Some Properties of Plastidic Cytochrome b-563

H. J. Lach and P. Böger

Fachbereich Biologie der Universität Konstanz

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Plastidic Cytochrome b-563

Cytochrome b-563 from spinach chloroplasts was isolated in a homogeneous and enzymatically active form. It has a low, but variable lipid content and a molecular weight of about 18,000 daltons.

Cytochrome b-563 is a tightly bound ("integral") protein of chloroplast thylakoids present in algae and higher plants. Apparently, it functions as a redox carrier in cyclic electron flow [1]. Isolation, which has been reported [2, 3], encounters the same difficulties as with the plastidic cytochrome b-559. For both proteins the preparative procedures have been improved by us [4, 5], yielding cytochrome b-563 in a homogeneous and enzymatically active form.

The preparation was carried out with ethanol-extracted chloroplast material from spinach (*Spinacia oleracea*, strain Atlanta) as described [4] (see refs. 4 and 5 for general methods and materials). In this case, however, the extracted chloroplast material equivalent to 100 mg of chlorophyll was suspended in about 12 ml of 4 M urea, including 2% Triton X-100 and 50 mM Tris-HCl buffer, pH 8.0 [N-tris-(hydroxymethyl)-aminomethane], taking care to omit any thiol-group containing reagents which deliberately leads to destruction of cytochrome b-559, but not of b-563. The suspension was gently sonified as published [4], the resultant supernatant chromatographed on a Biogel A-1.5 m column (Biorad, Munich) as specified [5] and eluted in the oxidized form with 4 M urea and 2% Triton X-100 buffered with 50 mM Tris-HCl, pH 8.0. The purest fraction (containing about 5 μM cytochrome) was determined by the absorption spectrum of its oxidized and reduced form (Fig. 1) and separate protein determination. Denatured cytochrome had the α-band peak shifted to 561 – 562 nm.

Homogeneity was controlled by analytical disc gel electrophoresis according to [6] as demonstrated in Fig. 2 To obtain the reproducible R_F value of 0.68, the gel had to be of 7.5% acrylamide only and to be polymerized by the catalytic riboflavin/tetramethyl-ethylene diamine (TEMED) couple. For molecular weight determination the protein was treated beforehand as indicated in the legend of Fig. 3, which caused more diffused bands in the gels as was observed with cytochrome b-559 [5]. However, under the conditions noted also the

Requests for reprints should be sent to Prof. Dr. P. Böger, Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, *D-7750 Konstanz*.

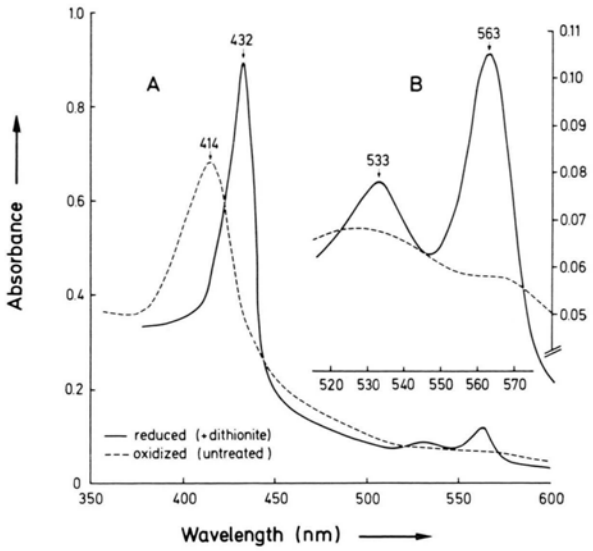

Fig. 1. Absorption spectra of isolated plastidic cytochrome b-563 from spinach in 4 M urea including 2% Triton X-100 and 50 mM Tris-HCl buffer, pH 8.0. An extinction coefficient of 20.7 $mM^{-1} \times cm^{-1}$, between 563 and 543 nm, was used [4]. The reduced form was produced with some particles of solid Na-dithionite added to a stoppered 1 ml cuvette.

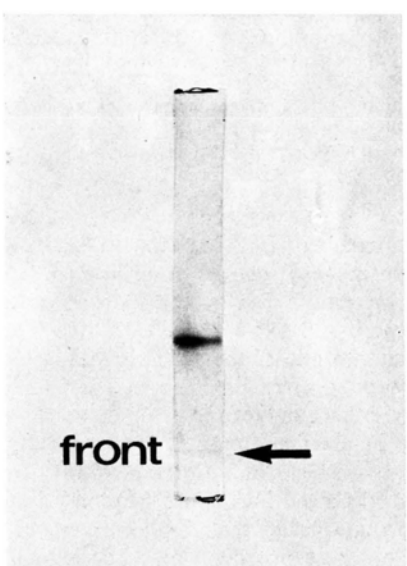

Fig. 2. Analytical polyacrylamide disc gel electrophoresis of spinach cytochrome b-563. The gel had 7.5% acrylamide and 0.5% Triton X-100. Electrophoresis was carried out for 5 h at 4 mA per gel tube in 10 mM Tris-HCl buffer, pH 8.0, including 80 mM glycine and 0.5% Triton X-100 with 30 μg of protein applied per gel.

