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The catalytic efficiency of soybean lipoxygenase-1 is enhanced at low gravity

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Abstract

Several cellular processes are modified when cells are placed under conditions of weightlessness. As yet, there is no coherent explanation for these observations, nor it is known which biomolecules might act as gravity sensors. Lipoxygenases generate leukotrienes and lipoxins from arachidonic acid, being responsible for many pharmacological and immunological effects, some of which are known to be affected by microgravity. In the course of the 28th parabolic flight campaign of the European Space Agency we measured the activity of pure soybean lipoxygenase-1 on linoleic acid, by a fibre optics spectrometer developed on purpose. It was found that microgravity reduced the apparent Michaelis–Menten constant (K_m) of the enzymatic reaction to one fourth with respect to the 1 *g* control, whereas, the catalytic constant (k_{cat}) was unaffected. Consequently, the catalytic efficiency of lipoxygenase-1 (k_{cat}/K_m) was approximately four-fold higher in flight than on ground. This unprecedented finding suggests that lipoxygenase-1 might be a molecular target for gravity.

Keywords: Catalysis; Enzymes; Humans; Lipoxygenase; Microgravity; Plants

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1. Introduction

Experiments in Space clearly show that several cellular processes, such as growth rates, signalling pathways and gene expression, are modified when cells are placed under conditions of weightlessness [1,2]. As yet, there is no coherent explanation for these observations, nor is it known which biomolecules might act as gravity sensors [1,2]. Recently, microtubule self-organisation has been shown to be gravity-dependent [3], suggesting that investigations at the molecular level might fill the gap between observation and understanding of Space effects. Cellular activities are mostly controlled by enzymes and pathological conditions can arise from alteration of just one of them [4]. Yet, biochemical investigations in microgravity are still very scant, because of the flight costs and of the need of instrumentation developed on purpose.

Lipoxygenases are a family of monomeric non-heme, non-sulfur iron dioxygenases which catalyze the conversion of unsaturated fatty acids into conjugated hydroperoxides [5]. Mammalian lipoxygenases have been implicated in the pathogenesis of several inflammatory conditions, in atherosclerosis, in brain ageing and in HIV infection [6]. In plants, lipoxygenases aid germination, participate in the synthesis of traumatin and jasmonic acid and in the response to abiotic stress [7]. Recently, they have been shown to initiate programmed death (apoptosis) of animal [8] and plant [9] cells. Interestingly, microgravity lowers the immunological response in humans and reduces the bone mass by inducing apoptosis [10,11]. Soybean lipoxygenase-1 (LOX-1) is prototypal for studying the lipoxygenases from tissues of different species, both in strucutural [12] and kinetic [13] investigations. Here, we took advantage of a fibre optics spectrometer developed on purpose, the EMEC (effect of microgravity on enzymatic catalysis) module, to measure the dioxygenation reaction by pure LOX-1 during the 28th parabolic flight campaign of the European Space Agency (ESA). The aim was to ascertain whether microgravity would affect enzyme catalysis.

2. Materials and methods

All chemicals were of the purest analytical grade. Linoleic acid and bovine serum albumin were from Sigma Chemical Co. (St. Louis, MO, USA). Lipoxygenase-1 (linoleate/oxygen oxidoreductase, EC 1.13.11.12, LOX-1) was purified to electrophoretic homogeneity from soybean (*Glycine max* (L.) Merrill, Williams) seeds as reported [14]. Protein concentration was determined according to [15], using bovine serum albumin as a standard.

A reduced gravity environment was obtained by flying a specially modified Airbus A300 Zero-G through a series of parabolic maneuvers, which result in approximately 20–25 s at a gravity level $= 10^{-2}$ g (the so-called 'microgravity phase') [16]. The parabolic flight campaign consisted of 30 parabolas per day for 3 consecutive days.

