Process-driven insights into LDH inhibition for next-generation enzyme-based anticancer screening

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Highlights

- LDH-A kinetics were fully characterized under physiological conditions.
- An ordered sequential mechanism with NADH as first binder was identified.
- Reference inhibitors showed distinct kinetic inhibition mechanisms.
- Kinetic fingerprints enable inhibitor discrimination for biosensing.

1. Introduction

Cancer cells undergo a profound metabolic reprogramming to sustain survival, rapid proliferation and uncontrolled growth. One of the most prominent features of this altered metabolic phenotype is the Warburg effect, characterized by an increased reliance on glycolysis even in the presence of oxygen. Lactate Dehydrogenase (LDH), particularly its isoform A, represents one of the main responsible of the described process: it catalyzes the conversion of pyruvate into lactate, while regenerating NAD^+, thereby maintaining the glycolytic flux required for tumor progression[1,2]. Due to its pivotal role in cancer metabolism and the relatively limited impact of its inhibition on normal and healthy tissues, LDH-A has emerged as an attractive therapeutic target. Several small-molecule inhibitors have been proposed; however, a comprehensive and quantitative understanding of LDH-A kinetics and inhibition mechanisms is still required to reliably evaluate inhibitor efficacy and support drug discovery efforts.

The present work aims to establish a detailed kinetics characterization of LDH by systematically investigating enzyme activity both in the absence and in the presence of well-known inhibitors. The primary objective is to construct a solid kinetic baseline that describes the intrinsic behavior of LDH-A and its response to inhibition. This kinetic framework is intended to serve as a foundation for the future development of a biosensing platform, capable of correlating the variations in enzymatic activity to inhibitor efficacy through predictive models. Ultimately, such a platform could significantly speed up the screening phase of novel anticancer drugs formulated as LHD-A inhibitors.

2. Methods

Kinetic studies were performed to determine both intrinsic and apparent kinetic parameters of LDH under physiologically relevant conditions. All the experiments were performed at 37°C and in 0.1 M pH 7.4 phosphate buffer solution, using a Jasco V-730 UV-Vis spectrophotometer. Enzymatic activity was monitored by recording the absorbance variation at 340 nm, which corresponds to NADH oxidation peak.

Initially, the intrinsic kinetic was evaluated in the absence of inhibitors to establish reliable reference parameters. Both the forward (pyruvate to lactate) and reverse reaction (lactate to pyruvate) were investigated by systematically varying either the substrate (pyruvate/lactate) or the cofactor ($NADH/NAD^+$) concentrations. Kinetic parameters were extracted from secondary plots based on Hanes-Woolf linearization [3].

Subsequently, apparent kinetic parameters for the forward reaction were determined in the presence of reference inhibitors, namely NHI2, Galloflavin, Gossypol, FX11 and sodium oxamate. Control experiments with DMSO, the solvent employed for inhibitors solubilization, were included as well, to evaluate its potential effects on the enzyme structure and activity. Apparent kinetic constants were

determined through Hanes-Woolf linearization method and validated through nonlinear fitting using OriginPro software.

3. Results and discussion

LDH exhibited an apparent Michaelis-Menten kinetic for both the forward and the reverse reaction. Analysis of the forward reaction, shown in Figure 1, revealed that variations in the maximum velocity were primarily influenced by NADH concentration, a trend also observed in the reverse reaction. These results supported an ordered sequential mechanism, in which the cofactor is the first to bind, inducing a conformational change that enables subsequent substrate binding. Based in this mechanism, real kinetic parameters were determined, revealing a higher catalytic efficiency and a greater selectivity of LDH-A towards the forward reaction. This behavior is consistent with the enzyme's physiological role in sustaining glycolysis under high metabolic demand.

The presence of reference inhibitors induced distinct kinetic responses, indicating multiple inhibition mechanisms. Specifically, sodium oxamate displayed competitive inhibition with respect to pyruvate, NHI2 followed a mixed-type inhibition mechanism, while FX11, galloflavin, and gossypol exhibited noncompetitive inhibition profiles. These findings demonstrate that kinetic analysis provides a powerful tool for discriminating among inhibitors based on their mechanistic fingerprints.

The reproducibility of the observed kinetic trends supports the robustness of the proposed model. Moreover, the ability to discriminate among inhibitors based on their kinetic fingerprints highlights the suitability of the approach for predictive, enzyme-based biosensing applications.

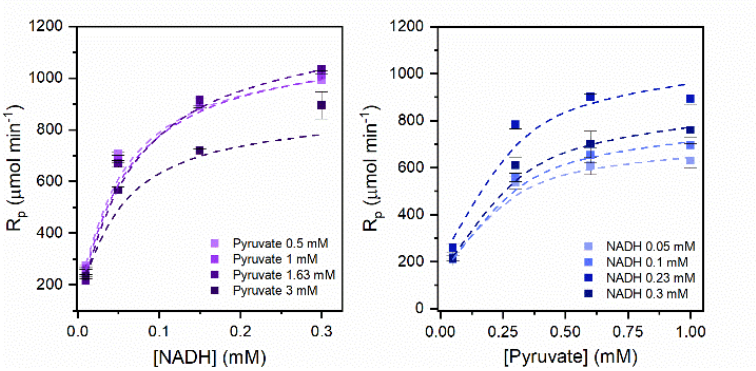

Figure 1. Representative kinetic analysis of the LDH forward reaction under varying NADH and pyruvate concentrations.

4. Conclusions

In this study, LDH-A was systematically characterized under physiologically relevant conditions, establishing a comprehensive kinetic description of its catalytic behavior. The enzyme follows an ordered sequential mechanism in which cofactor binding precedes substrate association, highlighting the central regulatory role of NADH in LDH catalysis. Distinct inhibition mechanisms were identified for a panel of reference inhibitors, enabling reliable discrimination based on kinetic signatures.

The kinetic constraints and mechanistic insights obtained in this work define essential requirements for future LDH immobilization strategies aimed at developing a solid-state platform for high-throughput inhibitor screening. Overall, this study provides a robust quantitative foundation for both LDH-targeted drug discovery and biosensor development.

References

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Keywords

LDH-A; enzyme kinetics; inhibition mechanisms; biosensing