BBA 43188

Electron spin resonance of non-haem iron in xanthine oxidase

Temperature-sensitive ESR signals due to iron in reduced xanthine oxidase were first reported by BRAY, PETTERSSON AND EHRENBERG[1]. Later, studies were made on the kinetics of the appearance and disappearance of the signal[2]. Iron in the enzyme has also been studied by magnetic susceptibility measurements[1,3], by Mössbauer resonance[4] and by optical rotatory dispersion and circular dichroism[5]. All the evidence points to a very strong resemblance between the iron of xanthine oxidase and that in other non-haem iron proteins[6], typified by, *e.g.*, spinach ferredoxin[7,8]. We now report on the ESR spectrum of milk xanthine oxidase recorded at helium temperatures. Similarities to spinach ferredoxin are confirmed though g values for the two proteins are by no means identical.

Xanthine oxidase was prepared either by the method of PALMER, BRAY AND BEINERT[9] or by that of HART AND BRAY[10]. Samples were reduced anaerobically in ESR tubes with taps[11]. With long reduction times using sodium dithionite as reducing agent, molybdenum signals became weak, which was advantageous for examining the iron signals. ESR measurements were made on a Varian V4502-15 X-band apparatus, blowing cold helium gas through the variable temperature fitting and measuring the sample temperature with a thermocouple. Integrations were performed by the method of WYARD[12] using copper-EDTA[1] as standard.

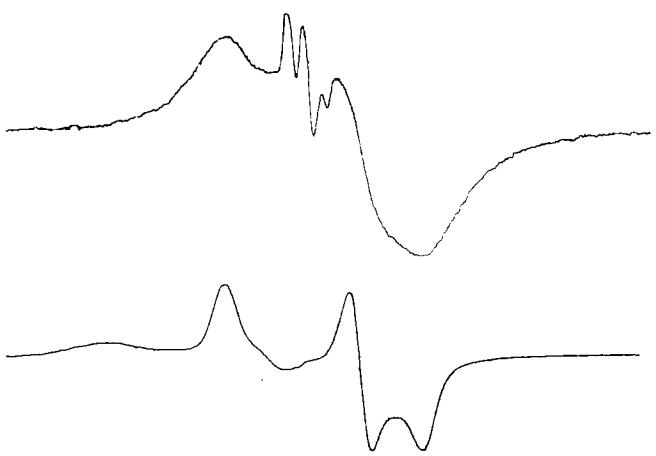

Fig. 1. X-band ESR spectrum of iron in reduced xanthine oxidase at 93 °K (top) and at about 11 °K (below). A weak molybdenum signal can be seen at the higher temperature but has disappeared, owing to saturation, at the lower temperature. A 4-h reduction with approx. 20 moles dithionite per mole enzyme, at pH 8.2 and 25° was employed. The sample had an activity per $A_{450\,m\mu}$ unit of 60 and was used at a concentration of 0.2 mM.

The spectrum of a sample of enzyme at both helium and nitrogen temperatures is shown in Fig. 1. The iron spectrum was unchanged, though somewhat less intense, when purine or salicylaldehyde was used for reduction. g values were g_z: 2.022, g_y: 1.935, g_x: 1.899, which may be compared with values for spinach ferredoxin[7,8,*].

* D. O. HALL, M. C. W. EVANS, F. R. WHATLEY AND J. F. GIBSON, personal communication.

A plot (Fig. 2) of integrated intensity of the signals against $1/T$ shows that copper-EDTA gives the expected straight line passing through the origin, as predicted by simple theory. Xanthine oxidase on the other hand, though it behaves ideally up to about 40 °K, shows progressively increasing deviations above this temperature. At

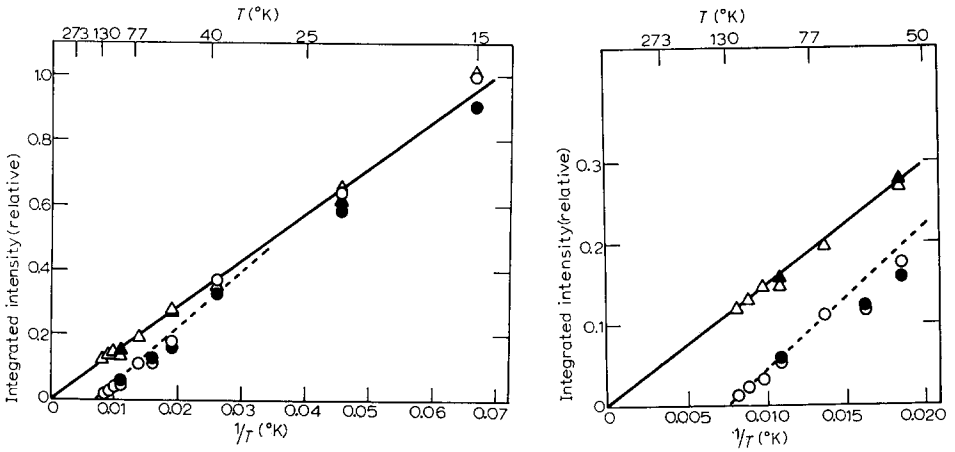

Fig. 2. Effect of temperature on signal intensity for xanthine oxidase and for Cu-EDTA. Integrated signal intensities, relative to the values at 15 °K, are plotted against the reciprocal of the absolute temperature. The graph on the right shows data at the higher temperatures on an expanded scale. Xanthine oxidase was reduced as in Fig. 1 and molybdenum signals, when observed, were not included in the integrations. △ and ▲, Cu-EDTA at low and high power, respectively; ○ and ●, xanthine oxidase at low and high power. Attenuations of microwave power ranging from 0 to 35 db were employed depending on the temperature. All results were normalized by multiplying by the factor: antilog$_{10}$ (db/20). At some temperatures two power levels differing by not less than 10 db were used to check that saturation effects were not serious. Measurements above 77 °K were carried out separately using nitrogen cooling in place of helium. To allow for changes in spectrometer sensitivity a correction was applied (30%) to all the data from the nitrogen series to bring the copper results in the two temperature ranges on to a single straight line.

93 °K the integrated intensity is only about 1/3 of the expected value, while the signal vanishes completely at about 130 °K*. Integration in the low-temperature region indicated that 2–3 of the 8 iron atoms of the enzyme molecule were participating in the signal, assuming 1 unpaired electron per iron atom. However, signal development under the conditions of reduction used was not necessarily optimal and copper-EDTA is by no means an ideal standard. Hence the participation of more of the iron atoms is not excluded.

We thank Professor F. R. WHATLEY and Dr. D. O. HALL for the sample of ferredoxin, Miss F. M. PICK for technical assistance and the Science Research Council for providing funds for liquid helium. Work at the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) was supported by grants from the Medical Research Council and the British Empire Cancer Campaign for Research,

* A sample of spinach ferredoxin prepared as in ref. 8 yielded a plot similar to that obtained for xanthine oxidase up to at least 77 °K.

and by the Public Health Service Research Grant No. CA-03188-09 from the National Cancer Institute, U.S. Public Health Service.

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Received October 30th, 1967
Revised manuscript received January 11th, 1968