Lipoxygenase activity was measured in the EMEC module, developed for ESA by Officine Galileo (Alenia Difesa, Florence, Italy). This is a spectrometer, which measures transmittance-absorbance at a single wavelength with performances higher than those of any conventional laboratory spectrometer [17]. It uses a Xenon arc lamp of 35 W, which lights two fused silica fibre optics bundles by means of two opposite Kohler illuminators. The EMEC contains eight cells, which allow to run eight different experiments in a completely automated way. Therefore, the activity of LOX-1 with four substrate concentrations was tested in six independent experiments during the ESA campaign. The dioxygenation of linoleic acid by LOX-1 was initiated by injecting the enzyme (at 8 nM final concentration) into the cell containing the substrate (15–120 μM) in 0.1 M sodium phosphate buffer, pH 7.0 [18]. In all experiments, the reaction buffer contained a saturating concentration (240 μM) of oxygen, as assessed by a YSI-5301 oxygen monitor (Yellow Springs Instrument, Yellow Springs, OH, USA). The formation of linoleic acid hydroperoxides was measured at 234 nm, over a period of 10 s [18]. The LOX-1 activity was measured on ground also in a conventional Lambda 18 spectrophotometer (Perkin-Elmer, Norwalk, CO, USA) and was compared to the activity measured in the EMEC

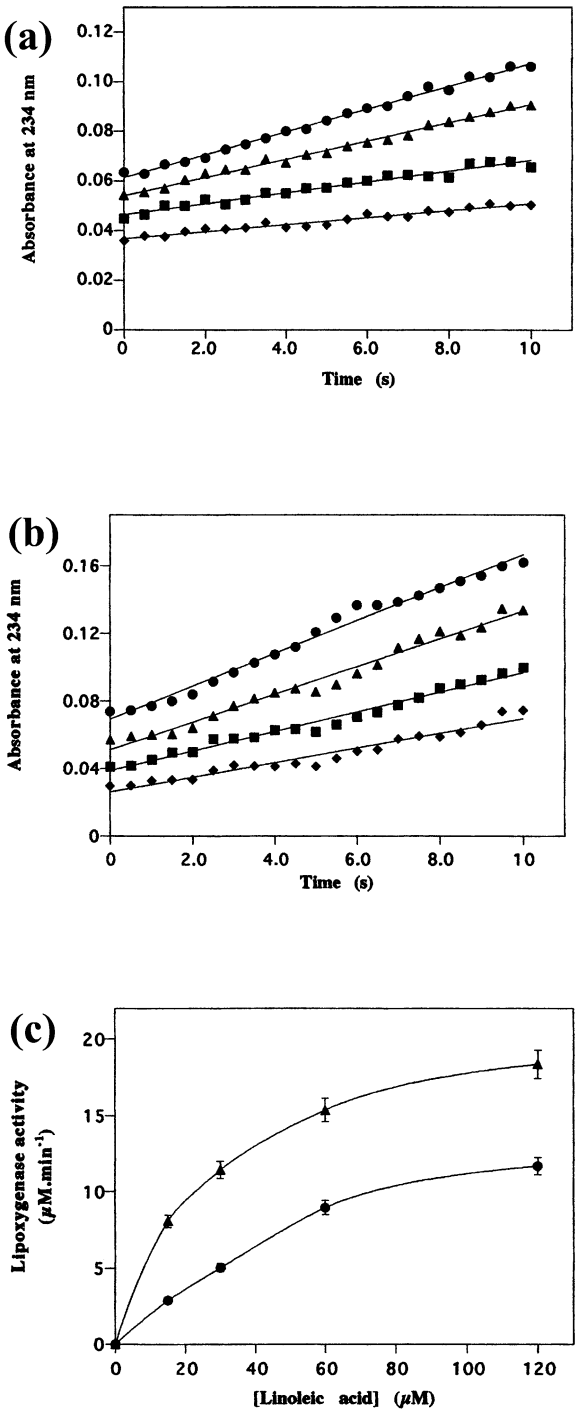

Fig. 1.

module. Each in flight experiment was performed during the microgravity phase of a parabola and on ground (1 g) controls were performed immediately before the parabolic flights, using the same batch of enzyme and substrate and the EMEC instrument. Apparent Michaelis–Menten (K_m) and maximum velocity (V_{max}) values were calculated by fitting the data to a Lineweaver–Burk plot [4,18], yielding straight lines with $r > 0.98$. The data reported in this paper are the mean ($\pm$S.D.) of six independent determinations. Statistical analysis was performed by the non-parametric Mann–Whitney test, elaborating experimental data by means of the InStat program (GraphPAD Software for Science, San Diego, CA, USA).

