Iron–Sulphur Centres in Mitochondria from *Arum maculatum* Spadix with Very High Rates of Cyanide-Resistant Respiration

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X-band electron-paramagnetic-resonance spectroscopy at 4.2–77 K combined with measurements of oxidation–reduction potential was used to identify iron–sulphur centres in *Arum maculatum* (cuckoo-pint) mitochondria. In the oxidized state a signal with a derivative maximum at $g = 2.02$ was assigned to succinate dehydrogenase centre S-3. Unreduced particles showed additional signals at $g = 2.04$ and 1.98 (at 9.2 GHz), which may be due to a spin–spin interaction. In the reduced state a prominent signal at $g = 1.93$ and 2.02 was resolved into at least three components that could be assigned to centres S-1 and S-2 of succinate dehydrogenase (midpoint potentials -7 and -240 mV respectively at pH 7.2) and a small amount of centre N-1b ($E_0' = -240$ mV) of NADH–ubiquinone reductase. In addition, changes in line shape around -10 mV indicated the presence of a fourth component in this signal. The latter was more readily reduced by NADH than by succinate, suggesting that it might be associated with the external NADH dehydrogenase. The iron–sulphur centres of NADH–ubiquinone reductase were present in an unusually low concentration, indicating that the alternative, non-phosphorylating, NADH dehydrogenase containing a low number of iron–sulphur centres may be responsible for most of the high rate of oxidation of NADH.

Mitochondria isolated from the mature spadix of *Arum maculatum* (cuckoo pint) (Prime, 1960) are capable of catalysing extremely high rates of respiration, a large proportion of which is mediated by a cyanide-resistant benzhydroxamic acid-sensitive ‘alternative’ oxidase rather than by cytochrome *c* oxidase (Henri & Nyns, 1975). *Arum* spadix mitochondria can oxidize exogenous NADH at extremely high rates (3000 nmol of O_2 /min per mg of protein) via a piericidin A-insensitive NADH dehydrogenase; in contrast, mammalian mitochondria can only oxidize NADH at rates of about 200–300 nmol of O_2 /min per mg of protein via the piericidin A-sensitive NADH–ubiquinone reductase located on the inner surface of the inner membrane. *Arum* spadix mitochondria, therefore, like other plant mitochondria, possess an exogenous NADH dehydrogenase that can transfer reducing equivalents from exogenous NADH to the oxidation chain by a piericidin A-insensitive non-phosphorylating route (Palmer, 1976). Little is known about the nature of this external NADH dehydrogenase, although Douce *et al.* (1973) have proposed that it may be a flavo-protein.

The e.p.r.† spectra of reduced mitochondria or submitochondrial particles show a number of signals,

† Abbreviations: e.p.r., electron paramagnetic resonance; Mops, 3-morpholinepropanesulphonic acid; Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino] ethanesulphonic acid.

centred around $g = 1.96$, in the temperature range 4.2–77 K. In heart and other types of animal mitochondria, the signals most clearly seen are those from the NADH–ubiquinone reductase (Complex I) segment of the respiratory chain (Orme-Johnson *et al.*, 1971, 1974; Ohnishi, 1976).§ The spectra of cyanide-sensitive mitochondria from plant tissue such as Jerusalem-artichoke (*Helianthus tuberosus*) tuber appear similar to those of the animal systems (Cammack & Palmer, 1973). By contrast, mitochondria from *Arum* spadix showed an intense signal at $g_{\parallel} = 2.02$ and $g_{\perp} = 1.93$. Over a wide temperature range the shape of this signal showed little variation. Signals corresponding to other iron–sulphur centres were present in relatively small amounts. On the basis of its signal parameters and response to substrate the $g = 1.93$ signal at 77 K was tentatively assigned by us (Cammack & Palmer, 1973) to NADH dehydrogenase centre N-1, with additional contributions from succinate dehydrogenase centre S-1.

