PRELIMINARY NOTES

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Molybdenum associated with NADH dehydrogenase in Complex I

In 1962, HATEFI *et al.*[1] reported in the ESR spectrum of Complex I, reduced with NADH and then shaken with air, two unidentified signals (*c* and *d* in Fig. 2 of ref. 1), in addition to a free-radical signal and the g = 1.94 signal now known to be due to an iron–sulphur protein. KAWAKITA AND OGURA[2] showed that on incubation of Complex I + III with NADH at 0°, the intensity of the g = 1.94 signal decreases with time and signals at g = 1.98 and g = 1.95 appear.

Very recently, D. V. DERVARTANIAN AND R. BRAMLETT (personal communication) showed that NADH dehydrogenase isolated from iron-deficient *Azotobacter vinelandii* contains molybdenum that is responsible for an asymmetric ESR signal at g = 1.95 under reducing conditions. This paper provides evidence for the fact, mentioned at a recent symposium[3], that the signals at g = 1.98 and g = 1.95 observed

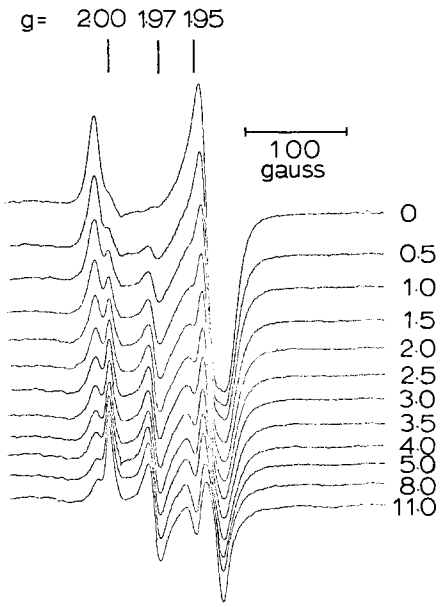

Fig. 1. Effect of incubation of Complex I (made according to ref. 1) with NADH at 22° on ESR spectra. Complex I (37 mg/ml) suspended in 0.66 M sucrose, 50 mM Tris–HCl (pH 8.0), and 1 mM histidine was mixed with NADH (6.2 mM) in an ESR cuvette and immediately frozen in liquid nitrogen. After the spectrum had been taken the cuvette was incubated in a water bath at 22°. At different time intervals (in minutes) as shown in the figure, ESR spectra were recorded with a Varian E-3 apparatus after rapid freezing of the sample. Conditions: temperature, −193°; modulation amplitude, 6.3 gauss; power, 63 mW.

by HATEFI *et al.*[1] and KAWAKITA AND OGURA[2] in reduced Complex I and Complex I + III, respectively, are also due to molybdenum.

Fig. 1 shows the ESR spectral changes on incubating Complex I with NADH. It can be seen that a signal at g = 1.97, with two components at g = 2.00 and about g = 1.95, appears while at the same time the intensity of the g = 1.94 signal is halved (Fig. 2). The intensity of its g = 2.01 component declines by about 80 %. The presence of rotenone (5 nmoles/mg protein) has no effect. These ESR spectral changes were correlated with a small increase in absorbance measured with the wavelength pair 460–510 nm, but 90 % of the initial decline in absorbance on adding NADH was still present after 6 min. Since excess NADH was used, the spectral changes cannot be due to partial reoxidation as HATEFI *et al.*[1] suggested (see also ref. 2).

The g = 1.97 signal is much less temperature dependent than the g = 1.94 signal, so that above −120° spectra of the g = 1.97 signal can be measured without interference of the iron–sulphur protein signal. This is shown in Fig. 3. The g = 1.97 signal has components at g = 2.00 (peak) and g = 1.95 (trough). These g values as well as the relative temperature independence of the signal intensities are characteristic for molybdenum (refs. 4 and 5).