3. Results

The LOX-1 activity, measured in the EMEC module, was linear over a 10-s period in the 15–120 μM substrate range, both on ground (Fig. 1a) and in flight (Fig. 1b). Interestingly, the absorbance at time 0 was almost identical for the respective linoleic acid concentrations in the ground and flight samples, suggesting that flight conditions (e.g. vibrations) did not affect the kinetics. Reaction rates calculated within this time window showed that LOX-1 activity depended on substrate concentration according to a typical Michaelis–Menten kinetics (Fig. 1c), yielding an apparent Michaelis–Menten constant (K_m) of 10.5 ± 0.5 and 2.6 ± 0.1 μM, on ground and in flight, respectively (Table 1). The apparent maximum velocity (V_{max}) was 22 ± 1 and 23 ± 1 μM min^{-1}, on ground and in flight, thus the catalytic efficiency of LOX-1 (k_{cat}/K_m) was 4.4×10^6 and 18.5×10^6 $M^{-1}.s^{-1}$, on ground and in flight, respectively (Table 1). The same kinetic parameters were obtained on ground in the EMEC module and in a conventional Lambda 18 spectropho-

Fig. 1. Activity of lipoxygenase-1 (LOX-1) on ground and in flight. Time-course of the dioxygenation of linoleic acid by LOX-1 in the EMEC module, on ground (a) or in flight (b). Linoleic acid was used at concentrations of 15 μM (diamonds), 30 μM (squares), 60 μM (triangles) or 120 μM (circles). (c) Dependence of LOX-1 activity on linoleic acid concentration, on ground (circles) or in flight (triangles).

Table 1
Kinetic parameters of the dioxygenation of linoleic acid by lipoxygenase-1 (LOX-1)

LOX-1	K_m (μM) V_{max} (μM.min^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1}s^{-1})
On ground (1 g) in Lambda 18	12.5 ± 0.6 25 ± 1	52	4.2×10^6
On ground (1 g) in EMEC	10.5 ± 0.5 22 ± 1	46	4.4×10^6
In flight ($= 10^{-2}$ g) in EMEC	2.6 ± 0.1* 23 ± 1	48	18.5×10^{6a}

[a] Denotes $P < 0.01$ compared to on-ground controls in EMEC ($n = 6$).

tometer (Table 1).

4. Discussion

Taking advantage of the EMEC module and of the 28th parabolic flight campaign of ESA, we showed for the first time that microgravity reduces the K_m of lipoxygenase activity on linoleate to one fourth of the 1 *g* control, without affecting the V_{max} (Table 1). Consequently, the catalytic efficiency of LOX-1 (k_{cat}/K_m) was approximately four-fold higher in flight than on ground (Table 1). The observation that K_m but not V_{max} was affected suggests that microgravity only facilitates the formation of the enzyme–substrate complex (ES). Therefore, gravity appears to affect the diffusion process, which occurs in enzyme catalysis. Indeed, in diffusion-controlled reactions or reaction steps, macroscopic concentration patterns can be formed from an initially homogeneous solution by way of non-linear dynamics processes [19]. Such processes lead to concentration (density) fluctuations, which are subject to a buoyancy force under gravity; this small, directional, gravity-driven molecular transport can affect molecule–molecule interaction, as shown in microtubule self-organisation [3,19]. On the other hand, the different dimensions of the molecules involved in microtubule self-organisation and in enzyme-substrate interactions leave open the possibility that other gravity-dependent factors might control LOX-1 catalysis in low gravity conditions, e.g. the partitioning of linoleic acid at the water/oil interface [20].

This unprecedented finding shown here for an enzyme suggests that lipoxygenase-1 might be a molecular target for gravity, the first yet described besides microtubules [3]. Since type-1 LOX is the main lipoxygenase in plants and shares with mammalian lipoxygenases several structural and mechanistic properties [21,22], these results could have a broader meaning. They might also form a biochemical background for the immunodepression and the bone mass reduction observed in humans during Space missions.

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