We report here the results of further measurements of the iron–sulphur components of *Arum maculatum* spadix mitochondria and submitochondrial particles, by using the technique of redox-potential measurements combined with e.p.r. spectroscopy, which has enabled us to make more definite assignments of some of the signals observed.

§ In referring to iron–sulphur centres the nomenclature of Ohnishi (1976) will be followed.

Experimental

Isolation of *Arum spadix* mitochondria

The inflorescences of *Arum maculatum* were collected from woodland locations in Kent just before the thermogenic stage (stage γ in the terminology of James & Beevers, 1950). The sterile portion of the spadix was removed just before the preparation of the mitochondria. The coloured epidermal tissue was not removed because of the large-scale nature of many of the preparations. The intact spadices were placed in isolating medium such that the ratio of tissue to medium was 100 g to 350 ml. The isolating medium contained 0.3 M-mannitol, 10 mM-EDTA, 10 mM-Mops, 2 mM- $\text{Na}_2\text{S}_2\text{O}_5$ and 0.1 % (w/v) bovine serum albumin at pH 7.4. The source of the reagents was the same as that described by Palmer & Kirk (1974). The tissue was homogenized with a Moulinex Mixer 66 (Alençon, France) for 45 s. The resulting homogenate was squeezed through three layers of muslin. Cell debris and starch were removed by centrifugation at 500 g for 5 min in a Sorvall RC-2B centrifuge. The mitochondria were then sedimented at 11 000 g for 15 min. The pellets of mitochondria were suspended in a medium containing 0.3 M-sucrose, 10 mM-EDTA, 10 mM-Tes (potassium salt) and 0.1 % bovine serum albumin at pH 7.4; 200 ml of this medium was added to the mitochondria derived from 100 g of spadices. The washed mitochondria were sedimented as before, and the final pellet was suspended in the medium used for washing to yield a final protein concentration of approx. 40 mg/ml. All operations were carried out at 4°C. Protein was determined after solubilization in 10 % (w/v) sodium deoxycholate, by the method of Lowry *et al.* (1951); bovine serum albumin was used as the standard.

Isolation of potato (*Solanum tuberosum*) mitochondria

Potato mitochondria were prepared by using the method previously described for Jerusalem-artichoke mitochondria (Palmer & Kirk, 1974), except that the starch was removed by centrifugation at 500 g for 5 min before sedimenting the mitochondria.

Preparation of *Arum* submitochondrial particles

Arum submitochondrial particles were prepared by freezing 10 ml batches of intact mitochondria (40 mg of protein/ml) for 12–18 h at 77 K, then thawing and sonicating by using a Dawe Soniprobe (model 7530A) tuned to an output of 4–5 A at setting 5 for two cycles of 25 s. The sample was immersed in an ice bath during sonication and allowed to cool to 4°C between the two cycles of sonication. The suspension was centrifuged at 48 000 g for 10 min to remove whole mitochondria, and the supernatant was centrifuged at 100 000 g for 1 h to yield a pellet of submitochondrial particles. This pellet was suspended in a small volume

of the medium used to wash the mitochondria to yield a suspension containing 40 mg of protein/ml.

Redox-potential titrations

Titration were carried out as described by Cammack *et al.* (1976) in an apparatus similar to that described by Dutton (1971). In this method a suspension of submitochondrial particles was adjusted to a particular redox potential, measured by a platinum electrode in the presence of mediators, with $\text{Na}_2\text{S}_2\text{O}_4$ as reductant or $\text{K}_3\text{Fe}(\text{CN})_6$ as oxidant. After an equilibration period of at least 1 min at 25°C, a sample of the suspension was withdrawn into an e.p.r. sample tube, under an atmosphere of argon, and frozen in an isopentane bath at –140°C. In this way a series of samples was obtained, poised at different redox potentials. Mediators, which were used in the appropriate potential regions, were those used by Cammack *et al.* (1976) and in addition, in the higher-potential range, *p*-benzoquinone ($E'_0 = 290$ mV) (Hopkin and Williams, Romford, Essex, U.K.), *NNN'*-tetramethyl-*p*-phenylenediamine ($E'_0 = 260$ mV), 2,6-dichlorophenol-indophenol ($E'_0 = 217$ mV), Methylene Blue ($E'_0 = 12$ mV) (all from BDH Chemicals, Poole, Dorset, U.K.), phenazine methosulphate ($E'_0 = 80$ mV), phenazine ethosulphate ($E'_0 = 55$ mV) (from Sigma Chemical Co., Kingston-upon-Thames, Surrey), and 2,5-dimethylbenzoquinone ($E'_0 = 180$ mV) (Eastman-Kodak Co., Rochester, NY, U.S.A.).

