The Effect of Detergents on Bovine Adrenal Ferredoxin

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The incubation of adrenal ferredoxin with various detergents in the presence of oxygen or ferricyanide leads to bleaching of the protein. The bleached preparation has the properties of apoferredoxin and it can be reconstituted with high yield by conventional methods.

The iron-sulphur protein from adrenocortical mitochondria (adrenodoxin, adrenal ferredoxin) is a component of the NADPH-dependent steroid-hydroxylation system (Omura *et al.*, 1966; Katagiri & Takemori, 1973). Adrenal ferredoxin forms complexes with phosphatidylcholine and phosphatidylethanolamine, and a hypothesis has been advanced that the interaction of ferredoxin and membrane lipids may be a physiologically important means of regulation of mineralocorticoid biosynthesis (Wickramasinghe, 1973). The interaction between detergents and proteins may be considered as a model of lipid-protein interaction. On the other hand, detergent binding to proteins is well known to be accompanied by conformational changes in the proteins (Tanford, 1968; Steinhardt & Reinolds, 1970). We therefore decided to investigate the interaction of detergents with adrenal ferredoxin.

Materials and Methods

Crystalline ferredoxin from bovine adrenal cortex was prepared as described by Suhara *et al.* (1972). The preparation was electrophoretically homogeneous and had a spectral purity index, E_{278}/E_{415} , of 0.86. Before the experiment the protein was dissolved in 50 mM-sodium/potassium phosphate buffer, pH 7.4, and subjected to gel filtration through Sephadex G-25 to remove $(\text{NH}_4)_2\text{SO}_4$. The reconstitution of adrenal ferredoxin was carried out as described by Suhara *et al.* (1974). Iron was determined by the method of Cameron (1965). DEAE-cellulose DE-52 was purchased from Whatman Biochemicals, Maidstone, Kent, U.K. Cetylpyridinium chloride was obtained from T. Schuchard, Munich, Germany. Triton X-100 was supplied by Sigma Chemical Co., St. Louis, MO, U.S.A. Sodium dodecyl sulphate, manufactured in the Chimreactiv, Shostkin, U.S.S.R., was recrystallized from ethanol. Other chemicals used were reagent grade. Detergents were dissolved in 50 mM buffer, pH 7.4. Optical spectra were recorded in 10 mm cells at 25°C. For experiments under anaerobic conditions Thunberg cuvettes were used which were evacuated to 12 Pa

(0.1 mmHg). Ferricyanide concentration was checked by using $\epsilon_{420} = 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$, and ferredoxin concentration was determined by using $\epsilon_{415} = 9.8 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

Results

Incubation of oxidized ferredoxin with detergents under aerobic conditions led to irreversible bleaching of the protein. The corresponding kinetic curves (Fig. 1) show that ionic detergents result in more effective bleaching than non-ionic Triton X-100. Ferredoxin decoloration takes place at detergent

Fig. 1. Bleaching of oxidized ferredoxin under aerobic conditions in the presence of detergents

The experiments were carried out in 50 mM-sodium/potassium phosphate buffer, pH 7.4, at 25°C. Curve 1, 15 mM-Triton X-100; curve 2, 17 mM-sodium dodecyl sulphate; curve 3, 14 mM-cetylpyridinium chloride; curve 4, 17 mM-sodium dodecyl sulphate, in the presence of 1.2 M-NaCl.

Fig. 2. Spectral changes in the oxidized ferredoxin under the effect of detergents

—, Native protein in phosphate buffer; ----, 2 min after addition of sodium dodecyl sulphate (0.5%); — · —, 6 h after addition of sodium dodecyl sulphate (0.5%). Similar changes were observed with cetylpyridinium chloride.

concentrations both higher and lower than the critical micelle concentration of each detergent. Buffer solutions of higher ionic strength induce faster decoloration (Fig. 1, curves 2 and 4). During protein bleaching all the spectral bands accounting for the iron-sulphur centre decrease to the same extent; fully decolorized ferredoxin exhibits u.v. absorption at 280 nm of the same pattern as that of the native protein. Fig. 2 shows the spectra of the starting protein and of the protein/detergent mixture immediately after detergent addition and the absorption of the fully bleached ferredoxin. Addition of the ionic detergents very rapidly results in some changes in the spectral bands corresponding to the iron-sulphur centre of the protein. The initial changes in the optical spectrum of ferredoxin induced by sodium dodecyl sulphate are reversible: the spectrum of native ferredoxin was observed after removal of the detergent by gel filtration of the mixture through Sephadex G-25. In the evacuated cells the ferredoxin decoloration proceeds more slowly than under aerobic conditions, the accelerating effect of oxygen being observable for reduced ferredoxin likewise. Decomposition of the reduced iron-sulphur centre is accompanied by disappearance of the e.p.r. (electron-paramagnetic-resonance) signal, recorded at 77 K.

