EFFECTS OF CHAOTROPIC AGENTS ON THE SPECTROSCOPIC PROPERTIES OF SPINACH FERREDOXIN

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SUMMARY

The "chaotropic agents" perclorate, tricloroacetate, thiocyanate, iodide, urea and guanidine HCl were found to cause striking changes in the EPR* and ORD* of purified spinach ferredoxin. At low concentrations of the agents the EPR signal became sharper, with small shifts in the apparent g-values which tended to approach those of another non-sulphur protein, adrenodoxin. At higher concentrations the EPR signal changed completely in shape, though still remaining centred around g = 1.95. After a few minutes this EPR signal disappeared entirely. The agents at high concentrations also caused changes in the ORD spectrum of ferredoxin, though these did not appear to correlate directly with the EPR effects. All these changes could be reversed by removal of the agent. As the concentration of the chaotropic agents was increased the ferredoxin became more unstable, especially in the reduced state.

The term "chaotropic agents" was used by Hatefi and Hanstein [1] to describe a group of compounds which, by altering the bulk properties of water, can cause membrane-bound proteins to become soluble. They include perchlorate, trichloroacetate, thiocyanate, iodide, urea and guanidine HCl. By the use of these agents a number of iron-sulphur proteins have been isolated in soluble form, apparently without denaturation [2,3]. On the other hand the rapid peroxidation of lipids in mitochondrial membranes in the presence of chaotropic agents has been attributed to the presence of the breakdown products of iron-sulphur proteins [4]. In view of the potential usefulness of this method of isolating proteins it is of interest to study the effects of chaotropic agents on purified iron-sulphur proteins, to determine whether any changes take place, and if so, whether they are reversible. This paper describes the effects of six chaotropic agents on spinach ferredoxin, studied by means of optical absorption, ORD and EPR spectroscopy.

*Abbreviations: EPR, electron paramagnetic resonance;
ORD, optical rotatory dispersion.

MATERIALS AND METHODS

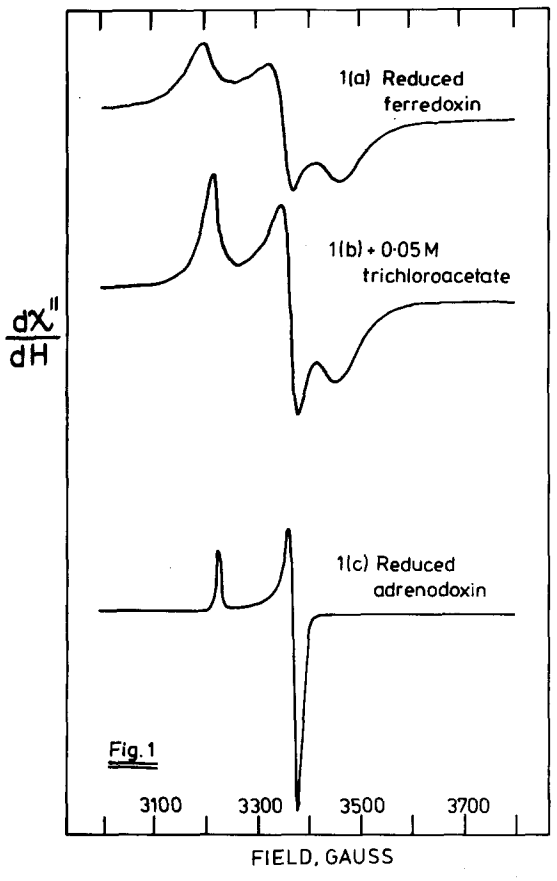
Spinach ferredoxin was prepared as described elsewhere [5]. The chaotropic agents were the purest products of BDH Chemicals Ltd. The buffer used was 20 mM potassium phosphate, pH 7.5, or 20 mM sodium HEPES (Calbiochem), pH 7.5. Samples for EPR measurements were reduced with 2 mM sodium dithionite for 1 min. before freezing. EPR measurements were made on a Varian E 4 spectrometer, using a liquid N_2 insert dewar. ORD spectra were recorded on a FICA Spectropol I spectropolarimeter.

