COMPLEX FORMATION BETWEEN FERREDOXIN AND NITRITE REDUCTASE

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1. Introduction

Assimilatory reduction of nitrite to ammonia in green algae and plants occurs in two stages: nitrate is first reduced to nitrite with NADH serving as the electron donor, and nitrite is then reduced to ammonia with reduced ferredoxin acting as the electron donor [1,2]. The 6 electron reduction of nitrite to ammonia is catalyzed by the enzyme ferredoxin:nitrite oxidoreductase (EC 1.6.6.4) which has been shown to contain siroheme and an Fe_2S_2 iron–sulfur center as prosthetic groups [3–6].

In oxygen-evolving photosynthetic organisms, reduced ferredoxin serves as the electron donor for the reduction of $NADP^+$ as well as of NO_2^- [7]. This process has been shown to involve a complex between ferredoxin and the flavoprotein, ferredoxin:$NADP^+$ oxidoreductase [8–10]. Other biological electron transfer reactions may also involve protein:protein interactions between ferredoxin and another electron carrier. For example, such interactions have recently been detected between a ferredoxin and cytochrome c_3 in the sulfate-reducting bacterium *Desulphovibrio gigas* [11]. The existence of complexes between ferredoxins and other enzymes and the known affinity of ferredoxin–Sepharose for ferredoxin:nitrite oxidoreductase [12,13] raised the possibility that a complex between ferredoxin and nitrite reductase can also form. We present here spectral evidence for the formation of such a complex.

2. Methods

Ferredoxin:nitrite oxidoreductase (nitrite reductase) was prepared from spinach as in [5] with the exception that chromatography on Sephacryl S-200 replaced chromatography on Sephadex G-200 as the final purification step. Diaphorase assays [14] showed the preparation to be completely free of ferredoxin:$NADP^+$ oxidoreductase, an enzyme known to form a complex with ferredoxin [8–10].

Ferredoxin was prepared from spinach by a modification of the procedure in [15]. Extinction coefficients of 39.7 mM^{-1} cm^{-1} (386 nm) for nitrite reductase(s) and 9.7 mM^{-1} cm^{-1} (420 nm) for ferredoxin [16] were used to measure the protein concentrations. Difference spectra were measured on an Aminco DW-2a spectrophotometer (monochromator half-band width = 1 nm) using dual compartment cells (Precision Scientific) as in [9]. The baseline was adjusted to zero with nitrite reductase in both cells prior to the addition of ferredoxin.

3. Results and discussion

Figure 1 shows the difference spectrum between

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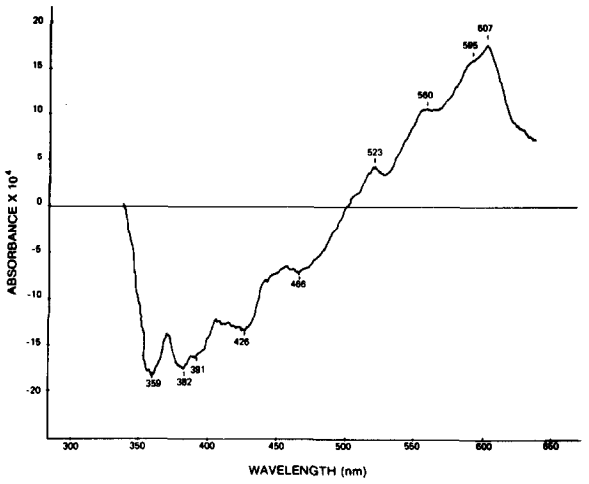

Fig.1. Difference spectrum of the nitrite reductase–ferredoxin complex. The reaction mixture contained 3.7 μM nitrite reductase and potassium phosphate buffer (pH 7.7, 0.08 M ionic strength).

a mixture of ferredoxin and nitrite reductase (1.2:1) and the sum of the spectra of equivalent amounts of ferredoxin and nitrite reductase separately. Presumably the difference spectrum results from interactions between the two proteins in the complex formed when both are present in the same reaction mixture. In all likelihood the features at 523 nm, 560 nm, 595 nm and 607 nm arise from perturbations of the siroheme group of nitrite reductase, as iron–sulfur proteins generally do not exhibit distinct spectral features in this region. The features in the difference spectrum at 382 nm and 391 nm may also arise from perturbation of the siroheme Soret band (found at 386 nm in uncomplexed nitrite reductase) during complex formation. The features at 426 nm and 466 nm can tentatively be attributed to alterations of the ferredoxin absorbance bands at 420 nm and 463 nm, although there may be contributions from the Fe_2S_2 center of nitrite reductase in this region as well. The feature at 359 nm cannot be uniquely assigned, at this time, to any of the chromophores present in the two proteins.

