# Oxygen induces fatty acid (*n*-6)-desaturation independently of temperature in *Acanthamoeba castellanii*

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**Abstract Induction of a microsomal oleate Δ12 (*n*-6) desaturase which is mainly responsible for an increase in membrane lipid unsaturation at low temperature has been observed in the free-living amoeba *Acanthamoeba castellanii*. In this study we show that the enzyme can also be regulated by oxygen independently of temperature in batch cultures grown to $O_2$-limitation. Raising the oxygen concentration from below the lower limit of detection ($< 0.1$ μM) to approximately air-saturation (230 μM), whilst maintaining the growth temperature constant (30°C), increased lipid unsaturation and elevated *n*-6-desaturase activity 2.3-fold. Addition of the protein synthesis inhibitor, anisomycin, showed that increased desaturase activity was due to new protein synthesis rather than activation of pre-existing enzyme. These observations are important for future studies of the mechanism of temperature adaptation in poikilotherms.**



*Key words:* Oleate (*n*-6)-desaturase; Oxygen; *Acanthamoeba castellanii*; Flow cytometry; Microsomal fraction

## 1. Introduction

One of the most common stresses that plants and micro-organisms have to cope with is that of extremes of temperature. There has been much work on the mechanisms of adaptation, which are necessary to allow for the effective functioning of biological membranes [1,11]. In *A. castellanii* the adaptive membrane response to chilling is necessary for continued phagocytosis [2,3]. Without an adaptive response, membrane lipids may undergo phase-separation with consequent deterioration of membrane performance and consequent cellular damage or death.

There are many strategies available to a poikilothermic organism for the optimization of the bilayer membrane structure at low viscosity with respect to the acyl chain order. Probably the most widespread is for the proportion of unsaturated acyl side chains to be increased. An increased 'unsaturation index' is usually a consequence of elevated fatty acid desaturase activity. A dramatic example of the importance of this adaptive mechanism comes from the work of Murata's group where genetic manipulation of organisms with desaturase genes has been shown to confer increased resistance to chilling stress [4,5].

There are several mechanisms by which desaturase activities can be increased [6]. Increased gene transcription to allow more enzyme protein to be made is particularly important in several recently studied cases, and we have shown that increased protein synthesis accounts for most of the raised desaturase activity during chilling adaptation in *Acanthamoeba castellanii* [7]. In this organism it is the *n*-6 desaturase which is induced with the consequent increased conversion of oleate to linoleate [8]. Although temperature triggers the response, the presence of oxygen is one requirement for maximal desaturase activity [9]. Because the increased solubility of oxygen at low temperatures in itself has been suggested to cause raised desaturation [10], we wished to differentiate any effect that oxygen concentrations might have in *Acanthamoeba* from that of lowered temperatures.

## 2. Methods

### 2.1. Organism and culture conditions

*Acanthamoeba castellanii* (Neff strain) was grown [12], either in shake flasks (200 ml culture) in 500 ml flasks; reciprocal shaking at 160 strokes $min^{-1}$) or in a 800-ml working vol. fermenter at 30°C. $O_2$ was measured with an Ingold 507 electrode. Maximum dissolved $O_2$ levels were calculated from solubility data [13].

### 2.2. Preparation of microsomal fractions

These were prepared as described previously [14].

### 2.3. Flow cytometry

Samples were analysed using a Skatron Argus Flow cytometer (Skatron, Tranby, Norway) using the FITC filter block [15]. Typically histograms were obtained from 5000 organisms after 5 min exposure to bis(1,3-dibutylbarbituric acid) trimethine oxonol.

## 3. Results and discussion

During growth in a fermenter, the dissolved $O_2$ concentration decreased after 27 h to below its detection limit with an oxygen electrode (i.e. $< 0.1$ μM) due to the very high rates of respiration. Moreover, at 48 h (when the culture was in the stationary phase), there was low *n*-6-desaturase activity and a consequent decrease in linoleate levels [3]. If we took *A. castellanii* cultures at 48 h and increased the partial pressure of the oxygen supplied from 20 to 60 kPa we were able to increase the dissolved oxygen levels when using partial pressures above 40 kPa. Dissolved $O_2$ concentrations were 3.5, 7, 74, 180 and 327 μM for sparging at 40, 45, 50, 55 and 60 kPa, respectively. (Air saturation for oxygen at 30°C was 230 μM.) Coincidentally, there was a noticeable increase in *n*-6 unsaturation in the cultures at $> 7$ μM dissolved $O_2$ (Fig. 1). Thus, for control cultures (supplied with air, but at $< 0.1$ μM $O_2$) total lipids contained 36% oleate and 10% linoleate while cultures with 327 μM $O_2$ contained 15% oleate and 32% linoleate. An increase in C20 polyenoate (derived from linoleate) unsaturation was also found (Fig. 1A). The unsaturation index was increased from 1.64 at $< 0.1$ μM $O_2$ to 2.2 at 330 μM $O_2$: half of this increase was attained when $O_2$ was 12.5 μM (Fig. 1B). Because we had to sparge vigorously in order to

