

## Article

# An Electrochemical Approach to Follow and Evaluate the Kinetic Catalysis of Ricin on hsDNA

George Oliveira \* and José Maurício Schneedorf

Department of Biochemistry, Federal University of Alfenas, Alfenas 37130-000, Brazil;  
jose.dasilva@unifal-mg.edu.br

\* Correspondence: george.oliveira@unifal-mg.edu.br

**Abstract:** International authorities classify the ricin toxin, present in castor seeds, as a potential agent for use in bioterrorism. Therefore, the detection, identification, and characterization of ricin are considered the first actions for its risk assessment during a suspected exposure, parallel to the development of therapeutic and medical countermeasures. In this study, we report the kinetic analysis of electro-oxidation of adenine released from hsDNA by the catalytic action of ricin by square wave voltammetry. The results suggest that ricin-mediated adenine release exhibited an unusual kinetic profile, with a progress curve controlled by the accumulation of the product and the values of the kinetic constants of  $46.6 \mu\text{M}$  for  $K_m$  and  $2000 \text{ min}^{-1}$  for  $k_{cat}$ , leading to a catalytic efficiency of  $7.1 \times 10^5 \text{ s}^{-1} \text{ M}^{-1}$ .

**Keywords:** ricin; square wave voltammetry; kinetic analysis; hsDNA

**Citation:** Oliveira, G.; Schneedorf, J.M. An Electrochemical Approach to Follow and Evaluate the Kinetic Catalysis of Ricin on hsDNA. *Life* **2021**, *11*, 405. <https://doi.org/10.3390/life11050405>

Academic Editor: Angela Tramonti and Roberto Contestabil

Received: 28 February 2021

Accepted: 19 April 2021

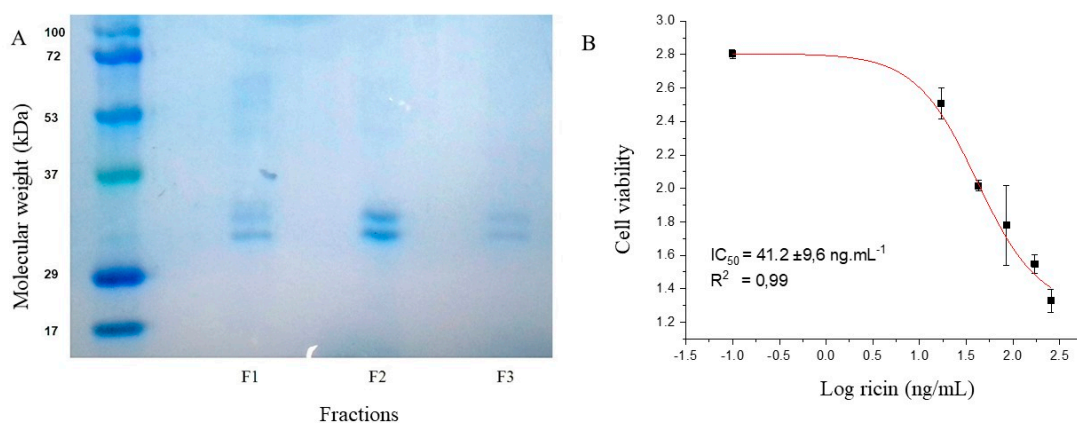
Published: 29 April 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

