Design of an enzyme-based bioreactor for anticancer drugs screening: kinetic evaluation of Lactate Dehydrogenase enzyme with different inhibitors

Chiara Vincenzi [a], Clarissa Cocuzza[a], Valentina Cauda [a], Debora Fino [a] & Marco Piumetti [a]

[a] Politecnico di Torino, Turin, Italy

E-mail: marco.piumetti@polito.it

Tumor cells usually undergo metabolic reprogramming, known as the Warburg effect, characterized by elevated lactate production even in the presence of oxygen. This shift is driven by lactate dehydrogenase (LDH), which converts pyruvate to lactate, thereby promoting tumor growth and proliferation[1],[2]. Selective inhibition of LDH represents a promising strategy to suppress tumor progression. This study aims to assess the inhibitory effects of various compounds on LDH activity, with the objective of developing an enzyme-based bioreactor for the efficient screening of potential anticancer agents.

To characterize LDH under different conditions, both kinetic and stability studies were performed. Kinetic experiments at 37°C using UV-Vis spectrophotometry monitored NADH oxidation at 340 nm while varying pyruvate and NADH concentrations in the presence of inhibitors (NHI-2, galloflavin, FX11, gossypol, and sodium oxamate). Kinetic parameters were determined using Hanes-Woolf plots and validated with Origin software. Molecular docking simulations identified inhibitor binding sites. Control experiments with DMSO also were included. Stability studies involved the incubation of LDH for 72 hours at 37°C both in the presence or in the absence of DMSO, followed by enzymatic activity assessments through UV-Vis spectrophotometry at predetermined time points.

Kinetic analyses revealed that LDH exhibits a kinetic response well described by Michaelis-Menten model when varying pyruvate concentration, while Hill's model is more appropriate to describe the reaction rate as a function of NADH concentration. Inhibitors such as Galloflavin, NHI-2, and FX11 demonstrated allosteric inhibition by binding to sites distinct from the active site, as confirmed by molecular simulations. In contrast, gossypol exhibited competitive inhibition with NADH, interacting with the ASN93 residue, while sodium oxamate acted competitively with pyruvate by binding to MET53.

This study integrates biochemical analysis with chemical engineering principles by combining kinetic modeling, molecular docking simulations, and enzyme stability assessments to optimize LDH-based biocatalytic processes. By leveraging concepts such as reaction kinetics and enzyme immobilization, it contributes to the development of a biosensing platform for the efficient screening of LDH inhibitors. This interdisciplinary approach, which bridges chemical reaction engineering, bioinformatics, and nanobiotechnology, holds potential for advancing pharmaceutical research and targeted cancer therapies. Future studies will focus on assessing the efficacy and selectivity of these inhibitors in pancreatic cancer and healthy cells.

Keywords: *anticancer drugs, enzyme inhibition, drug screening, lactate dehydrogenase.*

[1] Gan, J. et al, *Med. (United States)* **2018**, *97*, e13151

[2] Cocuzza, C. et al., *Microporous Mesoporous Mater.* **2024**, *376*, e113182