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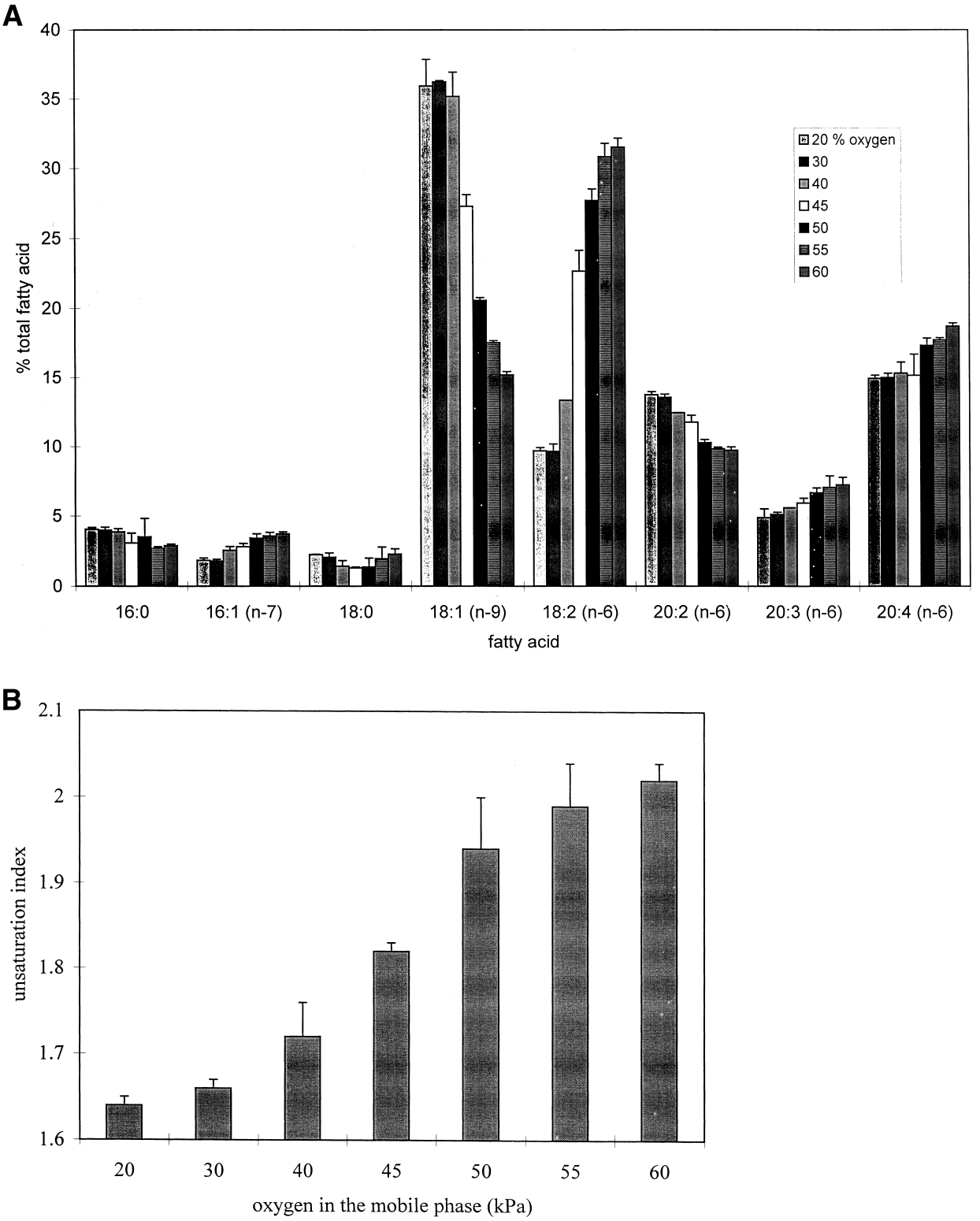


