ONE-SITE REACTIVITY OF HALOBACTERIAL 2Fe-FERREDOXIN AS A PLANT FERREDOXIN SUBSTITUTE

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1. Introduction

A 2Fe-ferredoxin isolated from several *Halobacterium* species was reported to resemble in many respects chloroplast-type ferredoxins [1,2]. Thus, optical characteristics, the EPR spectrum, and other physical properties resembled more closely plant rather than bacterial 2Fe-ferredoxins. Ferredoxin isolated from a *Halobacterium* of the Dead Sea cross-reacted to an extent of 15% with an algal ferredoxin (from *Spirulina maxima*), whereas it did not cross-react with the bacterial 2Fe-ferredoxins [3]. Moreover, a high degree of similarity was found between the amino acid sequences of halobacterial ferredoxins and that of the blue green alga *Nostoc muscorum* [4,5].

However, as has already been pointed out, functional differences exist between halobacterial and chloroplast type ferredoxins, in particular with respect to the ability of the former to substitute for the latter in the photoreduction of NADP by illuminated chloroplasts [1,2,6]. In the present study we investigated these functional differences and the mechanism underlying them in detail. Halobacterial ferredoxin is shown to be rather efficient in accepting electrons from the photosynthetically produced reductant, but rather poor in its further interaction with the flavoprotein.

2. Material and methods

Halobacterium of the Dead Sea and Swiss chard (*Beta vulgaris*) ferredoxins were prepared as previously described [2,7]. Their concentrations were determined using ϵ_{420} values of 9.1 and 9.8 mM^{-1} cm^{-1}, respectively. Lettuce chloroplasts [8] and partially purified (through the DEAE-cellulose column fractionation) ferredoxin-NADP-reductase [7] were prepared by published procedures. The flavoprotein concentration was determined using ϵ_{457} = 10 mM^{-1} cm^{-1}. Cytochrome *c* was purchased from Sigma.

NADP and cytochrome *c* photoreductions were measured as previously described [9]. Cytochrome *c* reduction by NADPH, catalyzed by ferredoxin-NADP-reductase was followed at 550 nm [10]. The photoreduction of ferredoxin by chloroplasts [11] and the formation of the ferredoxin-flavoprotein complex [12] were followed in an Aminco DW-2 Dual-Wavelength-Spectrophotometer.

3. Results

3.1. *Electron transport*

Fig.1 (left) illustrates that halobacterial ferredoxin can function well as an electron carrier in the photoreduction of cytochrome *c* catalysed by ferredoxin-supplemented chloroplasts. Its activity in this reaction compares well with that of the native plant ferredoxin. However, it is very inefficient in catalysing the photoinduced electron transfer to NADP (fig.1, centre). It thus seems that the interaction with the chloroplast-generated reductant functions well in the halobacterial ferredoxin but the interaction with the flavoprotein does not. This is corroborated by the finding that the efficiency of the halobacterial ferredoxin in catalysing the reduction of cytochrome *c* by NADPH, in the presence of the flavoprotein, was poor (fig.1, right). Furthermore, on turning off the light in the presence

