SUPEROXIDE DISMUTASE IN SOME OBLIGATELY ANAEROBIC BACTERIA

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1. Introduction

Substantial protection against oxygen toxicity is afforded to aerobic and facultatively anaerobic organisms by their possession of superoxide dismutase [1,2]. This enzyme was reportedly not present in obligately anaerobic bacteria [3], i.e. species which exhibit sensitivity to oxygen at 0.2 atm or less [1]. Thus it appeared that the distinction between the obligate and facultative anaerobe, formerly a somewhat arbitrary line drawn on a spectrum of aerotolerance, could be more satisfactorily defined in biochemical terms, viz.: the obligate anaerobe is devoid of superoxide dismutase, the facultative (aerotolerant) anaerobe contains this enzyme.

In this communication we report that possession of superoxide dismutase is not restricted to organisms capable of growth in air. We have found superoxide dismutase in moderately high specific activity in *Chlorobium thiosulfatophilum* and *Clostridium perfringens*, and have partially purified the cyanide-insensitive enzyme of the latter organism. Lesser activities of the enzyme were detected in *Chromatium* sp., *Desulfotomaculum nigrificans, Desulfovibrio desulfuricans* and some other species of *Clostridium*.

2. Experimental

Strains of *Clostridium butyricum* were kindly provided by Mr J. Wolf, University of Leeds, England, *Clostridium pasteurianum* ATCC 6013 was supplied by Mrs Winifred Ego, University of Hawaii, U.S.A. and *Clostridium perfringens* type A NCIB 11105 was a local isolate. *Escherichia coli* B and *E. coli* K-12 mutant AB1157 [4] were from our laboratory collection, and all other organisms were obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland. The *E. coli* strains were grown aerobically with shaking at 37°C in glucose (1%) nutrient broth (Oxoid Ltd., London, England) and all other organisms were grown anaerobically in batch culture, *Chlorobium thiosulfatophilum* NCIB 8346 with illumination at 30°C in CCT medium [5], *Chromatium* sp. NCIB 8348 with illumination at 30°C in the medium of Eymers and Wassink [6] supplemented with 0.05% yeast extract, *Clostridium* species at their optimum growth temperatures in Reinforced Clostridial Medium (Oxoid Ltd.), *Desulfotomaculum nigrificans* NCIB 8395 at 55°C in BETI broth [7] and *Desulfovibrio desulfuricans* NCIB 8307 at 30°C in medium C of Postgate [8]. The cultures were harvested in the late exponential phase by centrifuging at 12 000 × *g* for 20 min at 4°C. After washing in 0.05 M potassium phosphate buffer pH 7.0, the cells were resuspended in a small volume of this buffer and disrupted by passage at 20 000 psi through a chilled French pressure cell (Aminco, Silver Spring, Md., U.S.A.). Cell debris was removed by centrifugation at 26 000 × *g* for 30 min at 4°C. Protein was measured by the method of Lowry et al. [9]. Polyacrylamide gel disc electrophoresis of cell extracts was performed by the method of Davis [10], protein bands being located by staining with Coomassie Blue [11] and superoxide dismutase activity being located on duplicate gels by the method of Beauchamp and Fridovich [12]. Superoxide dismutase activity in crude and partially purified cell extracts was measured spectrophotometrically by the method of McCord and Fridovich [13]. This assay was complicated by the substantial soluble cytochrome *c* reductase activity present in many of the extracts.

This could be removed by prolonged dialysis, but it was found that the superoxide dismutase activity directly measurable after dialysis, equalled that indirectly assayable in the crude, undialysed extract by making allowance for the 'background' cytochrome *c* reduction (measured in the absence of the superoxide generating system of xanthine plus xanthine oxidase EC 1.2.3.2). One unit of superoxide dismutase activity is defined as that quantity of enzyme which would cause 50% inhibition of the rate of cytochrome *c* reduction by O_2^- generated by xanthine oxidase, though in the assay, stoichiometry was often not maintained at levels of inhibition greater than 40%. The superoxide dismutase of *Cl. perfringens* NCIB 11105 was purified 15-fold by a procedure (to be published elsewhere) involving precipitation in the 50 to 70% saturated ammonium sulphate fraction of the cell extract, followed by gel filtration through Sephadex G-75 and chromatography on DEAE-cellulose. Cytochrome *c* and enzymes were purchased from Sigma (London) Chemical Co., DEAE-cellulose from Whatman Biochemicals Ltd., Maidstone, England, Sephadex G-75 from Pharmacia (Great Britain) Ltd., London, and reagents for polyacrylamide gel electrophoresis from Eastman-Kodak, Rochester, N.Y., U.S.A.

3. Results

A preliminary survey of the distribution of superoxide dismutase in several obligately anaerobic bacteria, was carried out by visual inspection of polyacrylamide disc gel electrophoretograms made using samples of crude cell extracts, and treated to reveal the presence of bands of superoxide dismutase activity [12]. The enzyme was present in several of these organisms (table 1), *Chlorobium thiosulfatophilum* NCIB 8346 and *Clostridium perfringens* NCIB 11105 yielding particularly prominent bands of superoxide dismutase activity in this assay. Some activity was also discernible in a number of other species, including *Cl. acetobutylicum* and *Cl. pasteurianum* which McCord et al. [3] had reported to be devoid of the enzyme. Several other *Clostridium* species contained either no superoxide dismutase activity, or less than could be detected by this procedure, the findings being somewhat unpredictable in that different strains of the same species could differ in their contents of the enzyme (e.g. *Cl. butyricum*).