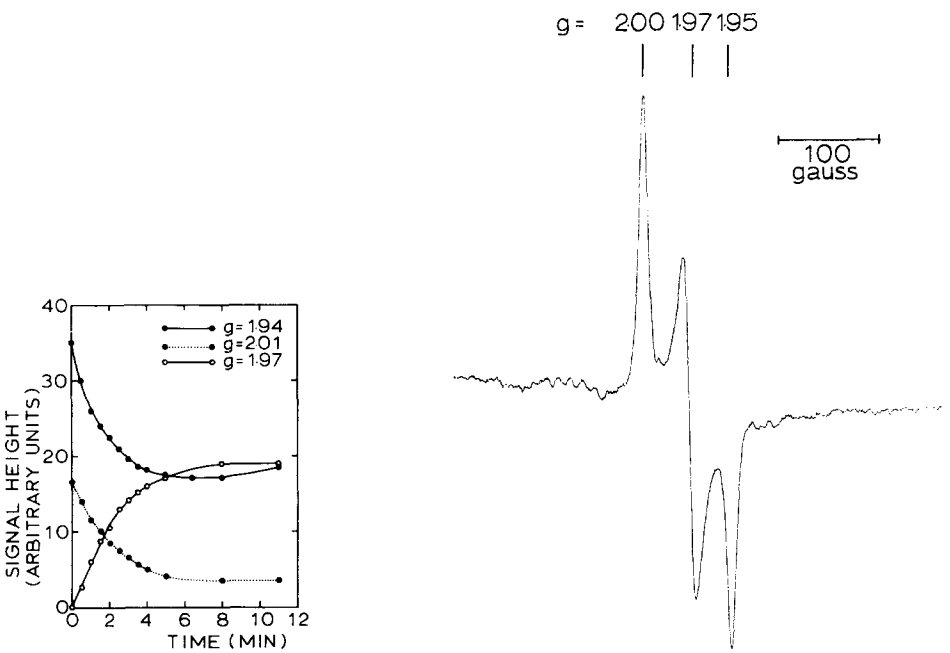

Fig. 2. Time course of spectral changes shown in Fig. 1. The peak height of the g = 2.01 top, the top *minus* trough of the g = 1.97 signal and the trough depth of the g = 1.94 signal are plotted against the incubation time. Because the g = 1.95 (trough) component of the g = 1.97 signal interferes with the top but not with the trough of the g = 1.94 signal, the trough depth of the latter signal was taken as a measure of the g = 1.94 signal intensity.

Fig. 3. ESR spectrum of the g = 1.97 signal, measured at −115°. Complex I was incubated with NADH as in Fig. 1 for 11 min at 22°. Conditions of measuring ESR spectrum were the same as in Fig. 1 except for the temperature, and the gain that was 5 times larger. At −193° the g = 1.97 signal showed saturation above 8 mW. At −115°, however, no saturation was observed even at 160 mW.

The amount of molybdenum visible in the ESR spectrum, determined by comparison of the signal intensity, by double integration, with that of a Cu(II)–EDTA standard, was 0.23 nmole Mo per mg protein. Taking into account that in xanthine oxidase[4] and aldehyde oxidase[5] maximally 20–25 % of the Mo present is visible in the ESR spectrum, it is likely that at least 1 nmole of Mo is present per mg protein, *i.e.* about 1 mole Mo per mole FMN. A preliminary analysis of molybdenum by atomic absorption shows the presence of about 1 nmole of Mo per mg of Complex I.

From Fig. 1 it can be seen that the trough of the g = 1.94 signal remaining at the end of the incubation is narrower than the original signal. In fact, other studies revealed that the g = 1.94 signal seen in the first trace of Fig. 1 is composed of two almost identical signals but with different contributions of the g = 2.01 component. It is evident from Fig. 2 that the decrease of the g = 1.94 signal and the appearance of the g = 1.97 signal are synchronous. The time course of these phenomena appears, however, far too slow to be important for the enzymic activities of Complex I.

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