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TOPIC(s) : Biocatalytic cascade reactions / (Chemo)enzymatic strategies

Developing inhibitors for human tyrosinase

AUTHORS
Inbal RUSH / TECHNION, BIOTECHNOLOGY AND FOOD ENGINEERING, ROOM 412, HAIFA

PURPOSE OF THE ABSTRACT
Tyrosinases are ubiquitous enzymes mainly responsible for the formation of melanin in skin pigmentation and in fruit and vegetable browning. Disorder in melanin formation has been linked to a variety of skin diseases in humans such as melasma, solar lentigo, and even melanoma. Therefore, tyrosinase inhibitors are highly warranted by the pharmaceutical, cosmetics, and food industries. Developing inhibitors for human tyrosinase is especially difficult due to the low expression yields in mammalian cells. The present research focuses on designing new peptide-based tyrosinase inhibitors using yeast surface display peptide libraries. Yeast surface display is an in vitro screening method utilizing flow-cytometry. It is a novel approach for this purpose, which will be used to screen and identify strong inhibitors for both human and bacterial tyrosinases. Initially, recombinant human and bacterial tyrosinases were expressed and purified in their active form, and utilized for the screening of several soluble rationally-designed peptides with potential inhibitory effect. Results showed a 4-fold lower IC50 value with human tyrosinase for peptide P8 (HSWMDWVPTPWAA), the most inhibiting peptide screened, compared to kojic acid, a commercially available tyrosinase inhibitor. Further characterization of soluble P8 with bacterial tyrosinase gave an IC50 value of 98 µM and a KD value of 4 µM, when measured using microscale thermophoresis analysis. Therefore, P8 serves as the peptide-library template in the yeast display system. To generate combinatorial libraries, four sets of sub-libraries, consisting of NNK codons in 3 or 4 adjacent positions in the P8 amino acid sequence were designed. In parallel, the conditions for the yeast display and flow-cytometry assay were calibrated, comprising an incubation temperature of 40°C and concentration of 0.5 µM of bacterial tyrosinase for binding. Optimization resulted in 60% expression levels of the displayed construct in flow cytometry analysis. Subsequent work will focus on optimizing binding of the libraries to human tyrosinase.
Development of small molecule inhibitors was also pursued. Several resorcinol-based hemiindigoid derivatives, were designed, synthesized and evaluated on human tyrosinase. Two compounds showed superior inhibition in both human melanoma cell lysates and purified human tyrosinase in comparison to kojic acid. In kinetic studies, a partial competitive inhibition mode was obtained from the Lineweaver-Burk correlation for both compounds, and Ki values were four orders of magnitude lower for the new compounds than for kojic acid, 0.55 and 0.25 µM versus 1100 µM, respectively. When the two compounds were tested as well with bacterial tyrosinase, IC50 values of 15 and 30 µM were defined, versus IC50 of 52 for kojic acid [1].

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS
tyrosinase | yeast surface display | inhibition | peptides

BIBLIOGRAPHY

[1] Roulier B#, Rush I#, Lazinski LM, P□r□s B, Olleik H, Royal G, Fishman A, Maresca M, Haudecoeur R. 2023. Eur J. Med. Chem. 246:114972. # equal contribution.