Algal Ferredoxin-NADP$^+$ Reductase with Different Molecular-Weight Forms

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Plastidic Ferredoxin-NADP$^+$ Reductase, Molecular Heterogeneity, Dimer Formation

Ferredoxin-NADP$^+$ reductase from the microalga *Bumilleriopsis* was isolated by a combination of affinity chromatography on a flavodoxin-Sepharose 4 B column and usual purification procedures. Both the elution pattern of the final gel filtration step and of the sodium dodecylsulfate (SDS) disc gel electrophoresis indicate that there are at least two different molecular-weight forms of the reductase, a monomeric form and a dimeric one.

Ferredoxin-NADP$^+$ reductase (Fd-NADP$^+$ reductase, oxidoreductase, EC 1.6.7.1) is the terminal redox carrier of the photosynthetic electron transport chain. It was first purified in a crystalline form from spinach leaves by Shin *et al.* [1]; nevertheless, it remained questionable whether this flavoprotein was electrophoretically homogeneous [2].

The easy formation of stoichiometric complexes between the reductase and ferredoxin or flavodoxin [3 – 5] was used advantageously to prepare homogeneous Fd-NADP$^+$ reductase from the alga *Bumilleriopsis filiformis.* Vischer (Xanthophyceae) by applying flavodoxin from *Peptostreptococcus elsdenii* immobilized on Sepharose 4 B according to [6]. The preparation of the reductase including affinity chromatography on immobilized flavodoxin has been described in [7]. Some more details are presented in this report. Fig. 1 A demonstrates the elution pattern of the final gel filtration step with Sephadex G-150. It clearly shows that two main fractions of the reductase with different molecular weights can be isolated. The molecular weight of the monomeric form (see below) was determined in separate Sephadex runs according to ref. 8 and was found to have 38,000 daltons. It could, therefore, be used as a marker protein

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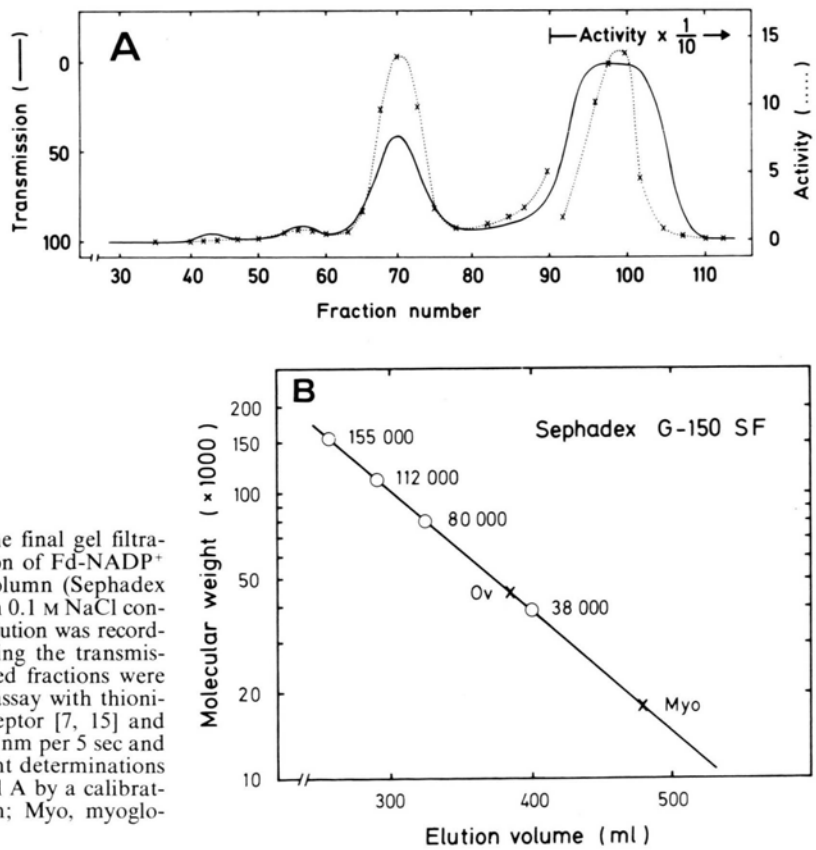

Fig. 1 A, B. (A) Elution diagram of the final gel filtration step (comp. [7]) in the preparation of Fd-NADP$^+$ reductase from *Bumilleriopsis.* The column (Sephadex G-150 superfine) was equilibrated with 0.1 M NaCl containing 50 mM Tris-HCl, pH 8.0. The elution was recorded with an LKB UviCord II, measuring the transmission at 280 nm. The activities of eluted fractions were determined by the transhydrogenase assay with thionicotinamide-NADP$^+$ as hydrogen acceptor [7, 15] and expressed as absorbance change at 400 nm per 5 sec and aliquot (of 10 µl). (B) Molecular-weight determinations of the eluted protein fractions of Fig. 1 A by a calibrated Sephadex column (Ov, ovalbumin; Myo, myoglobin).

