Thermal Stabilization of Lipases Bound to Solid-Phase Triazine-Scaffolded Biomimetic Ligands: A Preliminary Assessment

Diogo Ferreira-Faria[1,2] and M. Ângela Taipa[1,2*]

[1] Department of Bioengineering, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
[2] iBB - Institute for Bioengineering and Biosciences and i4HB - Institute for Health and Bioeconomy, Instituto-Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
* Correspondence: angela.taipa@tecnico.ulisboa.pt

Supplementary Materials

SDS-PAGE Analysis

SDS-PAGE electrophoresis was performed in a Mini-PROTEAN TetraSystem cell with power supply apparatus Power Pac 300 both from Bio-Rad. The resolving (12% (w/v) in acrylamide) and stacking (4% (w/v) in acrylamide) gels were assembled in the electrophoresis tank, and the chamber was properly filled with running buffer (192 mM glycine, 25 mM Tris, and 0,10% (w/v) SDS, pH 8.3). Samples were prepared by addition of 25 µL of Laemmli buffer from Bio-Rad (277.8 mM Tris-HCl pH 6.8, 4.4% LDS, 44.4% (w/v) glycerol, 0.02% bromophenol blue) and 5 µL of 1M dithiothreitol, into 20 µL of sample solution, boiled for 10 minutes in water at 90ºC, and loaded into each well. Molecular weight markers (Precision Plus ProteinTM Dual Color Standards (10–250 kDa) from Bio–Rad) were loaded into one well. Electrophoresis was performed at 90V for 2 hours. The gels were stained with a silver-staining method.

Gel Staining

The gels were placed in the fixation solution (30% (v/v) ethanol, 10% (v/v) acetic acid) for 2 hours at 20 ºC and then washed with ethanol 30% (v/v) for 10 minutes. After this, the gels were washed 2 x 10 minutes with MilliQ water was performed, followed by incubation with a solution of 0.8 mM sodium thiosulfate for 10 minutes. After washing 3 x 30 seconds with MilliQ water, the gels were incubated with freshly prepared 8.85 mM silver nitrate solution, for 30

minutes at 20 ºC, washed with MilliQ water for 1 minute and incubated with the developer solution (0.283 M sodium carbonate, 0.05% (v/v) formaldehyde), until the desired intensity/contrast was achieved. The developer solution was discarded and the gels were immersed in 5% (v/v) acetic acid solution. Agitation in each step was performed using a rotary shaker from Aralab (at 60 rpm).
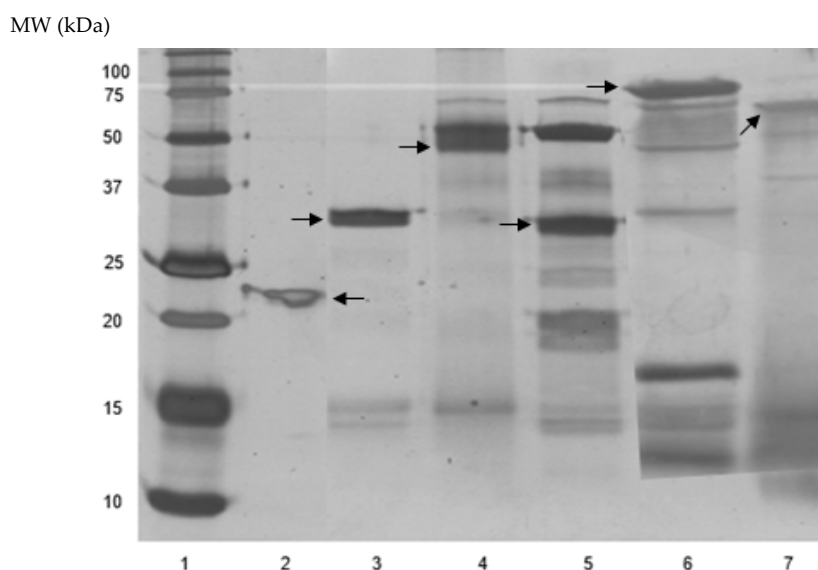