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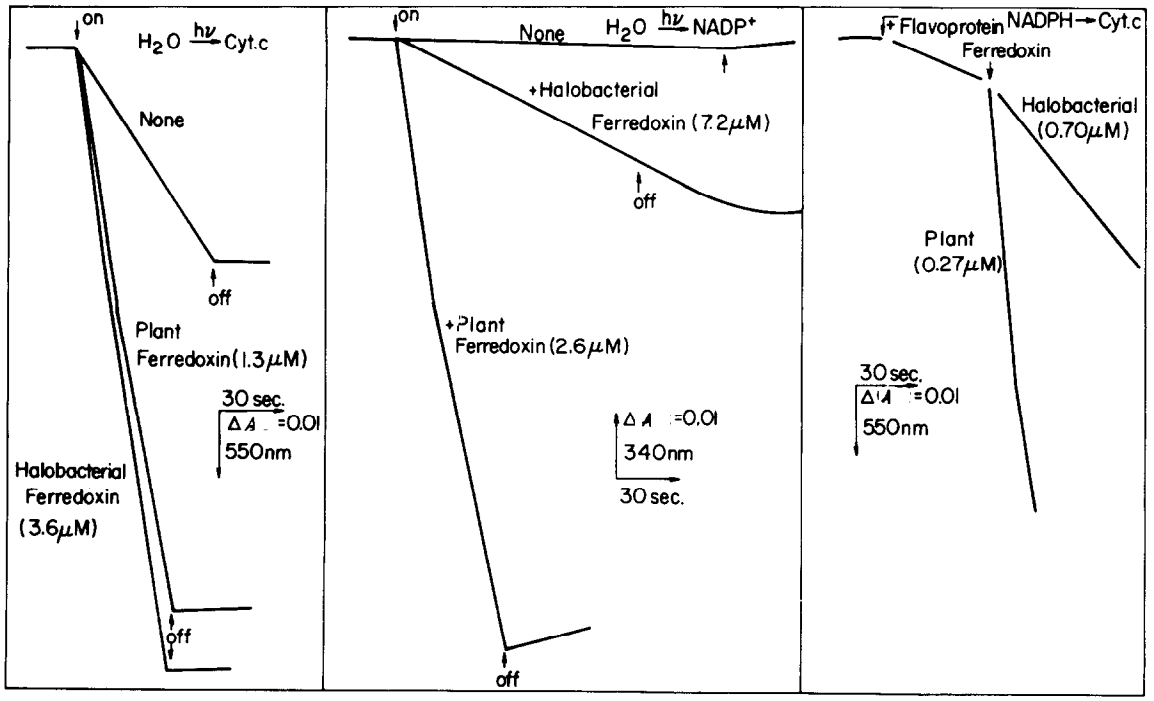

Fig.1. Competence of halobacterial ferredoxin in catalysing several plant ferredoxin dependent reactions. All reaction mixtures contained in a total volume of 3.0 ml: Tricine, pH 7.8, 20 mM; NaCl, 30 mM and the amount of ferredoxin indicated. The H_2O to cyt. *c* reaction contained in addition: cyt. *c*; 25 μM; and chloroplasts containing 3 μg of chlorophyll. The H_2O to NADP reaction contained in addition: $NADP^+$, 1 mM; and chloroplasts containing 21 μg chlorophyll. The NADPH to cyt. *c* reaction contained in addition: NADPH, 10 μM; cyt. *c* 10 μM; and ferredoxin-NADP-reductase (3 μl) to give the observed rate. Where indicated (on,off) illumination was with saturating red light provided by a 500 W slide projector filtered through a Schott RG 645 filter. The photomultiplier was protected from the actinic light with 1 cm of a saturated $CuSO_4$ solution.

of the halobacterial ferredoxin, but not the plant ferredoxin, reduction of NADP continued for some time in the dark (fig.1, centre) indicating that halobacterial ferredoxin was rather reduced in its light steady state. Thus, the interaction between reduced halobacterial ferredoxin and flavoprotein was considerably slower than that between photogenerated reductant and ferredoxin.

Table 1 lists the values of the apparent K_m for the two ferredoxins and of the V_{max} values determined

Table 1
Comparison of the apparent K_m and V_{max} values of plant and *Halobacterium* ferredoxin in three plant-ferredoxin catalysed reactions

Reaction	Apparent K_m of ferredoxin		Relative maximal activity
	Plant	*Halobacterium*	V_{max} (Halob.) / V_{max} (plant)
	μM	μM	
$H_2O \rightarrow$ cyt *c*	0.40	3.2	0.91
$H_2O \rightarrow$ NADP	2.2	2.6	0.08
NADPH → cyt *c*	0.29	1.7	0.14

The values were determined from Lineweaver–Burk plots of data obtained under the conditions specified in fig.1

for all of the above-mentioned reactions. It is evident from the table that the poor ability of the halobacterial ferredoxin to function in the two flavoprotein-requiring reactions is largely due to changes in V_{max}.