in Fig. 1 B. The data of Fig. 1 B suggest that the 80,000-dalton protein fraction represents the dimer of the reductase. The molecular weights of the other two fractions were estimated to be 112,000 and 155,000 daltons. The latter are arbitrary figures, since the amounts of these two forms are very low and weights > 80,000 daltons lay outside the calibrated scale of the Sephadex column. Therefore, only the two main fractions were checked further.

In order to determine molecular weight and purity of the apparent monomer and dimer forms, both were subjected to SDS-gel electrophoresis (Fig. 2). Both enzyme forms migrated as a single band corresponding to a molecular weight of approximately 38,000 daltons. When both enzyme forms were cross-linked with dimethyl suberimidate according to [9] only the higher molecular weight form exhibited two bands in subsequent SDS-gel electrophoresis. This suggests again that we were dealing with a dimer of the reductase. In passing, it should be noted

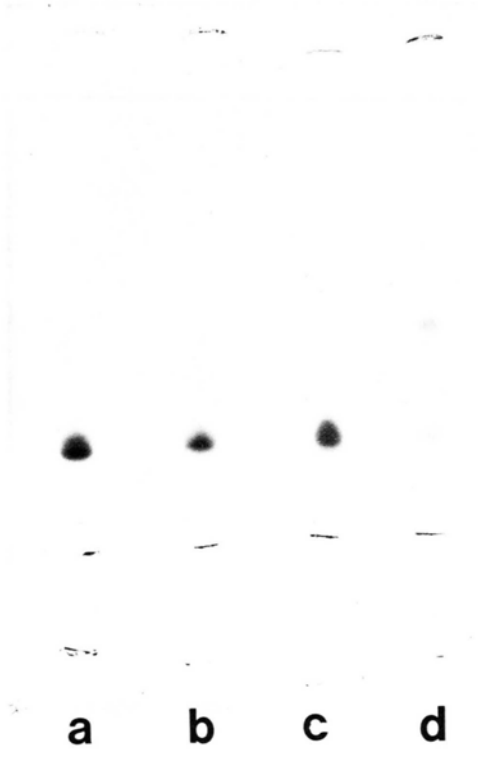

Fig. 2. SDS-disc gel electrophoresis of the 38,000 and 80,000 dalton enzyme species eluted from the Sephadex column of Fig. 1 A. Low-molecular weight form: not cross-linked (a), cross-linked (c); high-molecular form, not cross-linked (b), cross-linked (d). Both, cross-linking with dimethyl suberimidate and SDS electrophoresis were done according to ref. 9

that sometimes a small 27,000-dalton band was observed – particularly with older preparations – which most probably is due to (proteolytic) degradation. The absorption spectra of both forms were recorded immediately after elution from the Sephadex column. The most striking difference is between the absorbance ratios 414 to 318 nm being 3.3 for the monomer and 1.6 for the dimer. The ratio of 274/459 nm, however, is about equal — although not identical — for both forms, indicative of the same flavin content per protein.

Our results are compatible with the recent findings of Shin and Oshino [10] who also reported the isolation of two enzyme forms from spinach with different molecular weights. They used an affinity chromatography column with immobilized ferredoxin instead of flavodoxin. On the basis of gel filtration and SDS electrophoresis they concluded that the enzyme species of the higher molecular weight was a dimer of the reductase. The ratio of the absorbance maxima at 274 nm to 458 nm was estimated to be 7.5 for the monomer and 8.6 for the dimer. These values are clearly below those reported here for the reductase from *Bumilleriopsis*. Unfortunately, a comparison of additional spectral data is impossible since these authors confined themselves to the determination of the 274 nm to 458 nm ratio only.

Forms of Fd-$NADP^+$ reductases differing in molecular weight were also reported by Fredericks and Gehl [11] in crude spinach extracts. On further purification, which did not include affinity chromatography, the higher molecular-weight forms desintegrated, giving rise to a form of lower molecular weight estimated by gel filtration to be in the range of 50,000 daltons. Analysis of this purified reductase preparation by disc-gel electrophoresis demonstrated several unidentified contaminations as well as — assumed by the authors — two ionic forms of the flavoprotein [11]. Furthermore, by isoelectric focusing, five different ionic forms were found for the spinach reductase by Gozzer *et al.* [12], which were separated into two molecular-weight classes of 33,000 to 34,000 and 36,000 to 38,000 daltons, respectively (determined by SDS electrophoresis). Similar findings have previously been reported by Keirns and Wang [13]. However, their three different ionic forms of the spinach enzyme exhibited a uniform molecular weight of 37,000 daltons when determined by SDS-gel electrophoresis.

