IMMUNOLOGICAL COMPARISON OF FERREDOXINS

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1. Introduction

The biological significance of ferredoxins in various processes, and the increasing number of them that have been isolated and characterized from bacterial, plant and animal systems, have stimulated investigations on the physico-chemical properties of these proteins [1,2]. Antibodies prepared against *Clostridium*, spinach and swiss chard ferredoxins have been used to investigate the structure, function and localization of these ferredoxins [3–8]. This communication reports a comparison of the interaction of antibodies against ferredoxin from swiss chard, with ferredoxins isolated from higher plants, algae, bacteria and animals, and indicates an immunological correlation scale between these different types of ferredoxins.

2. Materials and methods

Ferredoxins were isolated essentially by the methods of Tagawa and Arnon [9] or of Rao et al. [10]. Spinach ferredoxin-NADP reductase was prepared by the method of Shin [11]. Apoferredoxins were prepared by precipitation with trichloroacetic acid [12] The preparation of antibodies and immunoglobulin fraction [7], and precipitation reactions [13] were as described previously. For assays of ferredoxin-mediated cytochrome *c* reduction, the reaction mixture consisted of 50 mM potassium phosphate, pH 7.8; 25 μM horse-heart cytochrome *c* (Boehringer); 0.5 μM ferredoxin-NADP reductase; and 50 μM NADPH, in a total volume of 1.0 ml; cytochrome *c* reduction was measured spectrophotometrically at 550 nm.

3. Results and discussion

Preliminary studies on immunoglobulins prepared against the two-iron ferredoxin from swiss chard indicated no interaction with the eight-iron ferredoxin from *Clostridium*, and a considerable interaction with the two-iron ferredoxin isolated from the blue-green alga *Phormidium persicinum* [7,8]. We have extended the investigation to a range of ferredoxins from plant, algal, bacterial and animal sources. All of these ferredoxins are of the two-iron type, except for the eight-iron ferredoxin and the four-iron high-potential iron-sulphur protein, both from *Chromatium*.

As described in table 1, the interaction between the antibody and various antigens was measured by; (i) a short-term precipitation consisting of a 1-hr incubation at 37°C, and (ii) a long-term precipitation, consisting of a 1-hr incubation at 37°C followed by an overnight incubation at 4°C [13].

From table 1 it can be seen that of the higher

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Table 1
Precipitin formation between ferredoxins and anti-swiss chard ferredoxin immunoglobulin.

Ferredoxin Species	Concentration μM	A_{280} short interaction	Long interaction
Higher plants			
Swiss chard (*Beta vulgaris*)	2.4	0.40	0.64
Swiss chard apoferredoxin	2.4	0.29	0.34
Spinach (*Spinacea oleracea*)	2.24	0.40	0.51
Spinach apoferredoxin	2.24	0.25	0.25
Alfalfa (*Medicago sativa*)	2.35	0.25	0.43
Parsley (*Petroselenum sativum*)	2.1	0.06	0.18
Maize (*Zea mays*)	2.2	0	0.18
Horsetail (*Equisetum telmateia*)	2.2	0	0.11
Green Algae			
Euglena gracilis	2.24	–	0.14
Scenedesmus obliquus	2.4	0	0.09
Bumilleriopsis filiformis	2.28	–	0.08
Blue-green Algae			
Spirulina maxima	2.3	0	0.16
Anabaena variabilis	2.38	0	0.17
Anacystis nidulans	2.52	0	0.13
Nostoc spp.	2.4	–	0.10
Animal			
Porcine adrenal	2.0	–	0.01
Bacteria			
Chromatium ferredoxin (8Fe)	2.5	0	0.02
Chromatium high potential iron protein	2.7	–	0.01

Precipitation reactions were carried out under standard conditions [13] for 'long interaction' and for only 1 hr at 37°C for 'short' incubation (see text). Control experiments for these ferredoxins with normal non-immunized rabbit immunoglobulin were almost zero.

plant ferredoxins, spinach and alfalfa interact strongly with the antibody, while parsley and maize interact more weakly. Ferredoxins from the primitive vascular plant *Equisetum* (horsetail) and the algae show only weak interactions, which agrees physico-chemical observations that *Equisetum* ferredoxin resembles those of the algae [14]. No significant interaction was observed with iron-sulphur proteins from non-plant sources.

