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Fluorescent compounds emit light that helps us study a key family of tumor markers.



The Department of Biochemistry and Molecular Biology has studied the enzymatic characterization of various fluorescent substrates that enable the detection of the activity of certain enzymes. Some variants of them are implicated in various types of cancer. This research is crucial because it enhances the reliability and accuracy of such studies.

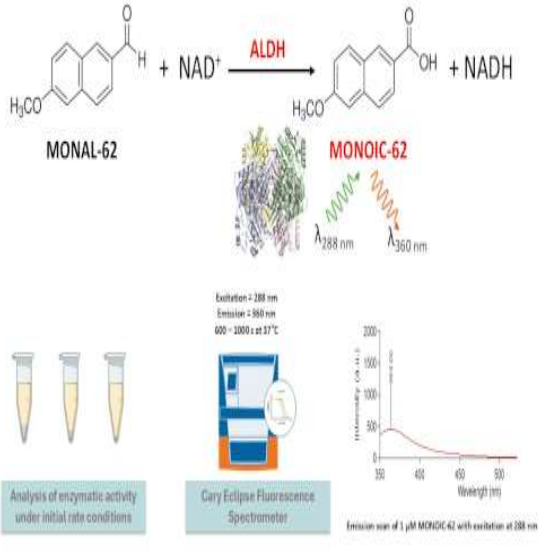
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Aldehyde dehydrogenases (ALDH) are enzymes specialized in oxidizing aldehydes to their corresponding carboxylic acids. They are involved in cellular defense mechanisms against oxidative stress and in the metabolism of vitamin A derivatives. Certain variants of these enzymes, known as isoforms, are highly upregulated in various types of cancer, particularly in subpopulations of tumor stem cells. For this reason, they are used as tumor markers and are promising candidates as therapeutic targets.Our research group has the methodology to produce recombinant human ALDH enzymes with high purity, allowing us to analyze in detail how they respond to different substrates and enzymatic inhibitors. In this work, we focused on the enzymatic characterization of various fluorescent substrates that, by emitting light and using a spectrofluorometer, enable us to detect ALDH activity with high sensitivity. The first compound we studied, BODIPY-aminoacetaldehyde, is already widely used in cancer

diagnostics, especially to detect chemoresistant cells via flow cytometry. However, until now, the behavior of this compound with each ALDH variant was unknown. We demonstrated that many ALDH isoforms oxidize BODIPY-aminoacetaldehyde, while others barely react, highlighting the limitations of the assay in identifying the ALDH isoforms responsible for the activity.

In the same study, we thoroughly characterized two naphthaldehyde isomers, named 6 methoxy-2-naphthaldehyde (MONAL-62) and 7-methoxy-1-naphthaldehyde (MONAL 71). With these compounds, we observe very marked differences among ALDH isoforms, allowing us to identify the origin of the detected activity. MONAL-62 stands out for its extraordinary sensitivity: we can detect minute amounts of ALDH activity, in the order of 2 attomol (10−18 mol) of enzyme per microliter, far below what was feasible with the most common assay to date. MONAL-71 is an even more specific substrate, making it a valuable tool for unequivocally identifying some ALDH isoforms. In parallel, we performed computational simulations of molecular interactions, which helped us understand these differences at the structural level, showing how the topology of the compound and the enzyme active site determine the proper substrate orientation and, therefore, enzymatic rate.

In collaboration with the pharmaceutical company Advanced BioDesign, and as part of another investigation, we characterized ALDH inhibitors DIMATE (S-methyl 4 (dimethylamino)-4-methylpent-2-ynethioate), currently in clinical trials, and its analog ABD0171. To analyze ALDH activity in triple-negative breast cancer cells, we combined the fluorescent substrates from this study with DIMATE and ABD0171 inhibitors to decipher which ALDH isoforms contribute most to the activity in cell extracts. With MONAL-62, these inhibitors completely blocked ALDH activity in cell extracts, which had not been possible with the classic assay. Overall, our methodology based on the use of sensitive and specific inhibitors provides a more reliable tool for measuring and distinguishing ALDH isoforms, with direct applications in basic and translational cancer research.



Enzymatic reaction of 6-methoxy-2-naphthaldehyde (MONAL-62) mediated by ALDH. *The methoxy (−OCH3) and aldehyde (−CHO) groups are both located at β positions of the aromatic naphthalene system. Enzymatic assays were carried out in 50 mM sodium pyrophosphate/NaOH, 0.5 mM EDTA, pH 8.1, in 1-mL quartz cuvette at 37°C, adding 150 µM NAD+ as a cofactor. The reaction was initiated by the addition of the aldehyde substrate and*

the fluorescence of MONOIC-62 product was monitored at 360 nm with excitation at 288 nm. Furthermore, 1 µM MONOIC-62 or MONOIC-71 was added as an internal standard to obtain the absolute reaction rate.

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References

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This work was carried out by Dr. Raquel Pequerul, together with students Daniela Covaleda, Andrés Sánchez-Vaca, and Laura Torres, from the research group in the Department of Biochemistry and Molecular Biology at the UAB, led by Drs. Jaume Farrés and Xavier Parés, in collaboration with Drs. Andrada Constantinescu and Mileidys Pérez-Alea from the pharmaceutical company Advanced BioDesign. We thank Dr. Salvador Bartolomé from the Genomics and Biomolecule Spectroscopy Service (SGiEB) for providing access to the facility and his support in using the spectrofluorometers.

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