E.p.r. spectra

These were recorded on a Varian E4 spectrometer. The samples were cooled either with a liquid- N_2 insert Dewar (77 K) or a liquid-helium transfer system (Oxford Instruments, Osney Mead, Oxford, U.K.) for variable temperatures down to 4.2 K. Because of a discrepancy between the temperature measured by the thermocouple and the temperature of the sample in the latter system, relatively high helium flow rates were used, and the temperature was calibrated with a carbon-resistance thermometer in the sample position.

Plots of the intensity of the various e.p.r. signals against applied potential were displayed on a Nicolet 1020A digital oscilloscope (Nicolet Instruments, Madison, WI, U.S.A.) interfaced to a HP 9830A calculator (Hewlett Packard Inc., Palo Alto, CA, U.S.A.). Theoretical curves, calculated from the Nernst equation, assuming a number of components of specified maximum signal intensities and midpoint potentials, were superimposed on the experimental points, and the parameters of the curves were adjusted to give the best fit. Good fits to the data were obtained assuming one-electron transfers (i.e. $n = 1$ in the Nernst equation).

Results

E.p.r. spectra of potato and Arum mitochondria

Fig. 1 shows a comparison of e.p.r. spectra recorded at 77K of reduced mitochondria from potato (*Solanum tuberosum*) tuber, which are typical of cyanide-sensitive plant mitochondria, and from *Arum maculatum* spadix. The succinate oxidase rates in the presence of ADP were approx. 150nmol of O_2 consumed/min per mg of protein for potato mitochondria and 900nmol of O_2 consumed/min per mg of protein for the *Arum* mitochondria.

The spectrum of potato mitochondria is similar to that seen in heart mitochondria (Orme-Johnson *et al.*, 1971) and probably is due mainly to centres N-1a and N-1b. The *Arum* mitochondria have a more intense signal at $g_{\parallel} = 2.02$, $g_{\perp} = 1.93$, which has a somewhat different line shape.

Fig. 1. *E.p.r. spectra of (a) mitochondria from potato tuber (12mg of protein/ml) and (b) Arum spadix (40mg of protein/ml) reduced with $Na_2S_2O_4$*

The ordinate is the first derivative of microwave absorption. The gain settings were adjusted to compensate for protein concentration. Spectra were measured at 77K with the following instrument settings: microwave power 20mW, frequency 9.26GHz, modulation amplitude 1mT, frequency 100KHz.

Fig. 2. *E.p.r. spectra, measured at 15K, of Arum spadix submitochondrial particles, poised at the following redox potentials: (a) -94mV, (b) -217mV, (c) -295mV*

The expanded regions were recorded at a fivefold-increased gain setting. Other instrument settings were as for Fig. 1.

Redox titrations of Arum spadix submitochondrial particles

Representative examples of e.p.r. spectra of samples poised at different redox potentials at 15K are shown in Fig. 2. The most obvious features were a signal in oxidized samples with a derivative maximum at $g = 2.02$ typical of HiPIP (high-potential iron-sulphur) proteins, and a signal at $g_{\parallel} = 2.02$, $g_{\perp} = 1.93$ which appears on reduction, typical of ferredoxin-type iron-sulphur proteins.

Spectra were recorded of samples poised at different potentials, over the temperature range 4.2–77K. The interpretation of the various features of the spectra, which are now described in detail, are summarized in Table 1.

