# INTERACTION OF L-DOPA DECARBOXYLASE WITH SUBSTRATES: A SPECTROPHOTOMETRIC STUDY

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## 1. Introduction

Mammalian L-DOPA decarboxylase (EC 4.1.1.26) is an enzyme with a broad specificity, which acts on the natural aromatic amino acids. It has been purified from kidney by Christenson et al. [1], by Borri Voltattorni et al. [2] and by Lancaster and Sourkes [3] and its spectral properties have been described [4].

While the interactions between enzyme and substrates have been studied with the bacterial amino acid decarboxylases [5], no data of this sort are available with the mammalian enzymes, which differ from the others in the value of pH optimum; furthermore the mechanism of action of DOPA decarboxylase, notwith-standing the biochemical and pharmacological importance of the enzyme, has received little attention.

We have studied the interaction of L-DOPA decarboxylase from hog kidney with its substrates by means of spectrophotometry, and we have been able to provide evidence for at least three enzyme-substrate or enzyme-product complexes; a quite unusual absorbance spectrum has been observed for two of them.

## 2. Materials and methods

The enzyme has been purified according to Borri Voltattorni et al. [2].

The absorption of the purified enzyme solution at pH 6.8 at wavelengths higher than 300 nm was routinely checked and found equal to that previously described [4], with a very constant absorbance ratio at 335 and 420 nm. The spectra were measured on a thermostated Beckmann DB-GT spectrophotometer, connected to a Beckmann 10 inch recorder. The substrates and products tested were purchased from Sigma Chem. Co. and from Fluka AG.

## 3. Results and discussion

The addition of L-DOPA to an enzyme solution at pH 6.8, which is the pH optimum for this substrate [1], is accompanied by an immediate yellowing of the solution, which rapidly fades out. This colour change should be due to the formation of substrate-enzyme complexes, which rapidly disappear owing to the high rate of decarboxylation of L-DOPA. Therefore, in order to obtain the spectral data of the intermediate complexes, other substrates were tested, particularly those which have a lower maximal velocity and a good affinity for the enzyme.

Spectra were obtained at pH 8.5, which is the pH optimum for substrates other than DOPA [1]. At this pH the enzyme alone has a higher 335/420 nm absorbance ratio than that observed at pH 6.8. Addition of L-5-hydroxytryptophan strongly increases the absorbance at 420 nm, as shown in fig.1.


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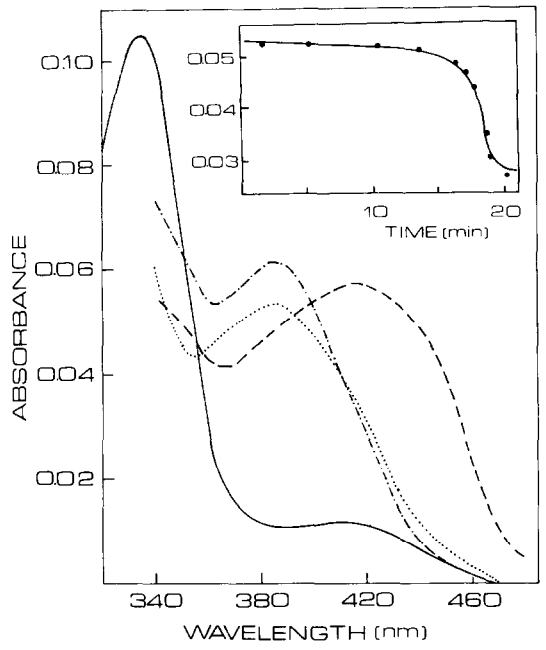



Fig.1. Spectra in Tris–HCl buffer, pH 8.5: (——) enzyme (1.45 mg/ml); (– – – –) enzyme plus 5 mM 5-hydroxy-L-tryptophan; (–.–.–.) enzyme plus 5 mM L-5-hydroxytryptophan (2 hr after substrate addition); (.....) enzyme plus 5 mM 5-hydroxytryptamine. The spectra are corrected for the absorption of the substrate or product; however the absorbance was too high to allow a precise measurements below 340 nm. In the inset: absorbance at 420 nm of enzyme plus 5 mM 5-hydroxy-L-tryptophan as a function of time at 20°C. The points represent theoretical values calculated as described in the text.

The same 420 nm peak is present after addition of L-phenylalanine, L-tyrosine, or L-tryptophan. Adding instead *m*-tyrosine or *o*-tyrosine a maximum at 385 nm is observed (fig.2). With L-5-hydroxytryptophan the absorbance is essentially invariant with pH in the range 6.8–8.6, but with *m*-tyrosine a decrease of pH brings out the appearance of a 430 nm peak and a decrease of the 385 nm band (fig.2). The midpoint of this spectral transition is at pH 7.6 (inset of fig.2).

All spectra, after different times (depending on the substrate) are converted into new ones, with a maximun at 385 nm, which do not undergo any further change in time (fig.1).

This spectral change can be observed also with *m*- and *o*-tyrosine; in this case there is no wavelength shift but only a decrease in intensity of the 385 nm band (fig.2).

The first spectra which appear upon addition of substrates to the enzyme, with a maximum at 420 (or 385) nm, and the spectra appearing subsequently with a maximum at 385 nm may be quite safely attributed respectively to substrate-enzyme and product–enzyme complexes.

In line with this view is the observation that the time and rate of disappearance of the first spectrum are clearly correlated with the kinetic parameters for the various substrates. The change in time of the 420 nm absorbance with L-5-hydroxytryptophan is shown as an example in the inset of fig.1; a good fit of the experimental curve is shown with points calculated by using the integrated form of the Michaelis Menten equation, assuming a $K_M$ of $1 \times 10^{-4}$ M (1) and a $V_{max}$ of 0.29 mM/min.

