Formation of Bis(μ-methanethiolato)bis(dinitrosyliron) from Parsley Ferredoxins and Nitrite

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The iron–sulphur nitrosyl complex $Fe_2(SMe)_2(NO)_4$ has been reported as a natural product formed upon storage of green plants in water containing relatively high natural levels of nitrite [1–3]. It has been reported [4] that although $Fe_2(SMe)_2(NO)_4$ cannot be detected in freshly harvested plant material, its concentration rises over a period of weeks to levels in the range 0.10–4.50 mg kg^{-1}, at the same time as the plant material becomes heavily contaminated with the mould *Geotrichum candidum*.

All reports of $Fe_2(SMe)_2(NO)_4$ as a natural product have come so far from Chinese sources and we are unaware of any confirmation from Western sources: furthermore, these reports leave some doubt concerning the identity of the plant material employed. In this communication we provide the first such confirmation of the formation of $Fe(SMe)_2(NO)_4$ from green plant material, using parsley, *Petroselinum crispum*, as the test material.

Incubation of parsley with 0.05 mol dm^{-3} sodium nitrite over a four week period, at room temperature in the dark, yielded $Fe_2(SMe)_2(NO)_4$, detected and identified by GC/MS. Control incubation with distilled water in the absence of sodium nitrite, provided no detectable $Fe_2(SMe)_2(NO)_4$: our conservative estimate of the detection limit is 10^{-9} g, equivalent to a concentration of $Fe_2(SMe)_2(NO)_4$ in the experimental cultures of 4×10^{-11} mol dm^{-3}. The presence of *Geotrichum candidum* does not appear to be significant, as samples containing sodium nitrite gave $Fe_2(SMe)_2(NO)_4$ whether or not inoculated with the culture: on the other hand, in the absence of added sodium nitrite no $Fe_2(SMe)_2(NO)_4$ was detected even in the presnece of *Geotrichum candidum*.

Our results provide confirmation of the very ready formation of $Fe(SMe)_2(NO)_4$ using a readily available and readily characterised plant species; but suggest that the role of *Geotrichum candidum* in $Fe_2(SMe)_2(NO)_4$ formation is at best marginal, as separate experiments have shown that this particular strain possesses no detectable nitrate reductase activity. Added nitrite is crucial to the formation of $Fe_2(SMe)_2(NO)_4$.

We have shown previously [5] that pre-formed synthetic models for both [2Fe–2S] and [4Fe–4S] iron–sulphur proteins react readily with nitrite to yield initially $[Fe(NO)_2(SH)_2]^-$, and thence $[Fe_4S_3(NO)_7]^-$, isolable in yields of around 40% based upon total iron. It is thus likely that the formation of $Fe_2(SMe)_2(NO)_4$ observed in the present work similarly depends upon nitrosylation of the iron–sulphur clusters in the parsley ferredoxins: the nature of the methylation step is still under investigation.

Experimental

Locally available parsley *Petroselinum crispum* (Mill.) was divided randomly into 150 g portions, washed, drained, and blanched by immersion for 4 min in boiling distilled water. Two portions were packed into beakers and just covered with distilled water (*ca.* 80 cm^3): two others were covered similarly with 0.05 dm^{-3} sodium nitrite solution.

Geotrichum candidum (Link), from a soil sample collected in Nagpur, India, was obtained from the Commonwealth Mycological Institute, Kew, as a freeze-dried culture, and was revived using a general nutrient medium. Portions of 0.5 cm^3 of a viable cell suspension in pH 7.0 phosphate buffer were added to one parsley aliquot in distilled water, and to one in 0.05 mol dm^{-3} sodium nitrite. The parsley portions were loosely covered and compressed using concentric beakers within the reaction vessels, and kept in the dark for four weeks.

After four weeks, each reaction mixture was worked up in the same manner. The solid material was separated and extracted with methylene chloride (4×100 cm^3): the aqueous phase was also extracted with methylene chloride (3×100 cm^3). The combined extracts were dried over anhydrous sodium sulphate, and reduced in volume to 5 cm^3. The resulting solution was chromatographed on silica (4 cm diameter × 10 cm length) with dry deoxygenated methylene chloride, and the yellow band was collected, and reduced to dryness. The residue was dissolved in dry deoxygenated hexane (20 cm^3) for GC/MS examination.

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GC/MS was performed using a Packard 437 gas chromatograph, interfaced to a VG 7070F double focussing mass spectrometer. A fused silica capillary column, 23 m x 0.32 mm i.d., coated with OV-101 methyl silicone phase, was used in the gas chromatograph, the column being inserted directly into the ion source of the mass spectrometer. GC injections ($1-5 \times 10^{-3}$ cm^3) were made using an on-column injection technique, with the column temperature held at 70 °C. The carrier gas was helium. After injection, the oven was held at 70 °C for 2 min, the temperature raised to 170 °C at 40 °C/min., programmed from 170 °C to 240 °C at 10 °C/min., and finally held at 240 °C for 2 min. Mass spectra were obtained every 2.5 s, using 1 s/deg scan rate for *m/z* 566 to *m/z* 42. The mass resolution was 800 (10% valley), and the accelerating voltage and electron energy 4 kV and 70 eV respectively. The trap current was 200 μA. Source and GC/MS interface temperatures were 250 °C. Mass spectral data were collected into a VG 2040 data system. Perfluorokerosene was used to calibrate the mass spectrometer; $Fe_2(SMe)_2[NO]_4$ was identified by comparison of both its retention time and mass spectrum with those obtained from an authentic synthetic sample [6].

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