This kind of pattern is characteristic of a heterophenotype in which the enzyme involved is dimeric[8].

The simplest explanation of a heterophenotype is that it is governed by a heterozygous genotype. However, in the present material all the 38 parasitic worms studied are characterized by heterophenotypes (table). It indicates that 2 loci are involved. This is supported by the observation, under identical experimental conditions, of a single band of GPI enzyme activity, with distinctly slower mobility, in a related species *Brugia pahangi* which is an animal filarial parasite.

Assuming the presence of 2 *Gpi* loci, the present results indicate the occurrence of a single allele for the *Gpi-1* locus and 2 alleles for the *Gpi-2* locus. Further evidence for the presence of 2 *Gpi* loci in subperiodic *Brugia malayi* rests with the finding of the other expected heterophenotypes in future material.

The absence of the heterozygous *Gpi-2*$^b$/*Gpi-2*$^c$ genotype could be due to the fact that the worms used in the present study were recovered from experimental animal hosts. As the parasites have been maintained in laboratory hosts, through passaging from one host to another, for about 20 years, it is reasonable to suggest that homozygosity within a single host could have been established. Experiments will be carried out in due course to infect a single jird with parasites of both GPI-phenotypes in the hope of recovering the heterozygous phenotype.

The GPI electrophoretic phenotypes in 3 species of cattle filarial parasites (*Onchocerca* spp.) have also been reported to possess multiple bands of enzyme activity[9]. The author, however, attributed the finding as 'probably due to oxidation during processing'.

Distribution of glucose phosphate isomerase (GPI) phenotypes in a subperiodic form of *Brugia malayi* from peninsular Malaysia

| Sex of worm | GPI-AB | GPI-AC |
|---|---|---|
| Male | 4 | 8 |
| Female | 13 | 13 |
| Total | 17 | 21 |

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## Inhibition of pig kidney dopa decarboxylase by coenzyme-5-hydroxytryptophan adducts[1]

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*Summary.* The effect of N-(5'-phosphopyridoxyl)-L-5-hydroxytryptophan, N-(5'-phosphopyridoxyl)-D-5-hydroxytryptophan and N-(5'-phosphopyridoxyl)-5-hydroxytryptamine on the reactivation of apoDopa decarboxylase to holoenzyme has been investigated. The different degree of inhibition exerted by these adducts has been interpreted on the basis of a different orientation of the 2 isomers of 5-HTP at the active of Dopa decarboxylase.

Phosphopyridoxyl-amino acids (PPxy-amino acids), products of reduction of Schiff bases formed between pyridoxal-P and amino acids, are adducts which combine both substrate and coenzyme into a single molecule and are structurally similar to the covalent intermediates in the reaction pathway of pyridoxal-P dependent enzymes. Their inhibitory effect on many pyridoxal-P dependent enzymes has been used to investigate aspects of the mechanism of catalysis[2-8].

Similar coenzyme-amino acids adducts behave as inhibitors of the recombination of apoDopa decarboxylase with pyridoxal-P[9]; moreover, kinetic studies on the nature of the inhibition caused by these adducts led to the assumption that in addition to a high-affinity coenzyme-binding active site, Dopa decarboxylase has at least 1 additional low-affinity pyridoxal-P binding site[10]. The adducts employed in these studies are derivatives of aromatic amino acids with a catechol-related structure.

Since 5-hydroxytryprophan (5-HTP) and its analogues also bind to the active site of the enzyme[11-13], we investigated the interaction of N-(5'-phosphopyridoxyl)-L-5-hydroxytryptophan (PPxy-L-5HTP), N-(5'-phosphopyridoxyl)-D-5-hydroxytryptophan (PPxy-D-5HTP) and N-(5'-phosphopyridoxyl)-5-hydroxytryptamine (PPxy-HT) with Dopa decarboxylase from pig kidney. The results allow the evaluation of the relative importance of the $\alpha$-carboxylate group for the binding of the 2 enantiomers of 5-HTP to Dopa decarboxylase and reinforce the concept that these may orientate differently at the active site of the enzyme, as previously suggested[14].

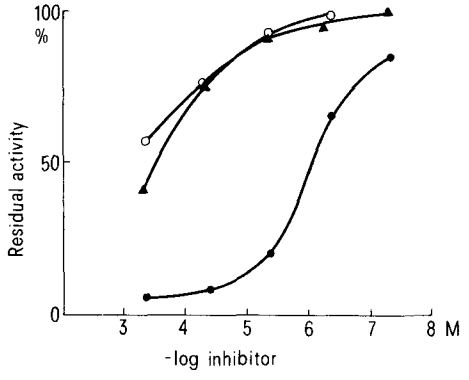
*Materials and methods.* PPXy-L-5HTP, PPxy-D-5HTP and PPxy-HT were synthesized following the general procedure of Ikawa[15]. Dopa decarboxylase (EC 4.1.1.28) from pig kidney was highly purified according to Borri Voltattorni et al.[16]. Two procedures were used to prepare apoenzyme. The first was by treatment with hydroxylamine, as previously described[17]. The second was as follows: holoenzyme was inactivated by incubating D-5HTP in 0.1 M Hepes pH 8.4 for 2 h at 37 °C and then dialyzed against 0.1 M potassium phosphate, pH 6.8. The resulting enzyme preparation has a residual activity ranging

from 0 to 4% of the original value, and after addition of $1 \times 10^{-6}$ M coenzyme exhibits activity ranging from 40 to 60% of the original.

