# EFFECT OF pH ON BOVINE ADRENAL MEDULLA DOPAMINE-β-HYDROXYLASE

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## 1. Introduction

The enzyme dopamine-α-hydroxylase (3,4-dihydroxy phenylethylamine, ascorbate: $O_2$ oxidoreductase (hydroxylating) EC 1.14.2.1) catalyzes the last step in norepinephrine biosynthesis, the convertion of dopamine to norepinephrine [1].

It is known that the hydroxylation reaction of dopamine-β-hydroxylase (DBH) implies a ping pong mechanism [2, 3], but no data demonstrating the effect of the hydrogen ion concentration upon the enzymatic reaction has been published to our knowledge. A study of the effect of pH could provide some information about the active center of the enzyme and the amino acids implicated in the enzymatic process.

## 2. Materials and methods

DBH was assayed according to the spectrophotometric procedure of Pisano et al. [4], as modified by us [5].

All determinations were performed using the enzyme obtained from bovine adrenal medulla which was purified by affinity chromatography [3].

$K_m$ and $V$ values were determined from Lineweaver–Burk plots [6]. Six different tyramine concentrations, ranging from 0.2 to 10 mM, were used. The cosubstrate, ascorbate, remained at a constant, saturating concentration of 10 mM.

For each experiment the actual pH of the reaction mixture was controlled.

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## 3. Results

Fig. 1A shows DBH activity against pH on a range from pH 4.5 to 7.5 at various tyramine concentrations and a saturating fixed ascorbate concentration. Several points need to be made. Firstly, the pH curves are symmetrical when the tyramine concentration is below 1 mM. At 1 mM, a plateau appears and over a tyramine concentration of 1 mM the pH curves become asym-

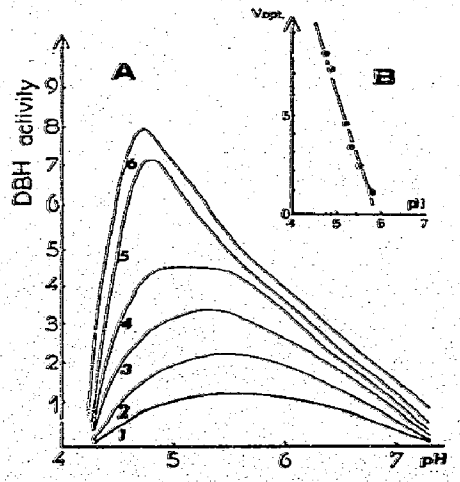


Fig. 1. A) DBH activities versus pH. DBH activities were determined at tyramine concentrations of: 1 : 0.2 mM; 2 : 0.5 mM; 3 : 1 mM; 4 : 2 mM; 5 : 5 mM; 6 : 10 mM. The buffers used were 0.5 M acetate buffer from pH 4.5 to pH 5.5 and 0.1 phosphate buffer from pH 5.0 to pH 7.5. (Curves were exactly superposed thus eliminating buffer action.) Each assay was performed with 1 μg of enzyme (specific activity of enzyme preparation: 100 μmoles/30 min/mg of enzyme). DBH activity is expressed as μmoles of octopamine formed per 30 min and per 50 μg of enzyme. B) $V_{opt}$ versus pH. DBH activities found at the optimum pH were plotted versus optimum pH for each curve. Each point corresponds to the tyramine concentration used in fig. 1A.



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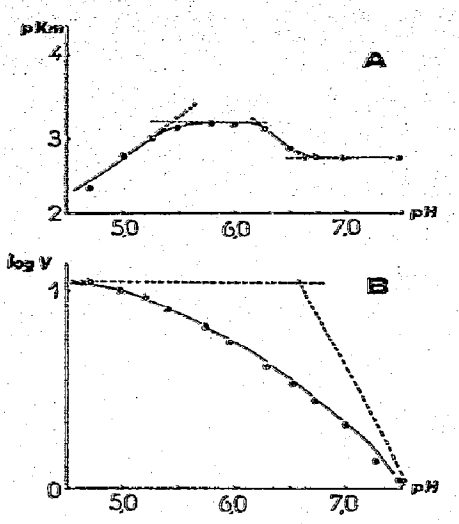


Fig. 2. Effect of pH on $K_m$ and $V$ of DBH. $pK_m(\log(K_m)^{-1})$ and log $V$ were determined as described in the Methods.

metrical. Secondly, it is relevant to note that the optimum pH was progressively shifted towards a lower pH when the tyramine concentration was raised. In fig. 1B, DBH activities at the optimum were plotted against pH at different tyramine concentrations. The relation between both factors seems to be linear.

When reciprocal rates were plotted against reciprocal tyramine concentrations at various pH's, it could be observed that the $V$ and the $K_m$ for tyramine varied as a function of pH as shown in fig. 2.

According to Dixon [7], the observed pH effects can be interpreted in terms of the pH values of groups situated in the free enzyme, in the free substrate, or in the enzyme–substrate complex. Groups situated in the free substrate can be excluded here since the $pK$ of the amino group of tyramine is 9.4 and the $pK$ for the acidic group of ascorbate is 3.0.

The downward bend of the $pK_m$–pH curve at pH 5.4 gives a $pK$ of the free enzyme. The slope of the curve, successively +1 and 0 is indicative of the ionisation of a single group. On the basis of the values, one could assume that this ionizable group is one carboxylic group. This group could participate in the fixation of tyramine to the active center since the log $V$–pH curve does not show intersecting lines at this $pK$ value.

