Investigating the biochemical background for histamine intolerance:
Degradation capacity of mixtures of biogenic amines by human, porcine and bovine Diamine Oxidase

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Introduction

Diamine Oxidase (EC 4.1.3.22; DAO) is accepted to be the main enzyme for degradation of foodborne biogenic amines in the intestine. Relative lack or inhibition of this enzyme has been linked with Histamine Intolerance (HIT), a condition marked by symptoms such as abdominal pain, diarrhea, rhinitis, uriticaria and headaches. Until now few data of total degradation capacity of biogenic amines of DAO are available.

We have thus designed novel fluorescence based activity assay using the diamine oxidase reaction side product, hydrogen peroxide (H_2O_2).

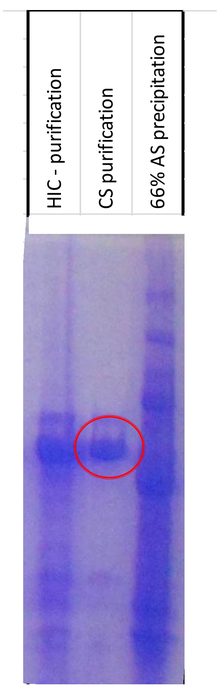
Purification of human diamine oxidase

Human placental and porcine/bovine kidney tissues are high-abundance sources of DAO. Tissue was homogenized in phosphate buffer pH 7.5 and subjected to a 33% / 66% fractionated ammonium sulfate precipitation.

The 66% pellet containing high DAO activity was further purified using a two step chromatographic strategy.

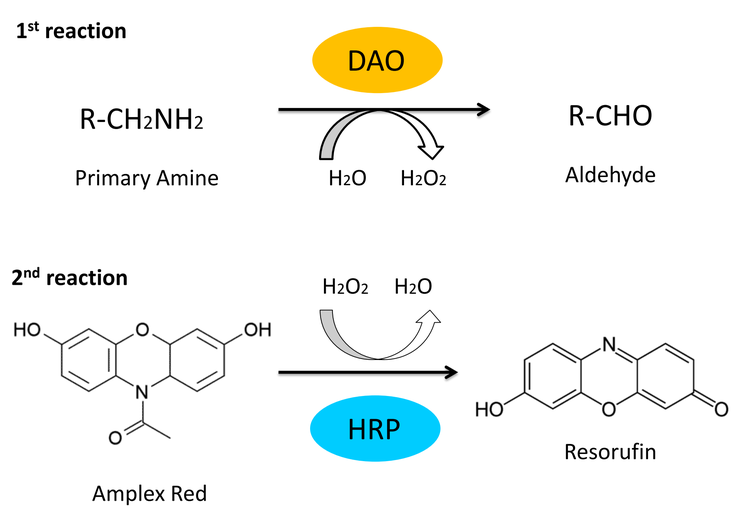
First step consisted of a hydrophobic interaction (HIC) FPLC using Phenyl Sepharose as a solid phase

This step was followed by a second affinity FPLC purification using Cellufine Sulfate (CS) as stationary phase.
This process yielded an enzymatic preparation with purity < 90%



Fluorescence based activity assay

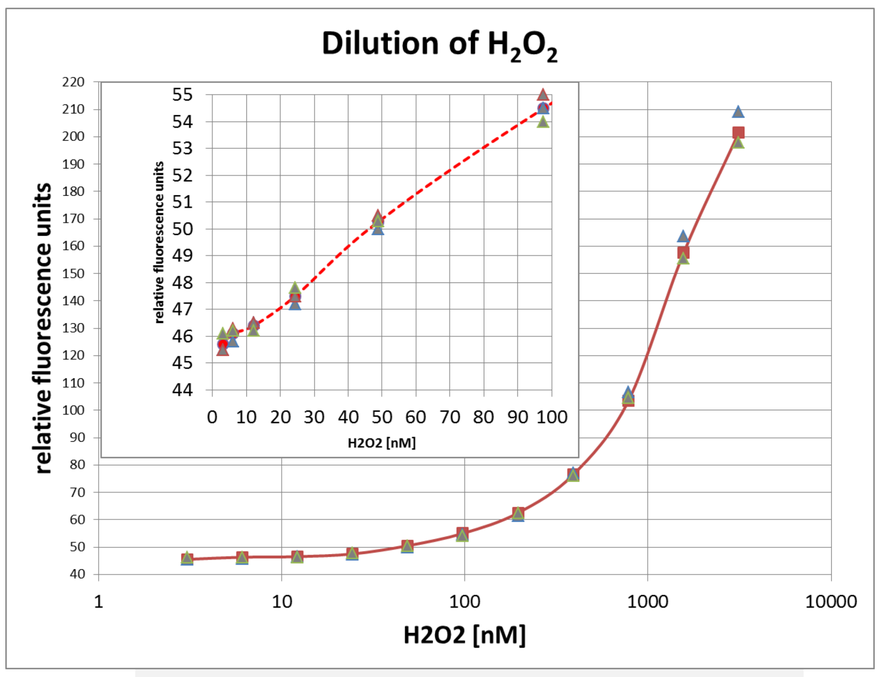
Physiological oxidation of primary amines to aldehydes produces an equimolar amount of hydrogen peroxide as a side product, which is used in this novel assay to oxidize Amplex Red (Invitrogen) to Resorufin, a reaction mediated by horseradish peroxidase.
Oxidation of nonfluorescent Amplex yields a highly fluorescent Resorufin with a $\lambda_{em/ex}$= 570/585nm