3.2. *Photoreduction of ferredoxin*

To further check the efficiency of halobacterial ferredoxin as an electron acceptor of the photoproduced reductant we monitored ferredoxin photoreduction directly in a dual wavelength spectrophotometer (fig.2). It is clear that halobacterial ferredoxin is reduced efficiently by the photochemically generated reductant. Upon turning off the light the reoxidation process of halobacterial ferredoxin is slower than that of plant ferredoxin. The slower reoxidation rate probably accounts for the fact that the steady-state redox level of the halobacterial ferredoxin in the light (in air) is more reduced than that of the plant ferredoxin. Thus, in the experiment of fig.2, the halobacterial ferredoxin was 98% reduced in the light, whereas the plant ferredoxin was only 42% reduced.

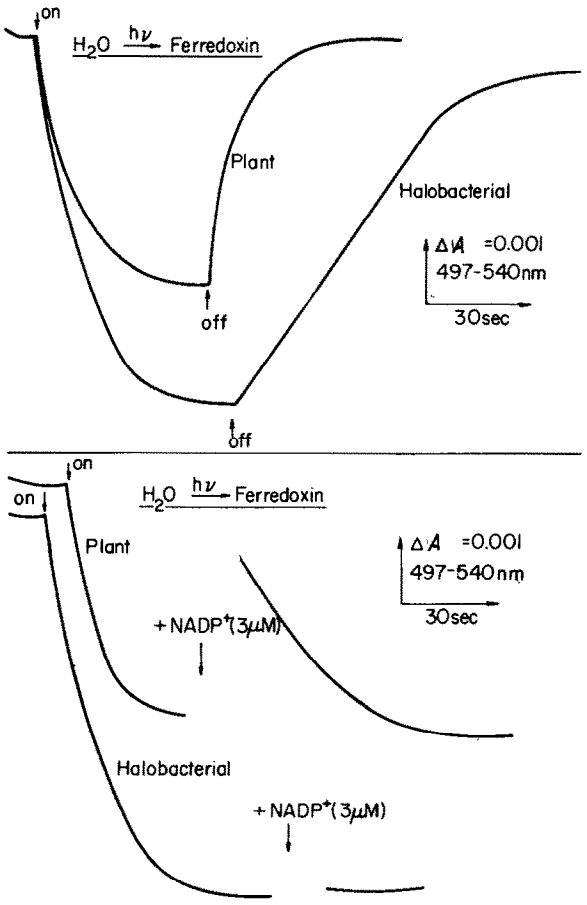

Fig.2. Photoreduction of halobacterial and plant ferredoxin. The reaction mixture contained in a total volume of 3.0 ml: Tricine, pH 7.8, 20 mM; NaCl, 30 mM; chloroplasts containing 37 μg of chlorophyll, and where indicated 2.5 μM halobacterial ferredoxin or 3.8 μM plant ferredoxin. Illumination as described under fig.1. Other details as described in section 2.

As was previously shown [11] addition of small amounts of NADP to the photoreduced plant ferredoxin in the light causes a rapid oxidation of photoreduced ferredoxin followed by its rereduction as the NADP is reduced. With halobacterial ferredoxin, NADP was incapable of causing the same oxidation-reduction cycle (fig.2).

3.3. *Complex formation*

The poor activity of halobacterial ferredoxin in flavoprotein-requiring reactions raised the possibility that this ferredoxin may be incapable of forming the 1:1 complex which occurs between plant ferredoxin and flavoprotein [13].

Fig.3 illustrates that this indeed seems to be the case. Halobacterial ferredoxin did not produce the typical difference spectrum of the complex, even though its absorption is essentially identical to that of plant ferredoxin [2].