The interaction of the apoferredoxins (lacking iron and labile sulphide) with the antibody was about half the extent of the interaction with the native ferredoxins. These results are similar to those reported for clostridial apoferredoxins [3,5] and suggest that at least a proportion of the antibody molecules are directed specifically at an antigenic determinant present only in the native ferredoxin.

We have studied the effect of the anti-swiss chard ferredoxin immunoglobulin on the ferredoxin-mediated reduction of cytochrome *c* in the presence of NADPH and ferredoxin-NADP reductase (table 2). A normal immunoglobulin fraction was used as a control. After 5 min incubation with the antibody, all of the ferredoxins from higher plants were inhibited to a considerable extent, including maize which failed to give a short-term precipitin reaction. Therefore it is clear that although this ferredoxin reacts with the antibody in a short time, there is a considerable delay before a three-dimensional precipitin lattice can be formed. Cytochrome *c* reduction mediated by the ferredoxins from the algae *Scenedesmus* and *Spirulina* was inhibited only at lower ferredoxin concentration, indicating that some of the antigenic sites of swiss chard ferredoxin may

Table 2
The effect of anti-swiss chard immunoglobulin on ferredoxin-mediated cytochrome *c* reduction by NADPH

Ferredoxin			Activity with globulin (% of control)	
Species	Concentration μM	Control activity	anti Fd. 1G.	Normal 1G.
Swiss chard	0.48	36.5	22	71
Spinach	0.56	37.8	50	106
	0.33	17.4	26	86
Alfalfa	0.56	41.4	41	103
Maize	0.55	19.5	63	106
Scenedesmus	0.54	40.1	84	84
	0.36	21.4	70	91
Spirulina	0.55	53.5	109	109
	0.37	35.7	69	106

Ferredoxin samples were preincubated for 5 min at 20°C with 0.2 ml of immunoglobulin fraction from immunized or non-immunized rabbits, as indicated, then assayed as described under Materials and methods. Activity is expressed in nmol cytochrome *c* reduced/min.

be missing in the algal species, so that only a fraction of the antibody molecules are active against them.

On the basis of the results of tables 1 and 2, we suggest that the ferredoxins can be separated into four groups differing in the degree of interaction with anti-swiss chard ferredoxin antibody; (a) swiss chard, spinach and alfalfa, which give a strong precipitin reaction under long and short-term conditions; (b) parsley and maize ferredoxin which exhibit a moderate interaction; (c) *Equisetum* and algal ferredoxins, which show no precipitin reaction in the short term and a weak interaction in the long term, and a slight inhibition of the activity in the enzyme assay; (d) the proteins from the photosynthetic bacterium *Chromatium* and the two-iron ferredoxin from pig adrenal, which show no significant interaction at all.

The fact that some ferredoxins show short-term precipitin reactions while others do not, may be due to the existence of binding sites on the ferredoxin with high and low affinities. Thus the antibodies might bind to certain sites on group (a) and (b) ferredoxins with high affinity, but bind to equivalent sites on group (c) ferredoxins with low affinity. Alternatively, groups (a) and (b) may have a binding site which is completely absent from group (c).

These studies demonstrate that, although the antibody is active with a wide range of two-iron plant ferredoxin species, there is a varying degree of specifictiy. This can be explained by a diversity of antigenic determinants on the ferredoxin molecule and different binding site specificities in the antibody population. Further investigations using anti-bacterial and anti-algal ferredoxin immunoglobulins may provide a more comprehensive study of the antigenic properties of ferredoxins.

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