The Uptake of Cu in Tyrosinase Affects the Monophenolase/Diphenolase Activity Ratio

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Introduction

- Tyrosinase (EC 1.14.18.1) is a type-3 Cu enzyme, distributed in all domains of life and performs two sequential reactions in the presence of molecular oxygen.
- The active site of tyrosinase is composed of six conserved His residues which coordinate CuA and CuB.
- We have recently determined the crystal structure of tyrosinase form Bacillus megaterium (TyrBm) and observed that Cu binding is variable in TyrBm.

Our objective was to study Cu accumulation and binding in TyrBm.

Cu concentration is a bottleneck of TyrBm's diphenolase activity

- By increasing Cu concentration from 0.1 µM to 10 µM, the monophenolase activity was improved by 4-fold while the diphenolase activity was improved by 8.5 fold.
- While the maximum monophenolase activity was reached at 3.5 µM Cu, the maximum diphenolase activity was reached at only 10 µM Cu.

We suggest that CuA entering the active site first meets the Met residues and is then transferred to the His60 which alters its conformation and transfers CuA into the active site.

Since the substitution of residues Asp205 and Phe197 had a significant effect on activity and Cu binding, we suggest that CuB passes only through Asp205 and Phe197.

Residues at the active site entrance control Cu uptake and determine the mono/diphenolase ratio

- Variants N205A and N205D have lower ability to bind Cu (conferring also by ICP-AES) and decreased activity on both substrates.
- In the crystal structure of WT TyrBm, Asn205 stabilizes His204, which coordinates CuB. However, no such interactions were observed in the structures of N205A and N205D.

The structure of F197A variant contained both Cu ions in the active site, in contrast to the structure of WT and has higher ability to bind copper according to the BCA-test.

Variant F197A has improved mono/diphenolase ratio by 2-fold.

Cu uptake

- The decrease in Cu concentration improves the mono/diphenolase ratio by 1.8 fold.
- Variants M61L and F197A improve the mono/diphenolase ratio. In both variants, the hydrophobic interactions with the substrates weakened and enabled easier substrate access. In the case of L-Tyr, this results in higher activity, but in the case of L-dopa this promotes suicide inactivation due to imperfect orientation.

Improving the ratio of mono/diphenolase activity

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