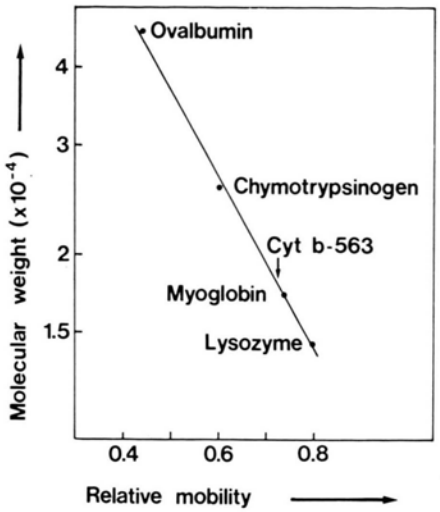

Fig. 3. Molecular weight determination of spinach cytochrome b-563 by disc gel electrophoresis. The protein was pretreated beforehand by 100 °C for 5 min in the presence of 1% sodium dodecylsulfate and 20 mM dithiothreitol, then incubated at 37 °C in the same medium for 12 h. The gel, 7.5% in polyacrylamide, contained 0.5% Triton X-100 and 4 mM dithiothreitol. Electrophoresis was done with 40 µg of pretreated protein per gel at 8 mA per tube for 5 h in Tris-HCl buffer, pH 8.0, with 0.2% Triton X-100 and 0.1% sodium dodecylsulfate. The latter concentrations were chosen from trials with 0.1 to 0.5% of both components and found to be optimal for marker proteins and cytochrome b-563 as well. Runs were performed separately for cytochrome and the group of marker proteins, respectively. The molecular weights of the marker proteins are: ovalbumin, 45,000; chymotrypsinogen 25,600; myoglobin, 17,800; lysozyme, 14,000 (all purchased from Serva, Heidelberg).

marker proteins (although with difficulty for chymotrypsinogen) could be clearly distinguished allowing determination of the molecular weight of cytochrome b-563 to be 18,000 ± 2,000. This figure corresponds to one of the subunits having a 20,000 molecular weight, as determined previously in gel runs by other authors [3]. We could not detect separate bands in our gels and, therefore, not determine fractions of 9,600 and 6,000 molecular weight, as reported by them for spinach cytochrome b-563. In addition, the lipid content of several preparations was not constant (33%) as reported, but was found variable from 5 – 20%. This neither had an influence on the spectrum nor on enzymic activity.

The protein was readily reduced by ferredoxin, to which electrons were donated from NADPH through ferredoxin-NADP reductase (EC 1.6.7.1) from the alga *Bumilleriopsis*. Details of this assay are described elsewhere [7]. The double reciprocal plot yielded straight lines between 0.13 and 1 µM ferredoxin assayed with a K_m of 0.6 µM for ferredoxin (Fig. 4). This is remarkably low and about the same value as found with soluble cytochrome c-553 from the alga just mentioned. This assay was not intended to indicate any physiological significance, but enzymatic reactivity of isolated cytochrome b-563 which was not shown by others [3]. This reactivity is lost by improper preparative performance, but in this case the protein can still be reduced by sodium dithionite.

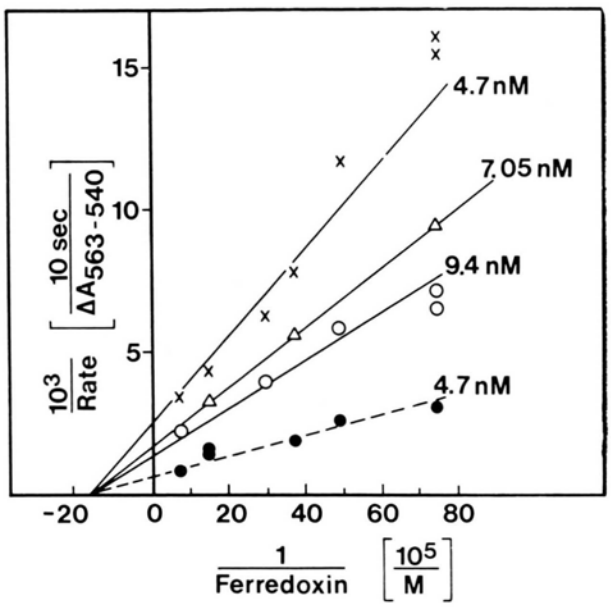

Fig. 4. Double-reciprocal plot of spinach cytochrome b-563 reduction by reduced ferredoxin. The reduction of ferredoxin was mediated by ferredoxin-NADP reductase and NADPH. The nmolar concentrations refer to the reductase; the cytochrome was 0.16 µM (solid lines) and 0.4 µM (dashed line). For the enzymatic assay the cytochrome b-563 was freed from urea by a 6×1 h dialysis *vs* 50 mM Tris-HCl, pH 8.0. Thereafter, the 2 – 3 µM cytochrome solution, still containing approx. 1.5% Triton X-100, was frozen by liquid nitrogen. Storage at −80 °C preserved activity for 2 – 3 months.

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