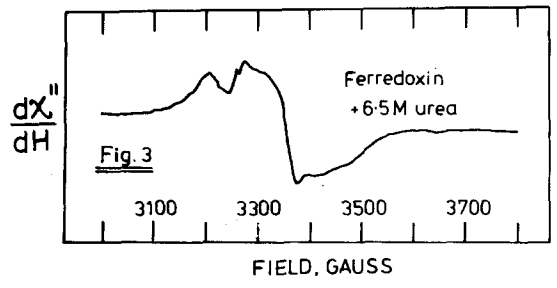
The reversal of the effects of chaotropic agents on reduced ferredoxin was demonstrated as follows: a ferredoxin soln. was mixed under argon with a small volume of chaotropic agent soln. and 40 µl of 50 mM sodium dithionite soln. (final volume 1 ml, ferredoxin approx. 0.5 mM). A 0.1 ml sample of the mixture was transferred to an EPR tube and frozen. The remainder of the solution was rapidly poured into 100 ml of aerobic phosphate buffer at 0°. The mixture was passed through a small (1 x 1 cm) column of DEAE-cellulose (Whatman DE23) and the absorbed ferredoxin was washed with 50 ml phosphate buffer and eluted with 0.8 M NaCl in phosphate. 0.1 ml samples were taken and reduced for EPR measurements.

RESULTS

Effects on the EPR spectrum: At concentrations of 10 - 50 mM (i.e. much lower than those used for extracting proteins from membranes, typically 0.4 - 1 M), the chaotropic agents were all found to cause specific changes in the EPR spectrum of reduced spinach ferredoxin (Fig. 1). These changes consisted of a decrease in linewidth and small shifts in the apparent g-values. This is illustrated in Fig. 1 (b) for trichloroacetate. The apparent g_x has moved down-field by 9 gauss, and g_y and g_z have moved up-field by 13 gauss and 18 gauss respectively. The effects of urea were somewhat smaller in magnitude than those of the other agents and were similar to those observed by Petering and Palmer [6].

Small effects of this type were observed in reduced spinach ferredoxin in the presence of methanol and iso-propanol by Coffman and Stavens [7]. As these





