A STABLE AND EASILY EXTRACTABLE PLANT-TYPE FERREDOXIN FROM THE BLUE-GREEN ALGA SPIRULINA MAXIMA.

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SUMMARY

The 2Fe + 2S plant-type ferredoxin from the blue-green alga Spirulina maxima can be easily extracted in high yield - 35 mg ferredoxin/100 g dried cells. The cells can be stored as a dried powder which is readily obtainable. The purified ferredoxin is very stable even aerobically at room temperature. It is much more stable than the five other plant-type ferredoxins with which it was compared. The biological activity of all ferredoxins was comparable.

Ferredoxins are being used to an increasing extent in biochemical experiments. They belong to the group of non-haem iron proteins known as iron-sulphur proteins, and they are now known to be electron carriers involved in numerous electron transfer reactions in soluble and membrane-bound systems[1,2]. An ideal ferredoxin would be stable in pure form, functional in various reactions and readily extractable from a cheap and available source of cells which can be easily stored. We think we have found a ferredoxin which fulfills these criteria better than any other ferredoxin so far isolated.

Spirulina maxima is a large, spiral-shaped, blue-green alga which grows on the surface of ponds whose water contains high amounts of bicarbonate giving it a high salinity and a high pH value [3-5]. It has a 60-70% protein content making it a rich source of protein food and it is easily digested. Indeed Spirulina spp.have been eaten for centuries by people around Lake Chad, Central Africa, and reportedly by Aztecs in Mexico. The organism from Chad has been characterized

as Spirulina platensis[6] but except for some minor morphological differences is analagous to Spirulina maxima from Mexico[7].

Spirulina spp. are now used experimentally as a food additive in human and poultry feeding programmes sponsored jointly by the Institut Francais du Petrole and a FAO/UNICEF/WHO combine in Algeria and Mexico[7]. The alga is cultivated in open ponds in the south of France (Antibes), in Algeria, and in Mexico with high yield (15-16 g dry weight/m^2/day, i.e. 16-18 tons/acre/year, under favourable conditions). It also grows naturally in salt pans in Lake Texcoco near Mexico City; 300 Kg/day is harvested at present and 1 ton/day is projected[7]. The dried cells from Mexico are available in large quantities at low cost and can be easily stored.

The dried cells were obtained from Sosa Texcoco S.A., Sullivan 51, Mexico 4 D.F., Mexico. They were extracted as described later to give a yield of 35 mg pure ferredoxin ($OD_{420/280}$= 0.57) per 100 g powder. This Spirulina ferredoxin was assayed chemically and biologically and compared with ferredoxins from Spinach (Spinacea oleracea), lucerne (Medicago sativa), maize (Zea mays), parsley (Petroselinum cristum) and a green alga (Scenedesmus obliquus). The relative stabilities of the ferredoxins were tested by storage for up to 7 weeks at - 196°C (liquid N_2), 4°C (refrigerator), and 21°C (room temperature).

Ferredoxin extraction.

All solutions contained 20 mM Tris-HCl pH 8.5 (buffer). 200 g dried Spirulina cells were suspended in 2 l of buffer plus 2 ml mercaptoethanol or 200 mg dithiothreitol. The suspension was then sonicated for 15 minutes at 0°C with a Dawe Soniprobe (Dawe Instruments, London, U.K.) at full power, about 4 amps. All subsequent steps were carried out at 0-4°C. The solid matter in the sonicate was centrifuged off in an MSE 6L at 2000 x g for one hour. 60g of Whatman DEAE-cellulose (DE 23) was stirred into the solution and the suspension was then

centrifuged at 1500 x g for 30 seconds and the supernatant discarded.

