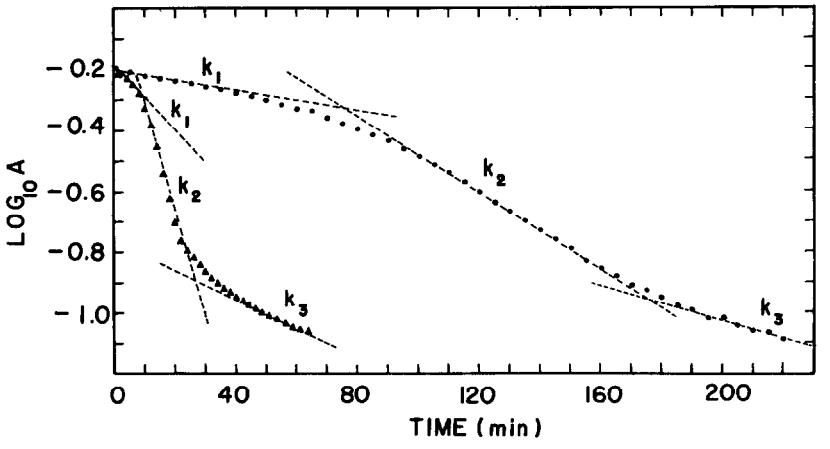

Fig. 1, Change in absorbance of adrenodoxin at 414 mμ due to urea and guanidine-HCl.

Protein concentration of adrenodoxin was 3.78 mg in 3.0 ml of 6 M urea or 4 M guanidine-HCl. Changes in absorbance at 414 mu were recorded by the use of a Zeiss spectrophotometer equipped with a recorder at 26° C.

●--● , adrenodoxin treated with urea

▲--▲ , adrenodoxin treated with guanidine-HCl

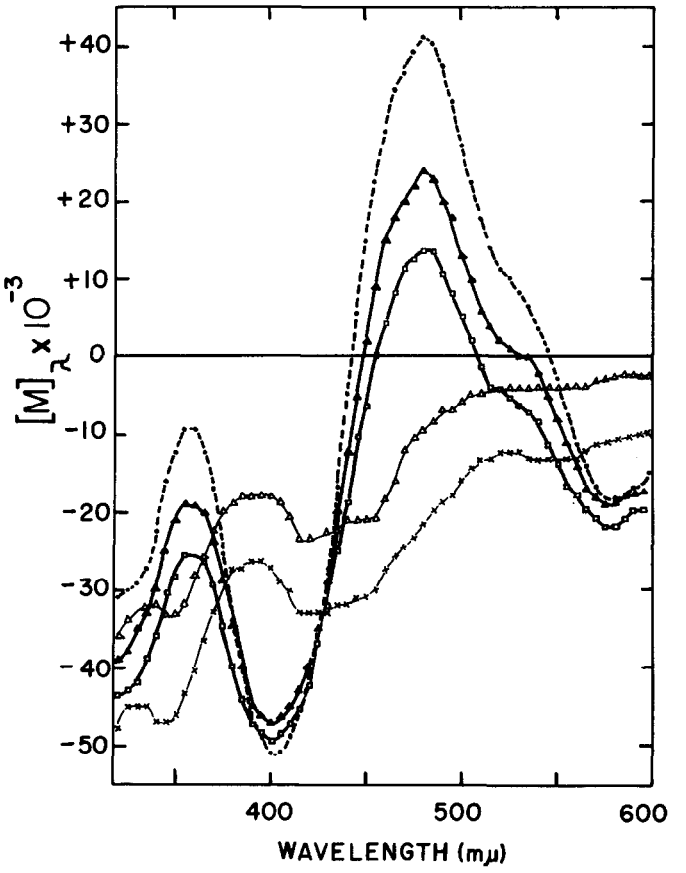

Fig. 2, Optical rotatory dispersion curves of urea and guanidine-HCl treated adrenodoxin.

Protein concentration of adrenodoxin used was 3.78 mg per 3.0 ml of 6 M urea or 4 M guanidine-HCl.

●---● , untreated sample
×—× , sample treated with 6 M urea for 2 min. at 26° C
△—△ , sample treated with 4 M guanidine-HCl for 2 min. at 26° C
▲—▲ , sample treated with 6 M urea for 2 min. at 26° C and dialyzed for 2 hours at 0° C
□—□ , sample treated with 4 M guanidine-HCl for 2 min. at 26° C and dialyzed for 2 hours at 0° C.

The measurements were performed by the use of a spectropolarimeer (Cary-60) with scanning speed of 10 min. for the indicated wavelength range at room temperature. Dialysis was carried out against 0.01 M phosphate buffer, pH 7.4. The denaturation was done at the same buffer.

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color of adrenodoxin. The activation energy of this irreversible process has been calculated to be 16 kcal per mole.