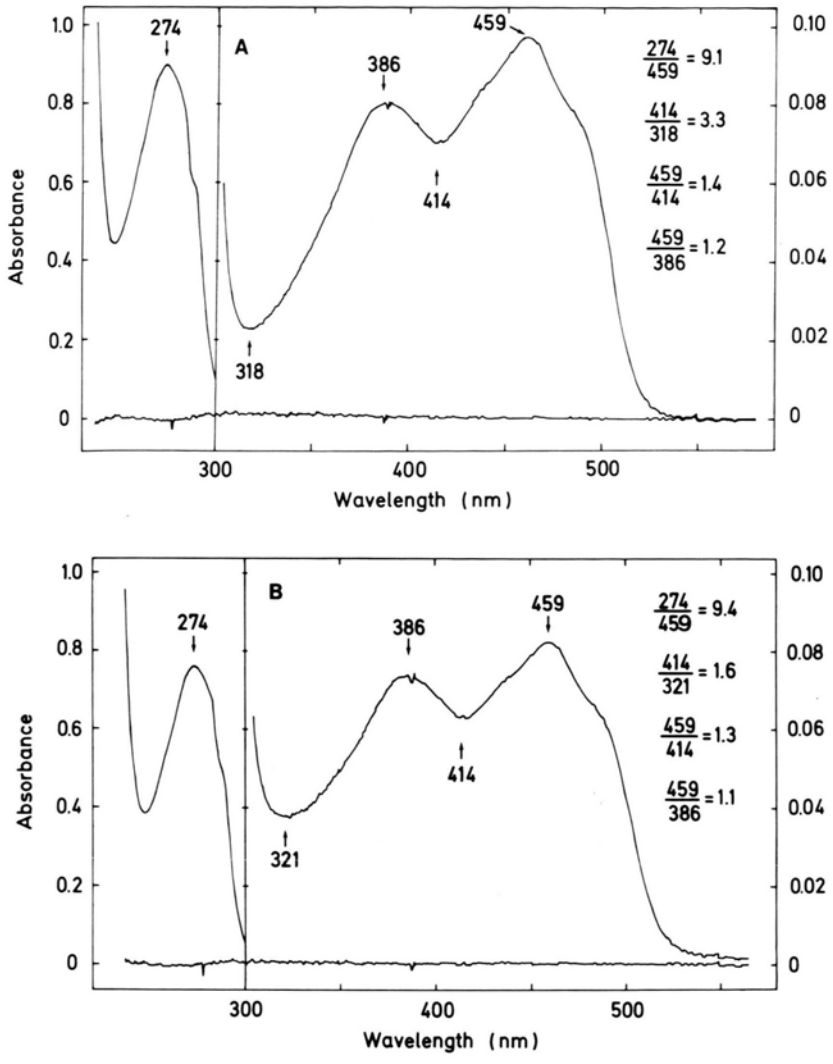

Fig. 3 A, B. Absorption spectra of the 38,000 dalton (A) and 80,000 dalton (B) enzyme species recorded after their elution from the Sephadex G-150 column (Spectrophotometer, Varian Super-Scan 3; protein dissolved in elution buffer as in Fig. 1 A, which also served as reference).

It remains an open question whether the occurrence of different forms of reductases is physiologically relevant or due to preparation factors. In this context, the work of Schneemann and Krogmann [14] should be mentioned, who described the isolation of a heterodimer of the reductase from spinach. The molecular weights of subunits were estimated (by SDS electrophoresis) to be 45,000 and 38,000 daltons. These bands apparently correspond to the enzyme classes with 33,000 and 38,000 daltons of Gozzer's preparation [12]. More detailed experiments are necessary to solve the apparent contradictions with respect to the heterogeneity of the reductase forms.

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Note added in proof:

Recently, Ellefson and Krogman [16] presented evidence that the different ionic enzyme forms are interconvertible and that differences in oxidation/reduction states are responsible for the appearance of enzyme forms with different isoelectric points. Another publication [17] suggested that beside the FAD-containing ferredoxin-$NADP^+$ reductase a membrane-bound form can be isolated without any detectable prosthetic group. However, is has to be substantiated that a native enzyme was isolated.

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