If all the spectral features present result from the formation of the same complex between ferredoxin and nitrite reductase then the change in absorbance produced by mixing the two proteins should show the same dependence on the ferredoxin:nitrite reductase ratio at all wavelengths. This is in fact the case, as is illustrated in fig.2 for two wavelengths (419 nm and 571 nm). All wavelengths examined showed the same dependence on ferredoxin:nitrite reductase ratio whether they were in a region where ferredoxin would be expected to be the primary absorbing species (e.g., 419 nm) or where nitrite reductase absorption would be expected to predominate (e.g., 571 nm is close to the 573 nm siroheme α band in the uncomplexed nitrite reductase).

The data in fig.2 allow a determination of the stoichiometry of the ferredoxin:nitrite reductase complex. The three lines in fig.2 show the expected behavior for 1:2 (dashed), a 1:1 (solid), and a 2:1

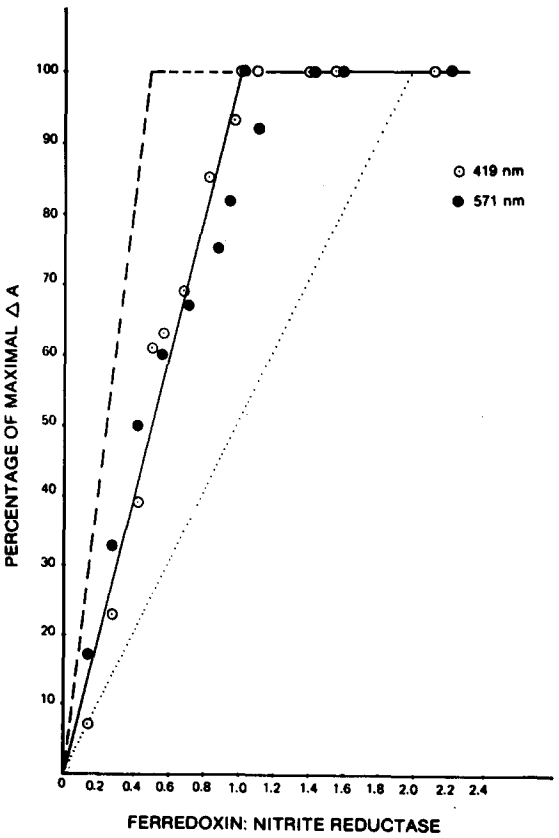

Fig.2. Titration of nitrite reductase with ferredoxin. Experimental conditions as in fig.1. The solid line represents the theoretical line for a 1:1 complex of ferredoxin with nitrite reductase, while the dashed and dotted lines represent those for 1:2 and 2:1 complexes, respectively.

(dotted) complexes (with negligably small dissociation constants) between ferredoxin and nitrite reductase. It is certainly possible to exclude a 2:1 complex from the data in fig.2. While it might be possible that the data in fig.2 represent a 1:2 complex of low affinity, the data are clearly most consistent with a 1:1 complex of high affinity between the two proteins. It perhaps should be mentioned that ferredoxin and ferredoxin:$NADP^+$ oxidoreductase also form a 1:1 complex [9].

The complex between ferredoxin and ferredoxin: $NADP^+$ oxidoreductase is stable only at low ionic strengths [8–10]. This fact and the fact that nitrite reductase can be eluted from a Sepharose–ferredoxin affinity column at high ionic strength suggested that the absorbance changes indicative of complex formation might be eliminated by raising the ionic strength. However, the difference spectrum shown in fig.1 was unaltered by the addition of NaCl up to 0.35 M. However, the two proteins could be separated from one another by applying the complex to a DEAE-cellulose column and subsequently eluting with NaCl.

The data presented in this paper and the reported binding of nitrite reductase to Sepharose–ferredoxin [12,13] support the idea that a complex between the two proteins can form. The possible role of this complex in the enzymic reduction of nitrite to NH_3 is under further investigation.

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