A dramatic change in the optical activity occurred after the

addition of urea or guanidine-HCl during the first phase of denaturation. The ORD spectrum of the untreated protein was destroyed within about 3 minutes resulting in the appearence of new multiple Cotton effects with the red shift of the principal effect (Fig. 2). The CD spectra during this process support the above observations (Fig. 3). At a reaction time of 70 minutes with urea or 25 minutes with guanidine HCl, the ORD became a plain dispersion and the CD disappeared in the visible region. However, the absorbance at 414 mu was about one half of that of the untreated protein. When the exposure to the denaturant was prolonged more than 70 minutes with urea or 25 minutes with

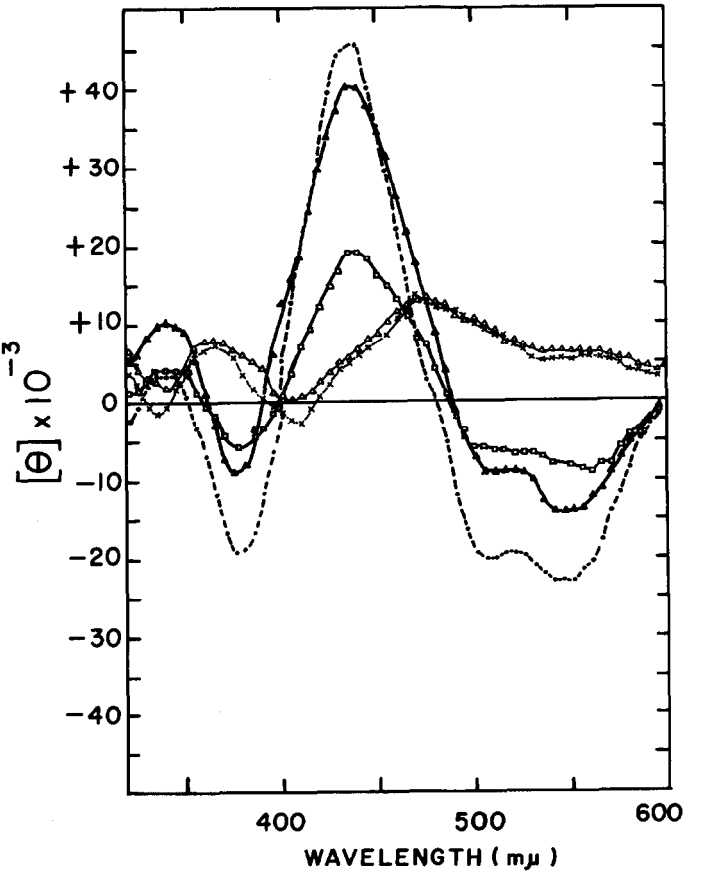

Fig. 3, Circular dichroism curves of adrenodoxin treated with urea and guanidine-HCl.

The experimental conditions and symbols used in the figure are same as in Fig. 2, except the spectropolarimeter was equipped with a CD attachment.

guanidine-HCl, the color of adrenodoxin disappeared finally resulting in a disordered structure (Table I).

As shown in Figures 2 and 3, the ORD and CD spectra of the urea and guanidine-HCl treated samples for 2 minutes at 26° C can be restored partially, if the denaturants are removed by dialysis at 0° C. Simultaneously, the absorption spectrum of the restored protein is comparable to that of the untreated one. When the restored protein from the above treatment was tested for the activity of NADPH-cytochrome c reduction in the presence of adrenodoxin reductase (2), it was found that the restored protein was enzymatically active. However, the prolonged treatment caused irreversible inactivation of the enzyme activity.

Stryer and Blout (7) demonstrated that optically inactive dye can display striking Cotton effects at its characteristic absorption bands when the dye is bound to helical poly-L-glutamic acid. Upon disruption of the helix, the Cotton effects are abolished, even though the dye remains bound. The same phenomenon, known as extrinsic Cotton effects, was found in heme proteins (8). A hypothesis to account for the origin of the asymmetry was implicated in changes of the protein conformation-induced optical rotation due to the asymmetric interaction with a chromophore (9). The present observations nicely

Table I. Moffitt Parameters of Urea and Guanidine-HCl treated Adrenodoxin

	Urea		Guanidine-HCl	
	70 min.	100 min.	100 min	140 min.
a_o	-510	-510	-670	-670
b_o	- 50	- 45	+110	+100

The experimental conditions were similar to those described in Figure 1, except the reaction time with the denaturants as indicated above.

fit to the hypothesis which can now be extended to iron-sulfur proteins, because a certain phase of denaturation process provides an intermediate state which exhibits original absorption spectrum but not optical activity. In view of the coordination structure of adrenodoxin as far as studied by chemical and physical approaches (6), it is most likely to be either binuclear tetrahedral or square planar configuration which is essentially symmetric. Therefore, the involvement of a pyramidal sulfur atom giving rise to optical activity is not valid for the configuration of the iron-sulfur linkage in adrenodoxin.

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