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Microgravity increases the affinity of lipoxygenases for free fatty acids

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Experiments in Space clearly show that various cellular processes, such as growth rates, signaling 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, neither is it known which biomolecules might act as gravity sensors [1,2]. Recently, microtubule self-organization 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].

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.12) generate leukotrienes and lipoxins from arachidonic acid, being responsible for many pharmacological and immunological effects, including programmed cell death (apoptosis) [5]. Interestingly, in humans microgravity lowers the immunological response and reduces the bone mass by inducing apoptosis [6,7].

We measured the dioxygenation of linoleic acid (15–120 μM) by pure soybean lipoxygenase-1 (LOX-1) (8 nM) in 0.1 M sodium phosphate buffer (pH 7.0), containing a saturating concentration (240 μM) of oxygen, as assessed by a YSI-5301 oxygen monitor (Yellow Springs Instrument, Yellow Springs, OH, USA). All experiments were performed at 25°C in a normoxic atmosphere on board a pressurized A300 Zero-G aircraft, during the 28th parabolic flight campaign of the European Space Agency (Bordeaux, 15–26 May 2000). Enzyme activity was assayed by the increase in absorbance at 234 nm over a linearity period of 10 s [5], using a fiber optics spectrometer developed for ESA by Officine Galileo (Florence, Italy): the EMEC (Effect of Microgravity on Enzymatic Catalysis) module [8]. In flight experiments were performed during the low gravity (so-called 'microgravity') phase of a parabola (20 ± 2 s at a gravity level of $10^{-2}g$). On ground ($1g$) controls were performed immediately before the parabolic flights, using the same batches of enzyme and substrate and the EMEC module as in flight. Apparent Michaelis–Menten (K_m) and maximum velocity (V_{max}) values were calculated by fitting the data to a Lineweaver–Burk plot [4]. It was found that microgravity reduced K_m to one fourth of the $1g$ control, without affecting V_{max} (Table 1). Consequently, the catalytic efficiency of lipoxygenase (k_{cat}/K_m) was approximately 4-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. 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 [9]. 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-organization [3,9].

This unprecedented finding shown here for an enzyme suggests that LOX-1 might be a molecular target for gravity, the first yet described besides microtubules [3]. Since the type-1 enzyme is the main lipoxygenase in plants and shares with mammalian lipoxygenases several structural and mechanistic properties [10], these results could have a broad validity.

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Table 1
Kinetic parameters of the dioxygenation of linoleic acid by 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 ($1g$)	10.5 ± 0.5	22 ± 1	46	4.4×10^6
In flight ($10^{-2}g$)	2.6 ± 0.1*	23 ± 1**	48**	18.5×10^6*

*Denotes $P < 0.01$, **denotes $P > 0.05$ compared to on ground controls, as calculated by the non-parametric Mann–Whitney test ($n = 6$).