Table 1
Qualitative demonstration of superoxide dismutase activity in extracts of some anaerobic bacteria[a]

Organism	Superoxide dismutase[b]
Photosynthetic anaerobes:	
Chlorobium thiosulfatophilum NCIB 8346	+
Chromatium sp. NCIB 8348	+
Sulphate reducers:	
Desulfotomaculum nigrificans NCIB 8395	+
Desulfovibrio desulfuricans NCIB 8307	+
Fermentative anaerobes:	
Clostridium acetobutylicum NCIB 6445	+
Clostridium acetobutylicum NCIB 8049	+
Clostridium acetobutylicum NCIB 8052	+
Clostridium beijerinckii NCIB 9362	+
Clostridium bifermentans NCIB 506	+
Clostridium butyricum SA I	+
Clostridium butyricum SA II	–
Clostridium butyricum CNRZ 528	+
Clostridium butyricum CNRZ 531	–
Clostridium pasteurianum ATCC 6013	+
Clostridium perfringens type A NCIB 11105	+
Clostridium sporogenes NCIB 532	+

[a] Organisms were grown, and extracts prepared, as described in Experimental. Crude cell extract (2 mg protein) was subjected to polyacrylamide gel electrophoresis and the band(s) of superoxide dismutase activity were visualised by the method of Beauchamp & Fridovich [12].

[b] Presence (+) of superoxide dismutase is contrasted with absence of sufficient activity of enzyme to be detectable in this assay (–).

Spectrophotometric assay of superoxide dismutase activity in crude cell extracts (2.0) enabled a comparison to be made between the specific activities of the enzyme in various anaerobes with those found in aerobically grown strains of *E. coli* (table 2). Though the enzyme was present in surprisingly high specific activity in *Chl. thiosulfatophilum* NCIB 8346 and *Cl. perfringens* NCIB 11105, in those other anaerobes possessing a superoxide dismutase, this was present at only 1 to 6% of the specific activity found in aerobic *E. coli*.

The partly purified preparation of superoxide dismutase from *Cl. perfringens* (2.0) migrated as two major protein bands on polyacrylamide gel electrophoresis, the enzyme activity coinciding with the greater of these bands (fig. 1). The nature and properties of

Table 2
Specific activities of superoxide dismutase in crude extracts of (a) aerobically grown *E. coli* and (b) several anaerobic bacteria

Organism	Specific activity of superoxide dismutase (units/mg of protein)[a]
Escherichia coli B	44.0
Escherichia coli K-12, mutant AB 1157	36.8
Chlorobium thiosulfatophilum NCIB 8346	14.0
Chromatium sp. NCIB 8348	0.6
Desulfotomaculum nigrificans NCIB 8395	2.6
Desulfovibrio desulfuricans NCIB 8307	0.6
Clostridium pasteurianum ATCC 6013	0.5
Clostridium perfringens NCIB 11105	15.6

[a] Superoxide dismutase activity was measured spectrophotometrically in undialysed, crude extracts, as described in Experimental.

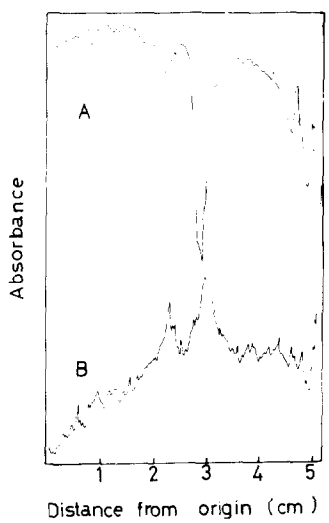

Fig. 1. Location of bands of (a) superoxide dismutase activity, and (b) protein, on duplicate polyacrylamide disc gel electrophoretograms of partly purified superoxide dismutase from *Cl. perfringens* NCIB 11105 (sp. activity = 234 units/mg protein). A. Gel 'negatively' stained to locate achromatic band of superoxide dismutase activity [12], and scanned at 560 nm. B. Gel 'positively' stained for protein with Coomassie Blue [11], and scanned at 609 nm. Peaks in A and B at 5.2 cm, coincide with location of front marker dye (bromophenol blue); the achromatic 'troughs' at 4.5 to 5 cm in trace A are artefactual, again being attributable to the marker dye. The gels were scanned using a Gilford 2400-S recording spectrophotometer.

this clostridial superoxide dismutase, which was not inhibited by 10^{-3} M cyanide, are now being studied.

4. Discussion

Though an organism can evidently contain quite substantial superoxide dismutase activity and yet be incapable of growth in air, this does not mean that the enzyme renders it no significant service. Even obligate anaerobes display a spectrum of oxygen sensitivity ranging from those bacteria for which oxygen is apparently bactericidal at very low concentrations to those which can tolerate limited exposure to air, and for which atmospheric oxygen may, in the short term, be reversibly bacteriostatic rather than bactericidal [14]. This range of oxygen sensitivity is displayed within the genus *Clostridium; Cl. perfringens* is noted for its comparative oxygen tolerance, whilst even *Cl. acetobutylicum* and *Cl. pasteurianum* can survive transient encounter with low concentrations of oxygen [15]. The present study should therefore now be extended to other species of *Clostridium* to determine whether there is any correlation between their differing degrees of oxygen tolerance and their contents of superoxide dismutase, with species such as *Cl. carnis, Cl histolyticum* and *Cl. tertium* which are relatively oxygen tolerant, being compared with more exacting species such as *Cl. oedematiens* type D [16].

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