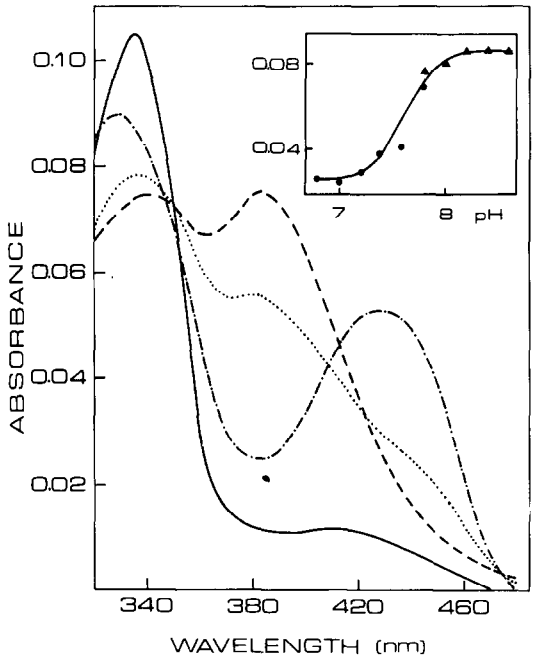



Fig.2. Spectra in Tris–HCl buffer, pH 8.5: (——) enzyme (1.45 mg/ml); (– – – –) enzyme plus 2.5 mM DL-m-tyrosine; (.....) enzyme plus 2.5 DL-m-tyrosine (2 hr after the addition of the substrate). Spectrum in phosphate buffer pH 6.8: (–.–.–.) enzyme plus 3.6 mM DL-M-tyrosine. In the inset: absorbance at 385 nm of the enzyme plus 3.6 mM DL-M-tyrosine as a function of pH. (●——●): phosphate buffer; (▲——▲): triethanolamine–HCl buffer.

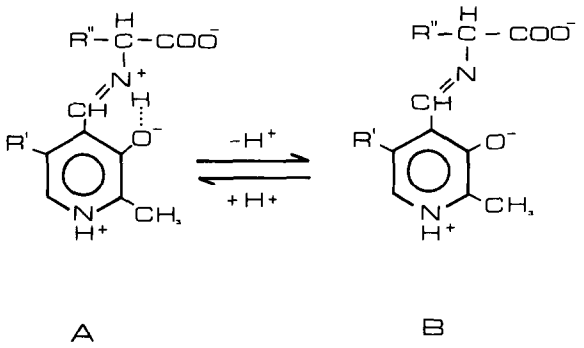


Fig.3. Proposed structures for coenzyme–substrate complexes absorbing at 420 nm (A) and 385 nm (B).

The identification of the complex, originating the second spectrum, with a product–enzyme complex is confirmed by the fact that addition of the amine products to the enzyme produces the same spectra (fig.1).

Structures of the different intermediate complexes can be proposed on the basis of their spectral characteristics. The 420 or 430 nm peak indicates a substrate–coenzyme complex which has the structure of a 4′-N-protonated Schiff base (compound *A* of Fig.3) [5].

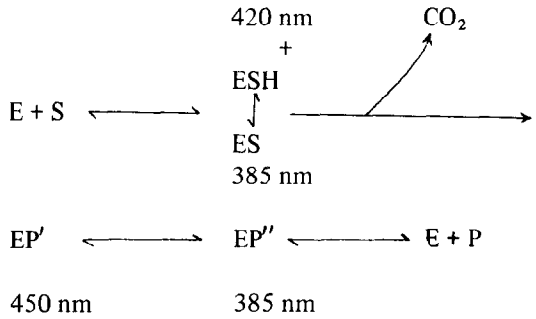
The interpretation of the 385 nm peak, shown by *m*-tyrosine and *o*-tyrosine and by product–enzyme complexes is more difficult, since this is the first example, as far as we know, of such an absorbance for complexes of pyridoxal-phosphate–enzymes. However a peak in the 380 nm region is shown by semicarbazide-pyridoxal-5′-phosphate–enzyme complexes [6], and, more important, by model compounds studied by Martell (compound XII, [7]), which are 1-N-protonated-4′-N-unprotonated Schiff bases; accordingly, the structure of our intermediate should be the one indicated for compound B in fig.3.

This interpretation is supported by the fact that the 385 nm peak is shown by the high pH form of the *m*-tyrosine–enzyme complex; the spectral transition of this complex should therefore be caused by a deprotonation of the 4′ nitrogen.

The presence of another complex, absorbing in the 450 nm region, is suggested by a pronounced shoulder on the absorption band of the L-5-hydroxytryptophan-enzyme complex (fig.1) and of the *m*-tyramine-enzyme complex (fig.2).

A 450 nm absorbing species has been detected previously in other $B_6$ dependent enzymes [8], but the structure responsible for it is still unknown. The fact that in DOPA-decarboxylase this complex appears either together with the first substrate–enzyme complex, or with the enzyme–product complex (depending on the nature of the substrate) suggests that this compound is located in the middle of the reaction pathway.

As a whole these data suggest the following sequence of intermediate complexes:




The difference of the spectra obtained at pH 8.5 respectively with L-5-hydroxytryptophan and M-tyrosine suggests that the pK values of the $ESH^+ \rightarrow ES$ transition may very significantly, depending on the nature of the substrate. Considering the behaviour of the 450 nm peak, also the rate limiting step seems to vary for different substrates. These considerations, together with the well known difference in pH optimum for DOPA and other substrates [1], suggest that for DOPA decarboxylase even limited variations in substrate structure originate rather large variations in the properties of the intermediate substrate-enzyme complexes.

## References

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