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Enzyme catalysis in microgravity: an intricate problem to be solved

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There are several reports indicating that weightlessness can affect certain biochemical processes. Most of the data are limited to cellular level, and the microgravity effects appear to be often contrasting [1]. It was to identify the physical–chemical link between microgravity and its potential cell sensors that about ten years ago we started the EMEC (Effect of Microgravity on Enzyme Catalysis) project, with the financial support from the European Space Agency (ESA) and the Agenzia Spaziale Italiana. In 1996, we studied the isocitrate lyase (EC 3.1.3.1) reaction during the 6 min microgravity time (average value 10^{-5} *g*) obtained by the parabolic flight of the sounding rocket MASER 7 (April 16–May 3, 1996, Kiruna, Sweden). The comparison with the data of a simultaneous test performed on ground with the same experimental hardware (the EMEC module [2]) showed that microgravity does not affect the enzyme kinetic parameters: saturation velocity and Michaelis constant [3].

Recently, an experiment performed during the 28th ESA parabolic flight campaign (May 15–26, Bordeaux, France) showed that in microgravity, the Michaelis constant of soybean lipoxygenase-1 for linoleic acid is decreased to one fourth of the ground control [4].

During the same parabolic flight campaign, we studied both the cleavage and the condensation reactions of *Pinus pinea* isocitrate lyase. Technically, the experiment was hosted on board a A300 Zero-G aircraft which, by means of special flight maneuvers, allows 20 s microgravity periods (about 10^{-2} *g*), alternating with hypergravity phases (1.8 *g*) and longer standard *g* conditions. The cleavage reaction (isocitrate = succinate+glyoxylate) was assayed by way of chemical coupling with phenylhydrazine; the formation of glyoxylate phenylhydrazone was followed at 324 nm at varying *threo*-D_s-isocitrate concentrations (range 0.05–0.3 mol/dm^3). The condensation reaction (glyoxylate+succinate = isocitrate) was coupled with isocitric dehydrogenase ($NADP^+$) and the reduction of the coenzyme was monitored at the same wavelength. The condensation reaction assay consisted of 16 tests at four fixed concentrations of succinate (range 0.3–2.0 mol/dm^3) each at four varied glyoxylate concentrations (range 0.04–0.2 mol/dm^3). One session of tests was devoted to study the product inhibition by succinate in the cleavage reaction.

Table 1
Kinetic parameters ± standard error for the condensation and cleavage reactions of *Pinus pinea* isocitrate lyase

	Microgravity	Standard *g*
Condensation		
$V_r/[E_T]$ (s^{-1})	11.7 ± 0.3	11.4 ± 0.3
K_s^Q (mM)	0.60 ± 0.25	0.54 ± 0.17
K_m^P (mM)	0.053 ± 0.020	0.063 ± 0.017
K_m^Q (mM)	0.088 ± 0.005	0.092 ± 0.004
Cleavage		
$V_f/[E_T]$ (s^{-1})	17.4 ± 0.2	17.5 ± 0.2
K_m^A (mM)	0.043 ± 0.002	0.039 ± 0.002

V_r and V_f are saturation velocities of the reverse and forward reactions, respectively. K_m indicates Michaelis constants for the superscript ligand (A = isocitrate); K_S is a dissociation constant; $[E_T]$ is total enzyme concentration.

Both in microgravity and in standard *g* controls, the enzyme reaction obeyed the same kinetic mechanism: Uni-Bi ordered, under steady-state assumptions (glyoxylate last product released), or Uni-Bi random under rapid equilibrium. The reverse reaction was consistent with the following initial velocity equation, typical of both mechanisms but with a different meaning of the kinetic constants [5]:

$$\frac{v}{V_r} = \frac{[P]\cdot[Q]}{K_S^Q K_m^P + K_m^Q[P] + K_m^P[Q] + [P]\cdot[Q]}$$

where P indicates succinate and Q glyoxylate (more details are given in Table 1).

The values of the kinetic constants as determined in microgravity and at 1 *g* are compared in Table 1; the parameters for the cleavage reaction are also reported.

Our results indicate that in either direction, isocitrate lyase reaction is not affected by microgravity.

To explain the microgravity effect observed on lipoxygenase affinity for linoleic acid, the authors [4] suggested that gravity may affect the reaction–diffusion process which occurs in enzyme catalysis, supporting their reasoning in the same way as Papaseit et al. [6] justified the observation that microtubule self-organization is gravity dependent. Our negative results, which come out from two distinct microgravity experiments, and from the analysis of both the forward and reverse enzyme reactions, prove that such theoretical considerations do not have general validity. Therefore, it is now necessary to understand why some reactions are sensitive, whereas others appear to be totally immune from the influence of the gravitational field. Probably, there are some other critical factors to be taken into account – mass ratio, density and/or nature of the reactants, catalytic mechanism, reversibility itself of the reaction, and/or time – to explain the opposite results observed. We think this kind of experimentation deserves to be continued and intensified, because through this way we could get new insights in understanding the biophysical and molecular basis of both the catalytic mechanisms and the striking efficiency of the enzymes.

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