Figure S1. SDS-PAGE analysis of different enzyme preparations. Lane 1: Precision Plus Protein™ Dual Color Standards (kDa); lane 2: cutinase (10 μg of total protein); lane 3: CVL (10 μg of total protein); lane 4: AOL (30 μg of total protein); lane 5: RML (30 μg of total protein); lane 6: RNL (30 μg of total protein); lane 7: CRL (35 μg of total protein). The arrows indicate the band with the molecular weight corresponding to the lipase protein in each sample.
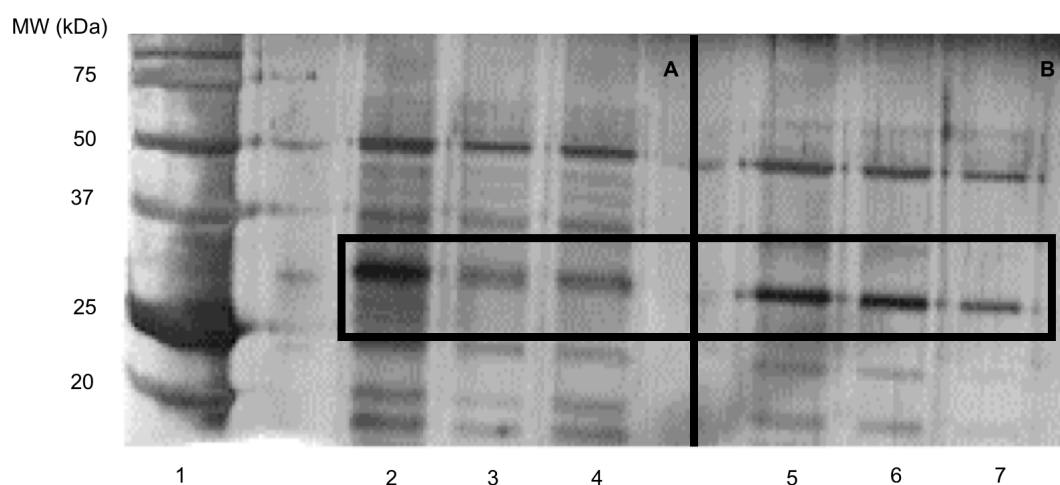

Figure S2. SDS-PAGE analysis of the load and fractions collected in the adsorption assay of RML. Lane 1: Molecular Weight Markers. Assay at pH 7 (**A**): lane 2: load (8 μg of total protein); lane 3: breakthrough (4 μg of total protein); lane 4: first wash (3 μg of total protein). Assay at pH 8 (**B**): lane 5: load (12 μg of total protein); lane 6: breakthrough (3 μg of total protein); lane 7 (8 μg of total protein): wash 1. The box surrounds the molecular weight zone of RML.
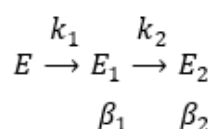
Enzyme Deactivation

Enzyme deactivation kinetics can often be described by first-order exponential decays:

$$\frac{A_t}{A_0} = \exp\left(-k_{d,obs}\, t\right) \tag{S1}$$

where A_t and A_0 represent the activities at time t and time zero, respectively, whereas $k_{d,obs}$ is the observed rate constant. Some enzymes, however, do not follow this simple mechanism and more complex models are adopted. The descriptive equations of such deactivation models are more complex and multi-parameter. Aymard and Belarbi found, however, that those equations could be simplified and reduced to a simple biexponential function by grouping individual rate constants [1].