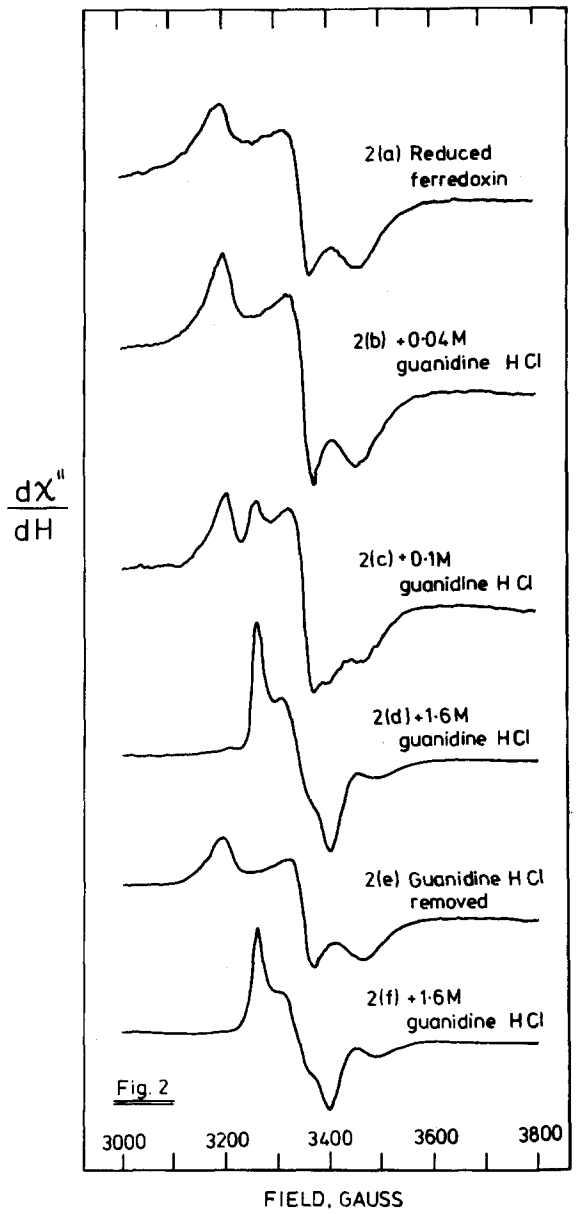


Fig. 1. EPR spectra of reduced spinach ferredoxin (0.52 mM) in the presence and absence of 0.05 M trichloroacetate compared with reduced adrenodoxin (0.29 mM). Measurements were made at 77° with the following spectrometer settings: microwave frequency, 9.17 GHz; microwave power, 20 mW; modulation amplitude, 4 gauss; gain 5×10^2 (for adrenodoxin, gain 8×10^1)

Fig. 2. EPR spectra of reduced spinach ferredoxin (0.6 mM) in the presence of varying concentrations of guanidine HCl. For spectra (e) and (f), the guanidine HCl was removed as described in the Materials and Methods section. Conditions of measurement were as for Fig. 1, except that for spectra (d) and (f) the gain setting was 2×10^2.

Fig. 3. EPR spectrum of reduced spinach ferredoxin (0.21 mM) in the presence of 6.5 M urea. Conditions of measurement were as for Fig. 1, except that the gain was 1.5×10^3.

workers pointed out, the changes in the spectrum, causing sharper linewidth and more axial symmetry, tend to make it resemble more closely the EPR spectrum of adrenodoxin (which is shown for comparison in Fig. 1c). Our recent Mössbauer evidence indicates that the iron-sulphur groups in these two proteins are very similar to each other [8].

No changes in the EPR signal of ferredoxin were observed in the presence of 1 M bromide, nitrate or phosphate. 4 M NaCl had a slight narrowing effect on the linewidths, and 2 M ammonium sulphate had a slight broadening effect.

At higher concentrations of the chaotropic agents a second, larger change was observed in the EPR spectrum. This was most easily seen with guanidine HCl (Fig. 2). As the concentration was raised to 1 M the sharpened spectrum (Fig. 2b) was replaced by a new signal centred around $g = 1.95$ (Fig. 2c & d). This appears to be a transient intermediate, since the signal disappeared in a few minutes at 20°, leaving no detectable EPR signal. These changes did not correspond with an irreversible denaturation of the protein, as the original spectrum could be restored to a large extent (though not completely) by removal of the guanidine HCl after reoxidation of the sample (Fig. 2e). On re-addition of guanidine HCl the same changes could be repeated (Fig. 2f).

With the other chaotropic agents no changes were observed in the EPR spectrum of ferredoxin until much higher concentrations were reached. With approx. 3 M trichloroacetate or perchlorate, 6.5 M urea or 70% v/v methanol, the spectrum began to resemble Fig. 2 (c). Fig. 3 illustrates the effect of 6.5 M urea. Petering and Palmer [6] did not observe this effect, probably because of rapid denaturation of the ferredoxin under these conditions. Attempts to obtain EPR signals with higher concentrations of the agents resulted in irreversible denaturation of the protein. Thus although it seems likely that the spectrum in all cases was tending to a spectrum similar to Fig. 2(d), this could only be observed with guanidine HCl.

Effects on the ORD. 1. Oxidized ferredoxin: We have observed changes in the ORD of oxidized spinach ferredoxin in the presence of all the chaotropic agents