The DE 23 to which the ferredoxin was adsorbed was washed three times with buffer and finally washed with 0.2M NaCl. The DE 23 was then transferred to a 30 x 4 cm chromatography column and washed with 0.25M NaCl to remove some of the blue material. The ferredoxin was eluted with 0.8M NaCl from the DE 23 as a dark brown solution contaminated with material adsorbing at 670 nm. 0.4g ammonium sulphate was then added per ml of solution and it was centrifuged at 23,000 x g for 1 hour. The greenish residue was discarded; the pink supernatant was desalted by passage through a 30 x 8 cm column of Sephadex G-25 and eluted with buffer. The eluate was adsorbed on a 40 x 2.5 cm column of DE 23 equilibrated with 0.2M NaCl and chromatographed with 0.35M NaCl. Fractions with an $OD_{420/280}$ ratio greater than 0.5 (mostly free of nucleic acids) were pooled and finally concentrated on a small (5 x 1 cm) column of DE 23. The ferredoxin was eluted in 0.8M NaCl as a dark reddish-brown solution and stored as the concentrated solution. The yield was 70 mg ferredoxin in 5 ml with an $OD_{420/280}$ ratio of about 0.57. The whole operation can be completed in less than two days. On adding ammonium sulphate to desalted ferredoxin until the appearance of turbidity and storing at 4°C, red crystals of ferredoxin are obtained. The shape of these crystals were similar to those of ferredoxins from other algae[8,9].

The ferredoxins from spinach and Scenedesmus were purified as described previously[10]. In the case of maize and lucerne the leaves were homogenized in buffer with "Polyklar AT" (insoluble polyvinylpyrrolidone as supplied by Gaf Ltd., Calder Street, Manchester) and mercaptoethanol, and the ferredoxin subsequently purified as described for spinach. The maize and lucerne were provided by Rothamsted Experimental Station and homogenized in 10kg batches using their IBP Pulper[11], and belt press[12]. The parsley ferredoxin[13] was provided by Dr. R.P.F. Gregory.

Ferredoxin Properties.

Purified Spirulina ferredoxin contains 2 atoms of Fe and 2 atoms of inorganic sulphur per mole, assuming a molecular weight of 12,000 and a molar extinction coefficient at 420 nm of 9,700 as is the case for spinach ferredoxin[14]. PCMB titration[15] gives a value of 10 per molecule suggesting that the molecule may contain six cysteine residues. The optical adsorption, EPR, CD and ORD spectra are all typical of plant ferredoxins[10].

In the biological assay for ferredoxin using spinach chloroplasts oxygen evolution coupled to NADP reduction was measured in an oxygen electrode (Rank Bros., Bottisham, Cambridge). This is a modified version of the spectrophotometric method of San Pietro[16]. Spirulina ferredoxin showed a close similarity to spinach ferredoxin. Indeed ferredoxins from all the six plants showed the same maximum rates of O_2 evolution at saturating amounts of ferredoxin in the reaction mixture. Spirulina and spinach ferredoxin also produced the same rates of CO_2 fixation in isolated chloroplasts[17].

Ferredoxin stability.

The stability of the six ferredoxins over a 7-week period was tested by storing samples in 0.1M NaCl plus 0.1M Tris-HCl, pH 8.5, in Parafilm-covered test tubes; no precautions were taken to keep the solutions anaerobic although we know that this does help retain the stability of ferredoxins. The ferredoxins were assayed by following their $OD_{420/280}$ ratios and their activities in the chloroplast NADP reduction system.

At liquid nitrogen temperatures all the ferredoxins showed a small loss in activity which was probably incurred during freezing and thawing. In the refrigerator at 4°C there was appreciable loss of activity of all ferredoxins - about 10-30% loss. At this temperature Spirulina ferredoxin was the most stable and parsley ferredoxin the least stable. At room

temperature the great stability of Spirulina ferredoxin became apparent; it still retained about 35% of its original activity after 7 weeks at 21°C, whereas the others (except for maize which still retained about 15% activity) had retained less than 5% of their original biological activity. If, however, special precautions are taken to keep ferredoxin solutions completely anaerobic they can be stored at 4°C for longer periods[18-20].

The stability of purified Spirulina ferredoxin, the ease of extraction from the dried cells, and the convenience of storage and cheapness of the cells, all seem to recommend this alga as an excellent source of a plant ferredoxin.

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