Overview of the 2 assay reactions

Experimental setup

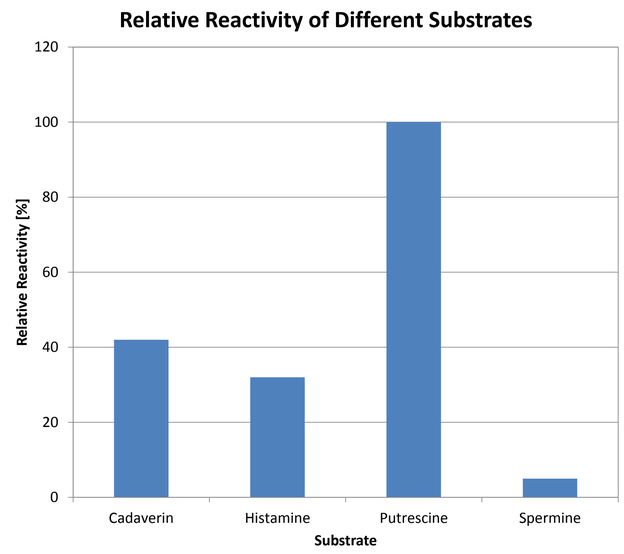
Assay was optimized for 96-well plates.
Detection limit was experimentally determined to be 20 nmol/l as shown in the figure below.
The dynamic range exceeds the maximum possible value that can be achieved by the enzyme.
Mean CV of triplicates was < 5%



Determination of the detection limit by a H_2O_2 serial dilution

Different Substrates for DAO

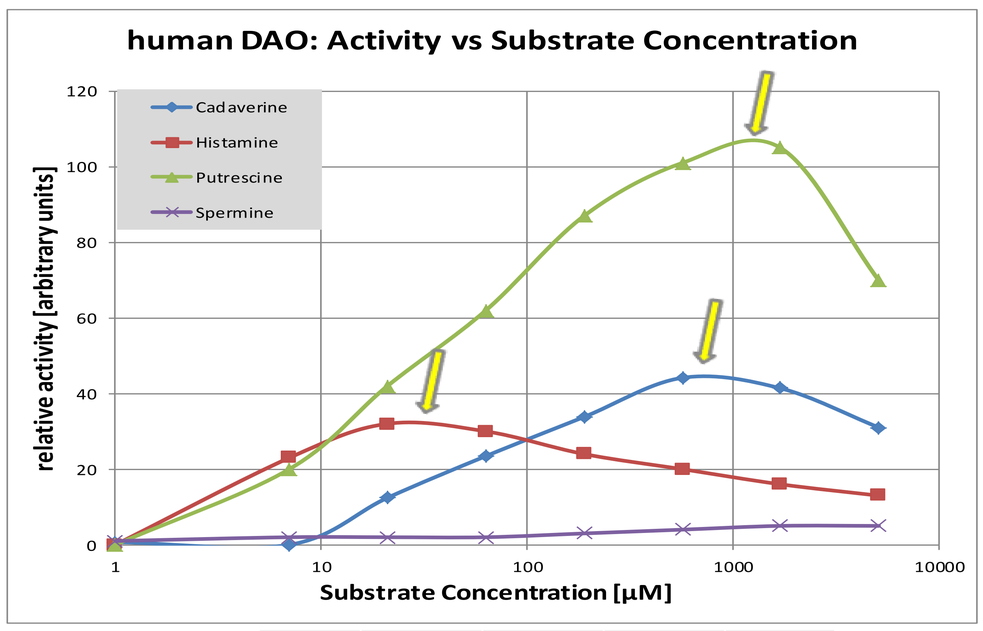
For testing the specific substrate activity of DAO, 6 different substrates (cadaverine, histamine, putrescine, serotonine, spermine, spermidine) were tested.
Different concentrations of substrate were incubated with DAO for 2 hours at 37 °C.
No activity of DAO could be determined with serotonine and spermidine as substrate.
Relative reactivity of h-DAO was 100%, 42%, 32%,5% for putrescine, cadaverine, histamine and spermine respectively.