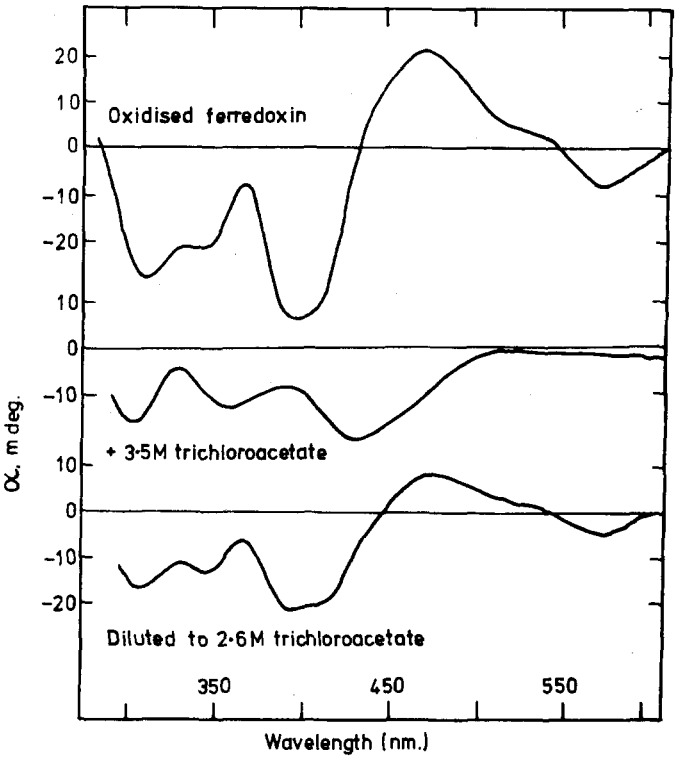


Fig. 4. ORD spectra of oxidized spinach ferredoxin (0.044 mM), alone and in the presence of 3.5 M trichloroacetate. The lower curve shows the effect of dilution with approx. $^1/_3$ volume of buffer; the amplitude has been corrected for dilution.

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and of methanol, similar to those previously observed by Padmabhan and Kimura [9] with urea and guanidine HCl. The concentration of the agents was very critical. Above a certain concentration of the agent a rapid change occurred in the shape of the ORD spectrum. This change could be reversed simply by diluting the sample with the buffer, so that the concentration of the agent was decreased. This is illustrated for trichloroacetate in Fig. 4. A similar effect of urea was reported to be dependent on salt concentration [6].

High concentrations of chaotropic agents (> 1 M) caused a gradual change of the ORD spectrum into that of the apo-protein; this change was accompanied by loss of the optical absorption at 420 nm and release of H_2S, and was probably due to denaturation of the ferredoxin.

2. Reduced ferredoxin: Surprisingly there were no specific changes in the ORD of reduced ferredoxin corresponding to the small changes in the EPR spectrum produced by low concentrations of all the chaotropic agents, or the large change produced by 1 M guanidine HCl. With high concentrations of the chaotropic agents at 20° the ORD spectrum showed denaturation of the ferredoxin over the course of a few minutes. This was much more rapid than the corresponding effect on the oxidized protein.

DISCUSSION

This study indicates that a number of different effects are induced by chaotropic agents on the physico-chemical properties of spinach ferredoxin. It is probable that at least some of these effects would be observed with other iron-sulphur proteins. Preliminary studies in our laboratory have indicated that the agents can cause changes in the EPR spectrum of adrenodoxin.

The observation that organic solvents such as methanol can produce similar effects suggests that the basic cause is a change in the structure of water around the ferredoxin molecule. With some of the changes of the EPR spectrum, we were unable to detect corresponding changes in the ORD of reduced ferredoxin. It may be that the EPR rather than the optical spectrum is a more sensitive monitor of small changes in the iron-sulphur group. The EPR spectra were measured on frozen samples, and it is possible that the chaotropic agents might induce small changes in the spectrum (e.g. decreased linewidths) by alteration of the structure of the ice around the ferredoxin molecules. However the larger EPR changes (Fig. 2d & 3) and the ORD effects (Fig. 4) are most probably due to changes in the conformation of the ferredoxin molecule.

The observation that the effects on the EPR and ORD spectra are reversible suggests that iron-sulphur proteins can be isolated with chaotropic agents without undergoing irreversible structural alterations. However the ORD results indicate that ferredoxin is unstable in the presence of high concentrations of chaotropic agents, especially in the reduced form. If this is true of other iron-

sulphur proteins, it indicates that they are best isolated by chaotropic agents under conditions in which they are oxidized.

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