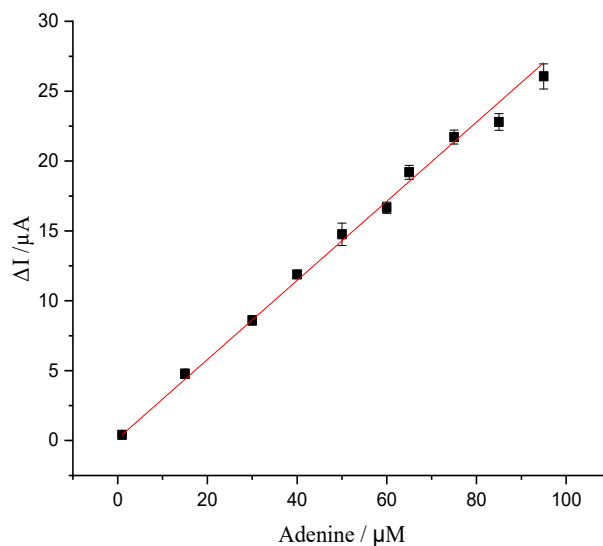


**Copyright:** © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

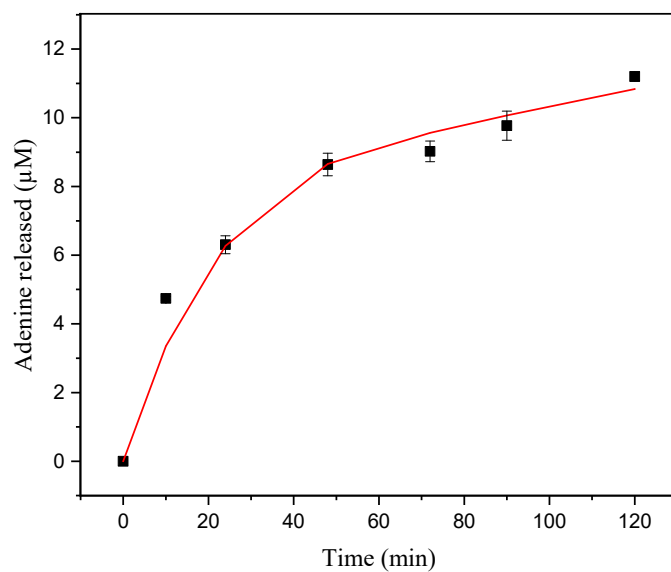
## Supplementary Materials:



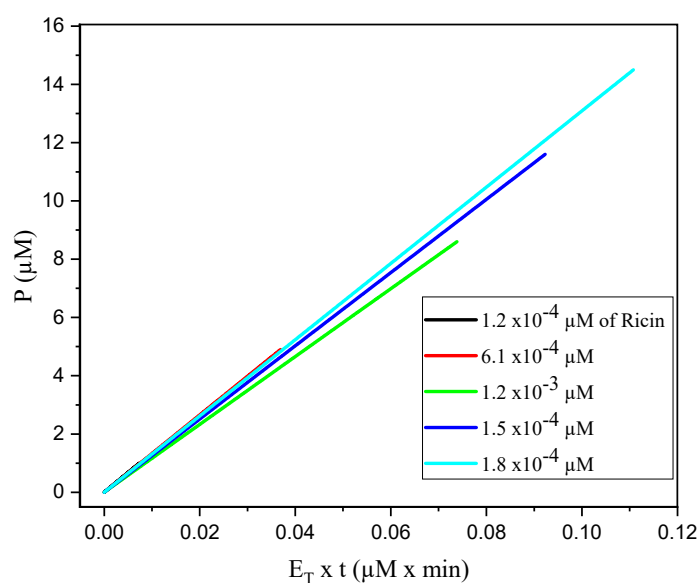
**Figure S1.** Purification and characterization of ricin. (A) SDS-PAGE (12 %) of samples under reduced conditions, (B) Effect of a pooled fraction of purified ricin on the viability of HEP2 cells for  $IC_{50}$  determination. The line represents a logistic equation fitted to the data.



**Figure S2.** Analytical curve of adenine obtained by successive additions of standard adenine aliquots in a range from 0 to 95  $\mu M$  in a solution containing hsDNA at 16  $\mu M$ .



**Figure S3.** Progress curve of adenine release from hsDNA (5  $\mu\text{M}$ ) catalyzed by ricin at 1.5 nM as obtained by HPLC. The solid curve represents the fit of eq 1 to the experimental data.



**Figure S4.** Selwyn's test (enzyme inactivation). Progress curves of adenine released from 16  $\mu\text{M}$  of hsDNA catalyzed by ricin at CPE in the acetate buffer pH 4.6 against the product of time with enzyme amount. The solid curve represents the linear fitting to the data.

**Table S1.** Ricin purification steps.

Purification Stage	Total Proteins (µg)	Total Activity µM.min <sup>-1</sup>	Specific Activity µM.min <sup>-1</sup> . µg <sup>-1</sup>	Purification Factor Times	Recovery (%)
Crude extract	1094.8	2.6	0.0024	1.0	100.0
Ammonium Sulfate (60%)	370.9	2.2	0.0059	2.4	82.6
Pre-purified extract (Sephadex G-50)	20.6	1.5	0.0726	30.0	56.6
Purified sample (Sephadex G-100)	4.6	1.3	0.2778	114.9	48.3