

DEPENDENCE OF BOVINE SERUM AMINE OXIDASE ACTIVITY ON pH

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The steady state oxidative deamination of spermine (SPM), spermidine (SPD), butylamine (BUA) and benzylamine (BZA) by bovine serum amine oxidase (BSAO) was studied at 37°C and 150 mM ionic strength, as function of the pH. In particular the dependence of k_c and K_m values was calculated from L.B. plots, obtained in the pH range 5.5 - 8.5, where no irreversible denaturation of enzyme was observed. The dependence of k_c on pH, reported in Fig.1, shows the enzyme typical bell-shaped behaviour, characterized by a maximum at pH 6.85. From these plots pK_a values of 6.5 and 7.2 were calculated for the substrates tested. The relative value of the k_c at the maximum were 1, 0.72, 0.55 and 0.44 for SPM, SPD, BUA and BZA respectively. The plots $\log k_c/K_m$ vs pH (Fig.2) are straight lines in the pH range 6.0 - 8.0 being the slopes for the four substrates 1.9 for SPM, 1.6 for SPD, 0.8 for BUA and 0.7 for BZA.

Discussion Different k_c values for SPM, SPD and BUA across the pH rate profile indicate that the corresponding step of the reaction is affected by the structure of the substrate. Therefore the enzyme re-oxidation, which is substrate independent, is not involved in the k_c control.

The ratio k_c/K_m was hypothesized to be equal to k_1 , which represents the kinetic rate constant of the interaction between the positive charged amines and the negative charged active site.

This hypothesis is now confirmed by the straight-lined trend of the plot. Furthermore the different slopes found appear follow the different number of positive charges of the three aliphatic substrates. This fact suggests that the number of the electrostatic interactions between the active site and the substrate modulates the catalytic efficiency of the BSAO at physiological conditions.

Fig. 1

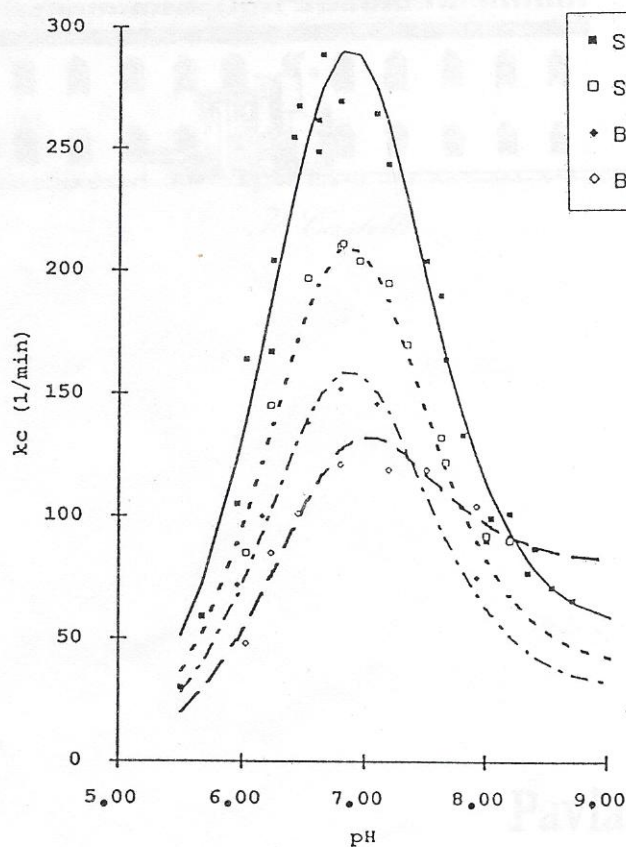


Fig. 2