relative activity of DAO, compared to Putrescine

Inhibition of DAO by high substrate concentrations

High concentrations of cadaverine, histamine and putrescine were mixed with DAO to evaluate the inhibition of DAO. Substrate inhibition occurred at 450 µM, 1000 µM, 50 µM for putrescine, cadaverine, and histamine respectively

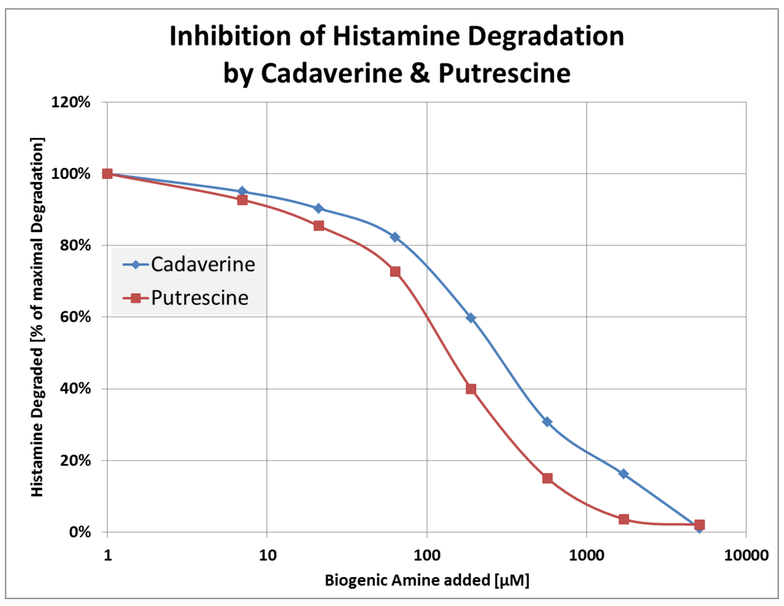


Inhibition of DAO at high Substrate concentrations

Influence of Histamine Degradation by Cadaverine and Putrescine

To test the influence of histamine degradation by cadaverine and putrescine, each of these substrates were added to the 100 mM histamine solution in increasing concentrations.

It can easily be seen that at equimolar concentrations only 60% and 75% (inhibition by putrescine and cadaverine respectively) of the histamine is degraded by DAO.
Taking into account that above mentioned biogenic amines normally occur in higher concentrations than histamine in certain food it is obvious that even rather low concentrations of histamine may provoke symptoms of intolerance when accompanied by putrescine or cadaverine.



Inhibition of degradation of Histamine in presence of Cadaverine And Putrescine

Conclusion

Based on the present findings, degradation activity of diamine oxidase as the core enzyme in histamine intolerance has to be reevaluated. In addition to histamine the content of other biogenic amines in food has to be considered for the evaluation of a diet.

As a consequence the symptom complex should be designated **"Biogenic Amines Intolerance Syndrome" (BAIS)** rather than "Histamine Intolerance" (HIT) to fully reflect to the biochemical background of the syndrome

References:

[1] Bachrach U in "Structure and Functions of Amine Oxidases", Bruno Mondovi (Ed), 1985: ISBN 0-8493-5869-8
[2] Maintz, L., Novak, N. 2007. Histamine and histamine intolerance. American Journal of Clinical Nutrition 85: 1185-1196
[3]Wüthrich, B. 2009. Histaminintoleranz: Fakt oder Fiktion? Allergologie 2:29-31