Table 1. *Iron-sulphur centres detected by e.p.r. spectroscopy of Arum maculatum submitochondrial particles*
Midpoint potentials are at pH 7.4 relative to the standard hydrogen electrode, and values are ± 15 mV. The nomenclature of Ohnishi (1976) is followed in the assignment of iron-sulphur centres. Abbreviation: QH $\cdot$, ubisemiquinone radical.

State	<i>g</i> values	Temperature (K)	Midpoint potential (mV)	Assignment
Reduced	2.02, 1.932, 1.922	77	-7	Succinate dehydrogenase centre S-1
Reduced	2.02, 1.93	18	-240	Succinate dehydrogenase centre S-2
Reduced	(.202), 1.933, 1.928	65	~ -20	Alternative NADH oxidation pathway?
Reduced	(2.03), 1.93	77	-240	NADH-ubiquinone reductase centre N-1b?
Reduced	2.05 (1.92)	18	-110	NADH-ubiquinone reductase centre N-2
Reduced	1.87	12	-275	NADH-ubiquinone reductase centre N-3 and N-4?
Oxidized	2.02, 2.00	12	85	Succinate dehydrogenase centre S-3
Oxidized	2.02, 2.00	18	-100	Oxidized 4Fe-4S centres?
Partially oxidized	2.038, 1.984	12		Spin-spin interaction between QH $\cdot$, and QH $\cdot$ or succinate dehydrogenase centre S-3

Signals at $g_{\perp} = 1.93$, $g_{\parallel} = 2.02$

The e.p.r. spectra of fully reduced submitochondrial particles at all temperatures from 77 K down to 4.2 K were dominated by this axial or near-axial signal (Fig. 3) as previously described (Cammack & Palmer, 1973). Oxidation-reduction titrations (Fig. 4) show that this signal consisted of at least two major components. When measured at 77 K (Fig. 4a) the signal titrated with a midpoint potential of -7 mV (± 6 mV; mean of five determinations). A minor component at -240 mV could also be detected in titrations of concentrated particles. When measured at 12 K (Fig. 4c) the component with the midpoint potential of -240 ± 7 mV was predominant. At an intermediate temperature, 24 K, both components could be seen in the titration curve (Fig. 4b). Clearly the first component has a slower electron-spin-relaxation, so that it is observable at higher temperatures and is highly saturated by microwave power at the lower temperatures at which the second component is observed.

The major signal observed at 77 K is clearly not due to centres N-1a or N-1b, which in heart mitochondria have midpoint potentials of -380 and -240 mV respectively (Ohnishi, 1975). It is possible that the -240 mV component of the $g = 1.93$ signal at 77 K is due to centre N-1b, although the amount is small, about 10–20% of the total signal, and is therefore difficult to determine quantitatively.

The most likely candidates for the major signals at $g = 1.93$ are centres S-1 and S-2 of succinate dehydrogenase. In particulate preparations of mammalian heart mitochondria, these have midpoint potentials

Fig. 3. Temperature-dependence of the e.p.r. spectrum of *Arum spadix* submitochondrial particles, reduced with $\text{Na}_2\text{S}_2\text{O}_4$. (a) 77 K; (b) 19 K; (c) 10 K. The expanded regions were recorded at a fivefold-increased gain setting. Other instrument settings were as for Fig. 1.

Fig. 4. Redox titration curves for the signal at $g = 1.93$, measured at (a) 77 K, (b) 24 K, (c) 12 K

The ordinate is the peak-to-amplitude of the signal at $g = 1.93$, measured as for Fig. 3, multiplied by the temperature to correct for transition probability. Samples poised at the various redox potentials were prepared as described in the Experimental section. Curves fitted to the points were calculated, assuming two components with varying proportions, in this case with midpoint potentials -5 mV and -235 mV .

