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Enzymology in supercooled water

RECENT years have witnessed an increasing interest in applications of low temperature techniques to studies of enzyme-catalysed reactions, the aims being the temporal resolution of the contributing reactions and the stabilisation of intermediate species. To overcome the low temperature limit set by the freezing point of the aqueous medium mixtures of water and various polar organic solvents have been used[1–4]. Although these aqueous mixtures affect the solvation interactions of the enzyme and its conformational stability[5], they can be shown to influence neither substrate specificity, nor the reaction pathway. We have investigated the feasibility of supercooled water to achieve conditions closer to the physiological ones. To circumvent freezing by heterogeneous nucleation, the aqueous phase containing enzyme, substrate and buffers was emulsified in an oil which was supersaturated with a water insoluble surfactant[6]. We here present the results on bacterial cytochrome P_{450}, a mono-oxygenase which has already been studied in mixed solvents[1,2].

Sorbitan tristearate was added to corn oil or safflower oil at 2.5% (w/v) and dissolved by gentle heating. An aliquot (1.5 ml) of an aqueous solution containing the enzyme in phosphate buffer, KCl and camphor (substrate) was emulsified in 3.5 ml of the oil phase at 10 °C. This was achieved by manual shaking, followed by microemulsification in a blender, until a droplet size of 1–5 μm diameter had been obtained. Optical spectra were recorded at temperatures down to −30 °C using an Aminco–Chance DW2 spectrophotometer. The various redox states of the substrate bound enzyme could be observed at low temperatures. The equilibrium constant governing the spin state equilibrium of the ferric cytochrome P_{405} was measured at several temperatures. The Van't Hoff ΔH at *p*H 7 agreed well with the corresponding value at ambient temperatures (10.3 kJ mol^{-1}) (ref. 7) but differed significantly from the low temperature value (30.1 kJ mol^{-1}) determined in a 50% aqueous ethylene glycol solution[8].

To investigate the possible stabilisation of labile species at subzero temperatures the unstable compound P_{450} ($Fe_S^{2+}.O_2$) was formed at 0 °C, quickly emulsified and cooled to −20 °C in the spectrophotometer. The absorption spectrum in Fig. 1 clearly shows the maxima at 418 and 552 nm, characteristic of the oxy-ferrocytochrome. Repetitive spectra taken over a period of 1 h showed no evidence of autoxidation. After heating the emulsion to 25 °C for 20 min and recording another spectrum at −20 °C, the compound had been totally transformed to Fe_S^{3+}. All spectra recorded in supercooled water were identical to those observed in normal conditions, except for a low temperature-induced sharpening of the peaks.

The preliminary experiments indicate that the extension of the accessible temperature range to the homogeneous nucleation temperature (approximately −40 °C) permits the stabilisation of enzyme-substrate complexes, and of labile intermediates and makes possible the temporal resolution of the enzyme catalysed reaction. A more detailed account of this work is in preparation.

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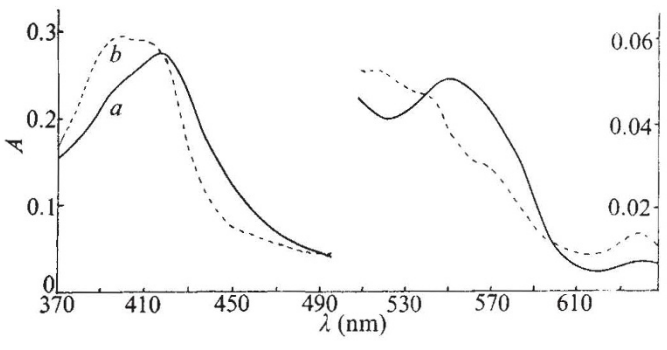

Fig. 1 Spectra of (*a*) oxy-ferrocytochrome P_{450} ($Fe_S^{2+}.O_2$) stabilised at −20 °C in emulsified supercooled water, and (*b*) the same sample after warming and maintaining at 20 °C for 40 min and subsequently recooling to −20 °C. The long wavelength part of the spectrum (right-hand ordinate) was recorded with a higher sensitivity.

Corrigenda

In 'Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland' by H. J. Chapman and S. Moorbath (*Nature* **268**, 41; 1977), line 4 in paragraph four should read . . . The isochron yields an age of 2680 ± 60 Myr (2σ), which . . . .

In 'Eighty thousand β-adrenoreceptors in a single cell' by D. Atlas, F. Hanski, A. Levitski (*Nature*, **268**, 145; 1977) the ordinate label of Fig. 1*c* should read . . . ($M^{-1} \times 10^{-14}$).

In 'Histone content of germinating pea embryo chromatin decreases as DNA replicates' by F. Grellett, M. Delseny and Y. Guitton (*Nature* **267**, 724; 1977) the labelling of the peaks in Fig. 1 should read (from left to right): H1 (30,500; 26,500; 24,800), H2B (16,800), H2A (15,100), H3 (13,500), H4 (10,500).