Decoloration of adrenal ferredoxin in the presence of detergents can be carried out with ferricyanide instead of oxygen. Addition of ferricyanide to the oxidized protein in the absence of sodium dodecyl sulphate does not bring about any decoloration for 1 h at least. However, in the presence of sodium dodecyl sulphate the protein is completely bleached within 2–3 min, and the ferricyanide is accordingly reduced. Titration of the oxidized ferredoxin solution in the presence of detergents indicates that each mol

of the protein can reduce approx. 8 mol of ferricyanide. Thus the decomposition of the iron-sulphur centre of the protein in the presence of detergents is caused by its non-specific oxidation. By chromatography on DEAE-cellulose DE-52 of protein preparations bleached by the detergents we obtained a main protein fraction lacking iron. Incubation of this fraction with mercaptoethanol, Fe^{2+} and Na_2S under the conditions described by Suhara *et al.* (1974) leads to the preparation having optical and e.p.r. spectra similar to those of the native adrenal ferredoxin. The yield of such reconstituted protein was about 75%. The preparation reconstituted by using ^{57}Fe has an e.p.r. spectrum similar to that published by Cammack *et al.* (1971).

Discussion

The bleaching of various ferredoxins in the presence of oxidants and urea studied by Petering *et al.* (1971) has essentially the same characteristics as those that we report here for detergent-containing media. It may be concluded from our data that the treatment of ferredoxin by the detergents results mainly in apoprotein formation. Thus the data presented probably mean that the iron-sulphur centre of ferredoxin is in a hydrophobic environment and that detergents disturb this environment. It is clear from the above-mentioned data that the interaction of detergents with the adrenal ferredoxin induces such changes in the protein conformation that its iron-sulphur centre is destroyed in the presence of oxidizing agents. Similar conformational changes probably occur on complexing of the cytochrome and ferredoxin, noted by Manabe & Kimura (1974). On formation of the complex, the process of iron-sulphur

centre decomposition and cytochrome reduction takes place, one molecule of ferredoxin reducing 9 molecules of cytochrome *c*. We have been able to confirm a large part of the data presented by Manabe & Kimura (1974) by using cytochrome *c* from adrenal cortex (S. S. Mardanyan, N. A. Grigoryan & R. M. Nalbandyan, unpublished work). However, the data in the present communication on the effect of detergents on adrenal ferredoxin are suggestive of non-specific oxidation of the iron-sulphur centre of the protein taking place after disturbance of its hydrophobic core. The cytochrome-ferredoxin complex is mainly bound by electrostatic forces (animal cytochrome *c* is a basic protein with pI 10.5, whereas animal ferredoxin is acidic with pI about 4). Chromophoric centres of both proteins are deep in the hydrophobic cores (Mukai *et al.*, 1973; Takano *et al.*, 1973), and after formation of the complex possibly these cores become involved in interaction. Cytochrome then serves as an oxidizing agent, and as a result of such oxidation the iron-sulphur centre is decomposed.

Though both proteins are located in the inner membrane of the adrenocortical mitochondrion, they probably cannot be neighbouring links in the respiratory or steroid-hydroxylating chains, since their redox potentials differ considerably [−270 mV and +250 mV for adrenal ferredoxin and cytochrome *c* respectively (Huang & Kimura, 1973; Mardanyan *et al.*, 1976)]. It is not clear at present whether the interaction of cytochrome with adrenal ferredoxin,

with the subsequent iron-sulphur-centre decomposition and cytochrome reduction observed *in vitro*, has physiological significance.

References

- Cameron, B. F. (1965) *Anal. Biochem.* **11**, 164–169
Cammack, R., Rao, K. K., Hall, D. O. & Johnson, C. E. (1971) *Biochem. J.* **125**, 849–856
Huang, J. J. & Kimura, T. (1973) *Biochemistry* **12**, 406–409
Katagiri, M. & Takemori, Sh. (1973) *Mol. Cell. Biochem.* **2**, 137–152
Manabe, T. & Kimura, T. (1974) *FEBS Lett.* **47**, 113–116
Mardanyan, S. S., Sarkisova, E. G., Osipova, E. A., Grigoryan, N. A. & Nalbandyan, R. M. (1976) *Bio-khimiya* **41**, 223–227
Mukai, K., Kimura, T., Hebert, J. & Kevan, L. (1973) *Biochim. Biophys. Acta* **295**, 49–56
Omura, T., Sanders, E., Estabrook, R. W., Cooper, D. I. & Rosenthal, O. (1966) *Arch. Biochem. Biophys.* **117**, 660–673
Petering, D., Fee, J. A. & Palmer, G. (1971) *J. Biol. Chem.* **246**, 643–653
Steinhardt, J. & Reynolds, J. A. (1970) *Multiple Equilibria in Proteins*, Academic Press, New York
Suhara, K., Takemori, Sh. & Katagiri, M. (1972) *Biochim. Biophys. Acta* **263**, 272–278
Suhara, K., Kanayama, K., Takemori, Sh. & Katagiri, M. (1974) *Biochim. Biophys. Acta* **336**, 309–317
Takano, T., Kallai, O. B., Swanson, R. & Dickerson, R. E. (1973) *J. Biol. Chem.* **248**, 5234–5255
Tanford, C. (1968) *Adv. Protein Chem.* **23**, 121–282
Wickramasinghe, R. H. (1973) *Cytobiology* **8**, 81–94