Fig. 5. E.p.r. spectra of *Arum spadix* submitochondrial particles (21 mg of protein/ml) adjusted to potentials of (a) 0 mV and (b) -113 mV

Spectra were recorded at 65 K with the following instrument settings: microwave power 20 mW, frequency 9.07 GHz, modulation amplitude 0.5 mT, frequency 100 KHz. Each spectrum was obtained by averaging four scans on a Nicolet 1020A digital oscilloscope.

of 0 mV and -260 mV respectively; centre S-1 is detected at 77 K, but centre S-2 is clearly discernible only below 20 K (Ohnishi *et al.*, 1976a).

Detailed examination of the spectra of the $g = 1.93$ signal at 77 K, in samples poised at different potentials, showed that there was a change in the line shape during the first stages of reduction (Fig. 5). Although small, this effect was consistently observed in titrations of five different particle preparations. It shows that there are two components, with slightly different midpoint potentials, appearing in this region. It is noteworthy that the existence of two such components could not easily be detected from the shape of the redox curve (Fig. 4a) alone. If the curve is the sum of two components with similar spectra they will not significantly alter its overall shape unless they are separated in midpoint potential by more than 40 mV. As the sample was progressively adjusted to more negative potentials, the derivative spectrum first showed two downward features at $g = 1.922$ and $g = 1.928$, the former frequently being more prominent than the latter. At lower redox potentials the downward feature at $g = 1.928$ became more prominent. This observation implies that there were two species present in comparable amounts. The species with the larger component at $g = 1.928$ had a midpoint potential lower, by 10–30 mV, than the other. Centre S-1 in pigeon heart particles has a rhombic spectrum at $g_x = 1.91$, $g_y = 1.93$, $g_z = 2.03$. It is perhaps to be expected that the g values in plant submitochondrial particles might be slightly different from these. Therefore one of the species observed in *Arum* might be centre S-1, and the other a modified or inactivated form. However, subsequent experiments on the reduction of the iron-sulphur centres with

different substrates provide another possible explanation.

Fig. 6 shows the effects of reduction by succinate and by NADH on the signals at $g_{\perp} = 1.93$ detected at 77 K. Reduction by NADH was relatively rapid, but reduction by succinate was slow, as shown by the small signal after reduction for 10 s at 20°C (Fig. 6a). It seems likely that the succinate dehydrogenase in these particles is largely in the inactive form (Singer *et al.*, 1973) and becomes activated by incubation with succinate. This would explain why only a small response to succinate was observed in previous experiments (Cammack & Palmer, 1973).

Slight differences in line shape, analogous to those seen in the redox titration, were consistently observed between samples reduced by succinate (Fig. 6b) and

those reduced by NADH (Fig. 6c). In succinate-reduced samples the minimum in the derivative spectrum was at $g = 1.922$. In NADH-reduced samples, the dip at $g = 1.928$, corresponding to the component with the slightly lower midpoint potential became more prominent (Fig. 6c). It seems likely therefore that the component with a downward feature at $g = 1.922$ is centre S-1 of succinate dehydrogenase. The component with the downward feature at $g = 1.928$, which is readily reduced by NADH, is most probably the component observed in the redox titrations with a midpoint about -20 mV, rather than centre N-1b which is present only in low concentrations. This implies that the feature at $g = 1.928$ is not due to an inactive form, but to a new type of iron-sulphur centre which may be associated with the rapid, piericidin-resistant, alternative oxidation pathway for NADH (Palmer & Coleman, 1974).

Other signals in reduced particles

On examining the e.p.r. spectra of submitochondrial particles at lower temperatures and an expanded scale (Fig. 3), additional signals could be observed. A derivative peak at $g_{\perp} = 2.05$, appearing below 25 K (see Fig. 3b) was attributed to centre N-2 of NADH-ubiquinone reductase (Cammack & Palmer, 1973). The $g_{\perp}$ of this signal would be hidden by the signals corresponding to the major components at $g = 1.93$. By using very concentrated particle preparations it was possible to obtain a rough estimate of -110 mV for the midpoint potential of this feature.