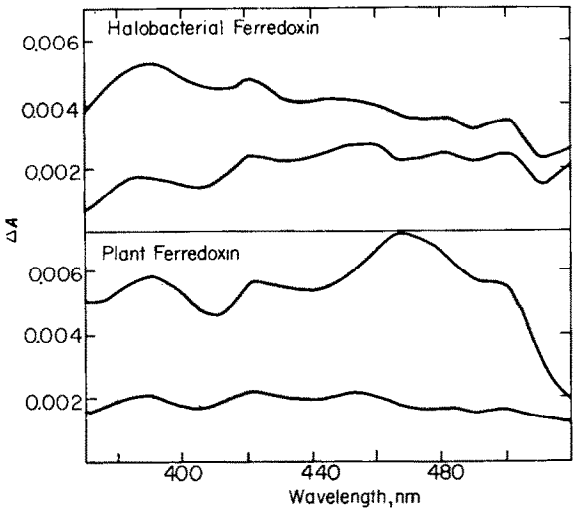

Fig.3. Inability of halobacterial ferredoxin to produce the typical difference spectrum of plant ferredoxin on combination with ferredoxin-NADP-reductase. Halobacterial (23 μM) or plant (21 μM) ferredoxin were placed in one side of two split cuvettes; and 20 μM ferredoxin-NADP-reductase in the other side. The baseline spectra presented on the bottom of each half figure were then recorded. One of the two cuvettes was then thoroughly mixed, and the difference spectrum presented as the top curve of each half figure was recorded.

4. Discussion

It is to our knowledge the first time that functional differences have been demonstrated in native 2Fe-ferredoxins with respect to the ability to interact with their two native partners in the photosynthetic system. Halobacterial ferredoxin is capable only of performing a partial reaction: it can accept electrons from the photochemically produced reductant but can only poorly donate electrons to the flavoprotein. The evidence indicates that this is due to the inability of flavoprotein ferredoxin-NADP-reductase to effectively interact with halobacterial ferredoxin. Thus, halobacterial ferredoxin cannot catalyse well the electron transfer reactions between photochemically produced reductant and flavoprotein, and the flavoprotein-mediated reaction between NADPH and cytochrome *c*, nor does it interact with the flavoprotein to form a 1:1 complex with the usual, visible absorption shifts. Nevertheless it is competent in serving as an electron acceptor to the photochemically generated reductant, and in catalysing the photochemical reduction of cytochrome *c*. The similarity in apparent K_m values of plant and *Halobacterium* ferredoxins for $NADP^+$ reduction (table 1) implies that the difference between them is not due to an impaired recognition of halobacterial ferredoxin by the enzyme. This is corroborated by the observation that halophilic ferredoxin acts as a competitive inhibitor of plant ferredoxin (unpublished results).

A similar effect, i.e. loss of the ability to catalyse many flavoprotein-requiring reactions, was observed in spinach ferredoxin when it was modified by trinitrophenylation [13,14], presumably at a lysyl residue. Also in that case the loss of activity caused by the modification was attributed to the inability of modified ferredoxin to effectively interact with flavoprotein. However, contrary to halobacterial ferredoxin, the trinitrophenylated ferredoxin was effective in catalysing the flavoprotein-dependent reaction between NADPH and cytochrome *c* [14]. It is interesting to note that ferredoxin of the Dead Sea *Halobacterium* is not only much more acidic than spinach ferredoxin, but also has 1 lysyl residue less than the latter. It is tempting to speculate that it is this lysyl residue in spinach ferredoxin which is responsible for the favorable interaction with flavoprotein. If so, this residue should be conserved in all 2Fe-ferredoxins from photosynthetic origin.

Finally, with respect to the evolutionary position of *Halobacterium*, it has recently been proposed [15] that its ferredoxin appears to have evolved from an ancestor of the blue-green algae and of chloroplasts. If this is the case, then the divergent event may have coincided with the loss of its ability to donate electrons in the photosynthetic system or with the development of this ability in chloroplast-type ferredoxins.

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