Fig. 1. Effects of increasing dissolved $O_2$ at 30°C in an $O_2$-limited culture of *Acanthamoeba castellanii* on whole cell fatty acid composition. Oxygen concentrations in a late-exponential phase culture were raised by stepwise increases of the partial pressure of $O_2$ in the supplied gas phase at 20-min intervals. Organisms were harvested, before each increase in $O_2$ concentration, lipids extracted [18,19] and methyl esters analysed [20,21]. A: Names of fatty acids are abbreviated with the first figure showing the number of carbon atoms and the second showing the number of double bonds; the positions of the double bonds were also determined. B: Unsaturation indices (mean numbers of double bonds per acyl chain). All values are means of four replicate determinations ± S.D.

increase the dissolved oxygen levels in the rapidly-respiring cell cultures, we checked that no damaged had occurred to *A. castellanii*. This was done by confirming exclusion of trimethine oxonol, an anionic membrane potential-sensitive flu-

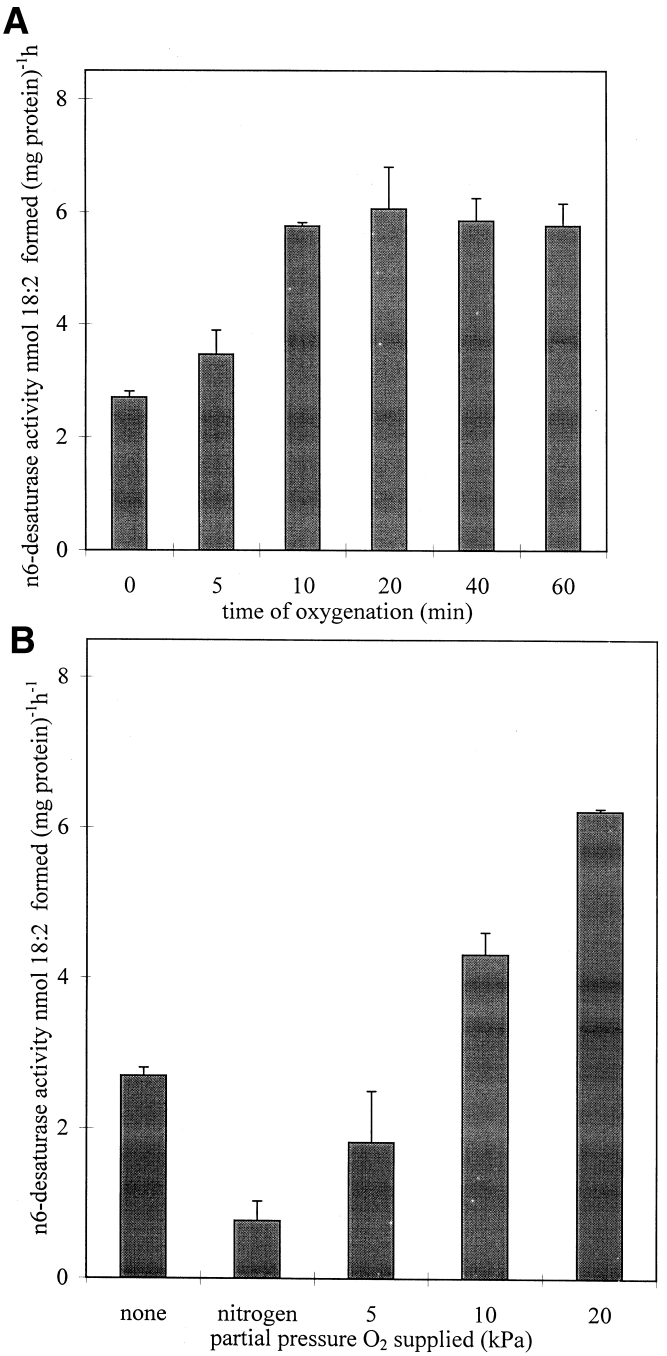


Fig. 2. Effects of increased $O_2$ in the culture medium of *A. castellanii* on *n*-6 (Δ12)-desaturase activities of subsequently isolated microsomal fractions. A: Effect of vigorous oxygenation of cultures (bubbling with 20 kPa $O_2$ in $N_2$ at 140 ml/min) on desaturation of oleate by microsomal membranes isolated subsequently. [1-$^{14}$C]Oleoyl CoA was used as substrate, and the reaction carried out as previously described. B: Effects of aerating cultures with gas mixtures containing different $O_2$ partial pressures on *n*-6-desaturase activity. The control culture was not aerated. Values are means ± S.D. from 3 experiments.

orophore and measuring cell-associated fluorescence by flow cytometry [16]. No decrease in viability [17] during the experimental period could be detected.