At lower temperatures (below 18 K), an additional signal at $g = 1.87$, together with a broad feature around $g = 2.09$, became visible. Although not prominent in Fig. 3(c), it is clearly seen in the expanded region of Fig. 4 of Cammack & Palmer (1973). The midpoint potential of the most prominent feature at $g = 1.87$ was estimated to be -275 mV. This signal does not correspond exactly to those of iron-sulphur centres detected in mammalian systems, but it most closely resembles (except in the exact g values) centres 3 and 4 of NADH-ubiquinone reductase (Orme-Johnson *et al.*, 1974) which have midpoint potentials -240 mV and -405 mV respectively, in submitochondrial particles (Ohnishi, 1975).

HiPIP-type signals

In the spectra of unreduced submitochondrial particles an intense e.p.r. signal was seen with a derivative peak at $g = 2.02$ (Fig. 2a). This type of signal is of the type expected for the higher $[\text{Fe}_4\text{S}_4(\text{RS})_4]^-$ oxidation state of a four-iron centre, similar to that in oxidized *Chromatium* HiPIP proteins. Signals of this type have been assigned to centre bc-3 in the ubiquinone-cytochrome *c* reductase region of the respiratory chain (Ruzicka & Beinert, 1974), and to centre S-3 in succinate dehydrogenase (Beinert *et al.*, 1975; Ohnishi *et al.*, 1976b). The former protein

Fig. 6. Reduction of iron-sulphur centres by succinate and by NADH

Submitochondrial particles (approx. 10 mg/ml) in aerated isolating medium were mixed in an e.p.r. tube with either 1 M-sodium succinate (final concn. 20 mM) or NADH (final concn. 20 mM) at 20°C, stirred vigorously with a syringe needle and then frozen in an isopentane bath at -140°C . The freezing time was approx. 2 s. Sample (a) was frozen 10 s after addition of succinate, (b) 60 s after adding succinate, and (c) 10 s after adding NADH. Spectra were recorded at 77 K under conditions similar to those described for Fig. 1.

would not be expected to be seen in the present experiments, as it is readily removed by the ultrasonic treatment used to prepare the submitochondrial particles (Ohnishi *et al.*, 1976c).

Centre S-3 is reported to have a midpoint potential in the region of 120 mV in mitochondria (Ohnishi *et al.*, 1976b) and 60 mV in a soluble preparation. The oxidizing and reducing agents (ferricyanide and dithionite) used in these titrations can have a destructive effect on the sensitive oxidized 4Fe-4S centres. To try to alleviate this problem, we first carried out a titration in which the oxidized particles, in the presence of suitable mediators, were progressively reduced with small additions of dithionite, no ferricyanide being added; in a second titration, particles reduced with ascorbate were progressively oxidized with ferricyanide. The results of these titrations are shown in Fig. 7. Clearly there are differences in behaviour on titrating in the two directions. On titration to higher potentials with ferricyanide there was a further gain in signal intensity around +300 mV, possibly owing to the 'super-oxidation' of other 4Fe-4S centres in the mitochondria; ferricyanide has been demonstrated to have this effect on bacterial ferredoxin (Sweeney *et al.*, 1974). Nevertheless, both titrations show a redox process with a midpoint potential about 85 mV, which is not greatly dissimilar from that of centre S-3. Further evidence for this assignment was provided by the temperature-dependence. The signal with a midpoint potential at +85 mV was only seen below 20 K, consistent with observations on centre S-3 (Ohnishi *et al.*, 1976c).