In the pH range from 6.0 to 7.5, the $pK_m$–pH curve shows a group of two $pK$ values, 6.2 and 6.6 forming a wave. At pH 6.2 the bend concave to the pH axis (slope decreases from 0 to −1 with increasing pH) corresponds to a $pK$ value for the enzyme. At pH 6.6 the bend convex to the pH axis (slope increases from −1 to 0 with increasing pH) corresponds to a $pK$ value for the enzyme–substrate complex. The two values 6.2 and 6.6 could correspond to the same group of the enzyme, the ionization of which is affected by the substrate binding so that its $pK$ is increased in the enzyme–substrate complex. This appears also in the log $V$–pH plots, where a $pK$ of 6.6 is found. In the case of the log $V$–pH curve, the downward bend at pH 6.6 introduces a change in the slope of the curve from 0 to −1 suggesting a proton dissociation, which must be that of the enzyme–substrate complex. The protonated form of this group is thus necessary to the enzymatic reaction. The $pK$ value of this group is in agreement with the assumption that an histidyl residue is involved in the hydroxylation reaction at the active center of the enzyme.

## 4. Discussion

It appears that DBH is sensitive to hydrogen ion concentration and that the enzyme has three $pK$ values (table 1). The $pK$ value of 5.4 could correspond to a carboxylic group, the ionization of which prevents the fixation of tyramine to the active center.

The $pK$ values of 6.2 and 6.6 are due to the same ionizing group of the protein. The value of 6.2 is due to the group of the free enzyme whereas the value of 6.6 is due to the group of the enzyme–substrate complex. Preliminary data (unpublished) from further experiments also suggest that an histidine residue participates in the enzymatic reaction. The slope of the $pK_m$–pH or the log $V$–pH curves changes by one unit (fig. 2), which means, according to Dixon [8] that one histidine residue participates in the enzymatic reaction.

The scheme of reactions required to explain this type of behaviour is shown in fig. 3.

It is relevant to note that no change of $K_m$ for the cosubstrate was observed when tyramine was held at a constant and saturating level, and ascorbate was varied. Thus, complexing of ascorbate could not be due to either an acidic group nor a basic group of the protein. The copper which is known to be associated with DBH [1] may be solely responsible for the transitory binding in the enzyme–ascorbate complex.

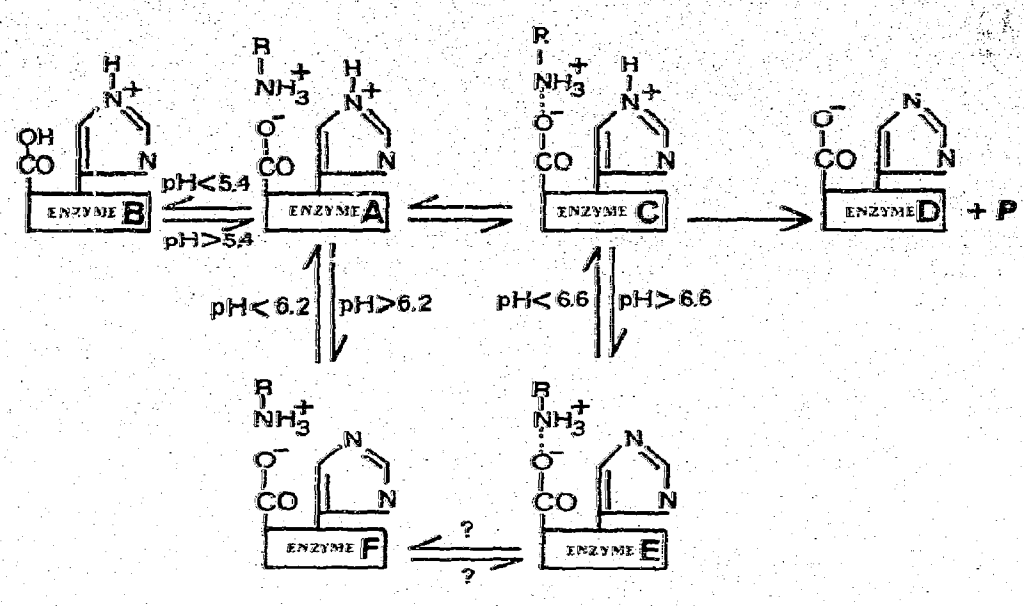


Fig. 3. Schematic illustration of the role of carboxylic group and of the histidyl residue according to pH values. $R-NH_3^+$ is the substrate and P reaction products. The carboxyl group of the free enzyme (A) is found unprotonated when the pH is greater than 5.4. For pH values lower than 5.4, the carboxyl group is protonated and the enzyme does not fix the $R-NH_3^+$ substrate (B). Over pH 5.4, the enzyme adsorbs the substrate (C) and the enzyme–substrate complex undergoes the enzymatic process (D + P). Over pH value of 6.6, the histidyl residue is unprotonated and the enzymatic reaction cannot occur (E). The histidyl residue of the free enzyme is unprotonated (F) for pH values over 6.2. From our data, it is not possible to know whether pH could influence the equilibrium between E and F.

It is interesting to point out that DBH activities are rather high in the acidic pH range. Moreover the *pK* of the histidinyl group is 6.6. DBH is localized in chromaffin granules in adrenal medulla [8]. It appears that acidic compounds are present in these particles, such as ATP and chromogranin proteins [9]. Thus, in these particles the microenvironment of DBH could be acidic and could create the conditions of optimum activity of the enzyme.

Table 1
*pK* values obtained from $pK_m$ and log $V$ plots as a function of pH.

| Curve | pK of free enzyme | pK of enzyme–substrate complex |
|---|---|---|
| $pK_m$ | 5.4<br>6.2 | 6.6 |
| log $V$ | – | 6.6 |

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