Since oxygen is a substrate for the *n*-6 desaturase reaction, then the increased lipid unsaturation observed (Fig. 1A) could have been due to increased $O_2$ availability as well as elevated enzyme activity. Therefore, we measured *n*-6 desaturase directly (under optimal in vitro conditions when oxygen was in excess) in microsomal fractions prepared from $O_2$-limited organisms exposed to increased dissolved $O_2$. In flask cultures we found it was not necessary to use such high partial pressures of $O_2$ as for the fermenter cultures. By sparging with 20 kPa $O_2$ at 140 ml/min we were able to achieve near saturation levels. This oxygenation increased detectable levels of fatty acid *n*-6-desaturase activity in a time-dependent manner up to 20 min, after which no further increase was seen (Fig. 2A). Moreover, as with the unsaturation index (Fig. 1B), the increase in desaturase activity was clearly dependent on

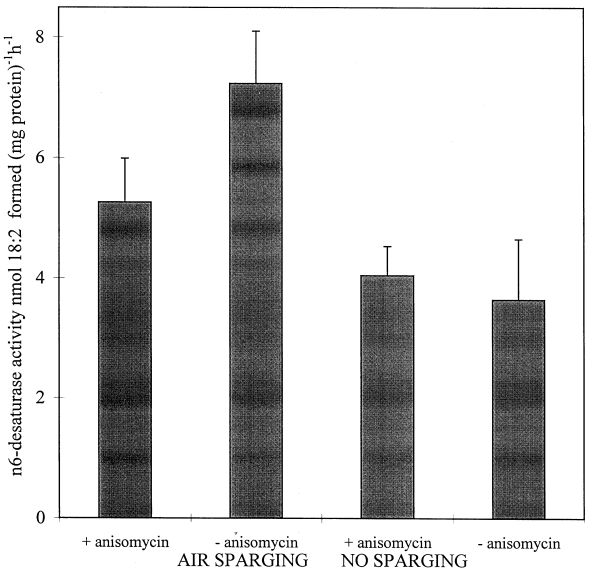


Fig. 3. Effects of anisomycin on *n*-6-desaturase activities [3] in microsomal fractions from $O_2$-limited *A. castellanii*. Cultures were oxygenated (20 kPa $O_2$ in $N_2$, 140 ml/min for 1 h) with or without anisomycin (0.1 mg/ml) before harvesting, homogenization and preparation of microsomes [2]. Control cultures were not oxygenated. Mean values are shown from 4 independent experiments ± S.D.

the oxygen concentration (Fig. 2B). These experiments show that it is possible to increase *n*-6-desaturase activity by oxygen without the need for a temperature shift.

For low temperature induction of *n*-6-desaturase activity, we had found previously that new protein synthesis was primarily responsible [7]. Accordingly, we used the same strategy to see whether a similar mechanism was used for oxygen-induced enzyme activity. We used anisomycin as an inhibitor of protein synthesis since this was the most effective compound available [7]. We measured total protein synthesis from $^{14}$C-leucine and confirmed that 0.1 mg/ml anisomycin was able to inhibit this about 60% under the experimental conditions used. Anisomycin had no effect on *n*-6-desaturase activity measured in non-sparged cultures (Fig. 3) nor did it affect endogenous lipid composition during the 1-h period of induction. When air sparging was used, the activity of the *n*-6 desaturase in the subsequently isolated membranes was increased from 3.6 to 7.1 nmol linoleate formed/mg protein/h (i.e. by 97%). For cultures containing anisomycin, oxygenation was only able to increase desaturase activity by 31%. The inhibition by anisomycin was similar to the total inhibition of protein synthesis, showing clearly that the new *n*-6-desaturase activity induced in the presence of oxygen was due to the de novo synthesis of enzyme and not to activation of previously existing desaturase.

Our experiments have shown that it is possible to induce a fatty acid desaturase by manipulation of dissolved oxygen levels under isothermal conditions. This finding is particularly important for temperature studies of organisms in a low-oxygen environment such as for protozoa such as *Acanthamoeba castellanii* with a high $O_2$ demand. For temperature-induced desaturases, it appears that the viscosity of the plasma membrane may be the sensing signal for increased gene transcription [10] and it will be important to elucidate the signal transduction pathway and molecular mechanisms required for the oxygen-induction of the *n*-6 desaturase which we have observed.

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