In all titrations with dithionite, a second component in the HiPIP-type signal was observed, with a midpoint potential around -100 mV. This signal could be observed up to 30 K. This signal might represent some new type of functional mitochondrial component, but it may represent a degradation product. Ferredoxins that contain 4Fe-4S centres are frequently found to show a HiPIP-type signal at $g = 2$ in the unreduced state (see Palmer & Sands, 1966). These signals are of variable intensity and may arise from centres that have been oxidized to the higher oxidation $[\text{Fe}_4\text{S}_4(\text{RS})_4]^-$ state by oxygen during preparation. The rather low midpoint potential of the second HiPIP component in *Arum* is consistent with potentials reported for these 'super-oxidized' ferredoxins. The HiPIP form of the four-iron ferredoxin from *Bacillus stearothermophilus* has a midpoint potential of about 0 mV (R. Cammack, unpublished work); the oxidized forms of *Desulphovibrio gigas* ferredoxins I and II have potentials of -50 and -130 mV respectively (Cammack *et al.*, 1977).

In some preparations of *Arum* mitochondria or submitochondrial particles, either as prepared or with small amounts of succinate added, a strong signal was seen, with apparent g values (at 9.2 GHz) of 2.04, 1.98 (Fig. 8a); these components were greatly diminished at temperatures above 20 K (Fig. 8b). The sample shows a small signal at $g = 1.93$ (Fig. 8c), which indicates that the potential of the system (assuming that the centres are in equilibrium) is probably between +30 and +80 mV. A similar signal has been observed in partly reduced heart mitochondria.

Fig. 7. Redox titration of the HiPIP-type iron-sulphur centres in *Arum spadix* submitochondrial particles

The ordinate is the peak-to-trough amplitude of the signal around $g = 2.1$, measured at 14 K. Titrations were carried out as described in the Experimental section. ●, reductive titration with $\text{Na}_2\text{S}_2\text{O}_4$; ○, oxidative titration with $\text{K}_3\text{Fe}(\text{CN})_6$ (see the Results section). Curves were calculated, assuming midpoint potentials +90 mV, -100 mV (solid line, reductive titration) and +280 mV (broken line, oxidative titration). The curve for the oxidative titration does not go to zero, because the HiPIP signal was not completely reduced by ascorbate.

Fig. 8. Spectra of a suspension of *Arum spadix* submitochondrial particles as prepared at a concentration of 40 mg of protein/ml, recorded (a) at 17 K, (b) at 26 K, and (c) at 26 K and at fivefold-increased gain setting. Other conditions of measurement were as described for Fig. 1.

dria and has been interpreted as being due to a dipolar interaction between an ubisemiquinone radical and another paramagnetic centre, either a semiquinone or HiPIP-type iron-sulphur centre (Ruzicka *et al.*, 1975). Moore *et al.* (1976) have observed a similar signal in mung-bean (*Phaseolus vulgaris*) mitochondria, and report a correlation between the intensity of the signal with the operation of the 'alternative oxidase' pathway. In the present case, it was difficult to make quantitative estimates on the signal, because the preparations tended to become anaerobic and the signal decreased.

Discussion

Since *Arum* spadix mitochondria show very high rates of oxidation of substrates such as NADH and succinate, they would be expected to contain the constituents of the respiratory pathways in large amounts. Therefore it is not surprising to find high concentrations of succinate dehydrogenase centres S-1, S-2 and S-3.

A striking feature of these spectra is the very low concentration of iron-sulphur centres of NADH-ubiquinone reductase (Complex I). This implies that NADH is oxidized by the external, piericidin A-

resistant, non-phosphorylating pathway. The situation is analogous to that in certain yeasts, such as *Candida utilis*, in which Complex I is at a low concentration under conditions of rapid growth, appearing only when the cells are in the resting phase (Grossman *et al.*, 1974). The present results are in agreement with the conclusions of Passam & Palmer (1972) that in *Arum* spadix mitochondria the oxidation of NADH via the 'alternative oxidase' occurs principally by a process that by-passes all the phosphorylation sites.

Examination of the line shape of the $g = 1.93$ signal at 77 K gives evidence for a second iron-sulphur centre, with a potential somewhat lower than -7 mV, that may be involved in the oxidation of NADH. The question as to whether it is a component of the external NADH dehydrogenase can only be resolved by isolation of the protein responsible. *Arum maculatum* spadix tissue, with its extremely high rates of respiration, provides a particularly suitable starting material for such work.

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