Rates of decarboxylation in a typical assay were determined according to Charteris and John[18] by adding apoenzyme to a reaction mixture containing $1 \times 10^{-6}$ M coenzyme and inhibitor at various concentrations in 0.05 M potassium phosphate pH 6.8. The reaction was started by addition of $5 \times 10^{-4}$ M L-Dopa and the reaction time was 5 min at 25 °C. In selected experiments, aimed at assessing binding reversibility of coenzyme and adducts to apoenzyme, different sequences of reacting coenzyme, adducts and apoenzyme were tested.

*Results and discussion.* The figure shows the effects of varying concentrations of PPxy-L-5HTP, PPxy-D-5HTP and PPxy-HT on the reactivation of apoenzyme to holoDopa decarboxylase. All of the tested compounds display, although to different extents, an inhibitory effect on reconstitution of Dopa decarboxylase with coenzyme resulting from their binding to the active site. In the range of the concentrations of PPxy-derivatives used, inhibition is not observed on holoDopa, thus indicating that these adducts are not able to displace pyridoxal-P from holoenzyme. Conversely, the coenzyme cannot cause a rapid displacement of the adduct from the enzyme once the inhibitor-enzyme complex is formed. Therefore, conventional kinetic methods cannot be used to determine dissociation constants for these adducts, since the determination of dissociation constants for inhibitors by kinetic methods assumes that the binding of the inhibitors is readily reversible. Thus, as suggested by Rudd and Thanassi[10] and our previous observations[9], the inhibition exerted by these adducts is under kinetic control, and the apparent affinities of coenzyme and adducts do not reflect their true affinities for the enzyme. For this reason, we measured the degree of inhibition of the adducts and these values can be reasonably regarded as indicative of the different affinity of these coenzyme-amino acid adducts for the enzyme.

In these experiments, one thing to be noted is the similarity in behavior between PPxy-L-5HTP and PPxy-HT. This could be due to the fact that the binding is not favored by the presence of the carboxylate group, as already observed in a study on the interaction of PPxy-derivatives with tyrosine decarboxylase[5] and the inhibitor binding to Dopa decarboxylase[13]. In this regard, it has been suggested that the carboxyl group causes some strain in the substrate-coenzyme-apoenzyme complexes. Since this group is the one eliminated during the catalytic process, the distortion caused by the carboxyl group is likely to affect the catalytic efficiency of the amino acid decarboxylases. On this basis, the behavior of PPxy-D-5HTP cannot be easily explained. PPxy-D-5HTP is a more effective inhibitor than the other 2 compounds: the concentration of PPxy-D-5HTP required to cause 50% inhibition is $\sim 10^{-7}$ M, while a concentration of $\sim 10^{-4}$ M is required to cause the same inhibition by PPxy-L-5HTP and PPxy-HT. One possible explanation is that the carboxyl group of D-5HTP could contribute to the binding by interacting with some groups on the enzyme molecule because of its different positioning as compared with the corresponding one of L-5HTP. This hypothesis is based on the new data on the interaction of D-5HTP with Dopa decarboxylase. Borri Voltattorni et al.[14] have recently provided evidence for the binding of D-5HTP to the active site of Dopa decarboxylase. The authors have also shown that D-5HTP does undergo a transamination reaction and does not behave as a substrate for the decarboxylase. According to Dunathan's hypothesis[19], and assuming that the side chain of the aromatic D-amino acid is oriented in the same relative position as that of the corresponding L-form, it has been suggested that the $\alpha$-carboxylate and the $\alpha$-hydrogen of L- and D-5HTP, respectively, are perpendicular to the plane of the pyridoxal-P ring. Therefore, the different inhibition exerted by PPxy-L-5HTP and PPxy-D-5HTP seems to strengthen, even if it does not prove, the suggested hypothesis of the different positioning of the 2 D- and L-forms of the amino acid. If this interpretation is correct, once again the use of PPxy-amino acids must be regarded as an useful tool to analyze the contribution of different functional groups of the substrate and the coenzyme to the binding. Results identical to those reported in the figure have been obtained using the enzyme resulting from the interaction of Dopa decarboxylase with D-5HTP. These data, while in agreement with the proposed transaminating mechanism exerted by Dopa decarboxylase on D-5HTP[14], make it possible to view the reaction of the enzyme with D-5HTP as a convenient way to generate apoenzyme.




Inhibitory action on recombination of apoDopa decarboxylase with PLP exerted by various concentrations of (▲) N-(5'-phosphopyridoxyl)-L-5-hydroxytryptophan, (●) N-(5'-phosphopyridoxyl)-D-5-hydroxytryptophan or (○) N-(5'-phosphopyridoxyl)-5-hydroxytryptamine. The data represent the average of 3 or 4 separate experiments.

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