Series-Type Models to Describe Enzyme Thermal Deactivation

Series-type models that are frequently used to describe thermal deactivation of immobilized enzymes include the model of Henley and Sadana [2]:

$$E \xrightarrow{k_1} E_1 \xrightarrow{k_2} E_2$$
$$\quad\quad \beta_1 \quad\; \beta_2$$

This model consists of a sequence of two first order steps (with rate constants k_1 and k_2) in which E is the initial active form of the enzyme, and E_1 and E_2 are the intermediate and final species, respectively. The specific activities of E_1 and E_2 relative to E are given by β_1 and β_2. According to this mechanism, the activity decay is described by the following equation:

$$\frac{A_t}{A_0} = \left(1 + \frac{\beta_1 k_1}{k_2 - k_1} - \frac{\beta_2 k_2}{k_2 - k_1}\right)e^{-k_1 t} - \left(\frac{\beta_1 k_1}{k_2 - k_1} - \frac{\beta_2 k_1}{k_2 - k_1}\right)e^{-k_2 t} + \beta_2 \tag{S2}$$

where A_t is the activity at a given time and A_0 is the initial activity.

Data from activity decays at 60 °C of enzymes bound to dipetide-mimic triazine-based synthetic ligands (*e.g.*, CRL bound to ligand 3/3') were fitted to the model of Henley and Sadana to provide a phenomenological description of the data as well as to guide the eye on the graphic representation of the inactivation curves. Fitting data to the formalism of this model does not mean, however, that it is the correct or most suitable model to describe, from a mechanistic point of view, the enzyme deactivation. Mechanistic elucidation would imply obtaining further data (*e.g.* deactivations at different temperatures). Nevertheless, the biexponential formalism is one that generally fits several different mechanistic descriptions including series models, concomitant models, competitive models and some more complex hybrid ones, as well-described in [1].

In many cases, the following formalism applies:

$$\frac{A_t}{A_0} = A e^{-\alpha t} + B e^{-\beta t} \tag{S3}$$

For a large number of cases, this formalism may be reduced to an even more simple three-parameter biexponential expression [1]:

$$\frac{A_t}{A_0} = A e^{-\alpha t} + (1 - A) e^{-\beta t} \tag{S4}$$

Such is the case of the series-type mechanism described by Eq. S2 when β_2 is 0.

Therefore, models with a fairly universal character can be used to describe thermal enzyme activity decay, irrespectively of the underlying mechanism, using parameters (such as *A* or *B* in equations S3 and S4) that can be complex functions of individual rate constants (Eq. S2) as well as apparent first-order rate constants (such as α and β from equations S3 and S4). Model simplifications of this type are of interest when little is known about the actual inactivation mechanisms, such as in the present work, to compare enzyme deactivation under similar conditions.

The values of parameters obtained by fitting the models of Aymard and Belarbi and of Henley and Sadana to the activity decay of different enzymes adsorbed to dipepetide-mimic triazine-scaffolded synthetic affinity ligands are presented in Table S1.

Table S1. Values obtained by fitting the models of Aymard and Belarbi and of Henley and Sadana to the activity decay of the different enzymes adsorbed to triazine-based synthetic affinity ligands**.**

Aymard and Belarbi [1]				
Enzyme – Ligand	**A**	**α (min^{-1})**	**B**	**β (min^{-1})**
CVL – 3'/11	0.580	0.0010	0.420	0.0256
CRL – 3/3'	0.431	0.0356	0.471	0.00
Henley and Sadana [2]				
Enzyme – Ligand	β_1	β_2	k_1 (min^{-1})	k_2 (min^{-1})
Cutinase – 3'/11	1.00	0.00	0.0500	0.0802
CRL – 3/3'	0.00	0.525	0.0584	0.606

References

[1]   Aymard, C.; Belarbi, A. Kinetics of thermal deactivation of enzymes: a simple three parameters phenomenological model can describe the decay of enzyme activity, irrespectively of the mechanism. *Enz. Microb. Technol*. **2000**, *27*, 612–618.

[2]   Henley, J.P.; Sadana, A. Categorization of enzyme deactivations using a series-type mechanism. *Enz. Microb. Technol*. **1985**, *7*, 50–60.