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INVESTIGATION OF SOME PROPERTIES AND GLYCINE INHIBITON OF L-TYROSINE DECARBOXYLASE FROM S. FEACALIS BY A pCO_2 MEMBRANE ELECTRODE

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ABSTRACT

In the present study, some kinetic properties of L-tyrosine decarboxylase were determined and the effect of glycine on the enzyme activity was investigated using a diffusion type CO_2 electrode. It was observed that the glycine is a weak inhibitor of the enzyme and although it has some disadvantages, pCO_2 electrode is a good tool for kinetic studies.

INTRODUCTION

L-tyrosine Decarboxylase (TDAE), EC. 4.1.1.25 is the bacterial counterpart of aromatic L-amino acid decarboxylases and is especially rich in S. Feacalis (Gale, 1946). TDAE is specific for L-tyrosine and does not decarboxylate D-tyrosine, tyrosine derivatives and other amino acids except L-Dopa (Epps, 1944). It requires pyridoxal 5'-phosphate (PLP) as coenzyme and is mostly used for L-tyrosine (Havas, 1982) or PLP (Chabnar, 1970; Hassan, 1981; Fonda, 1986) estimation in biological samples.

Mechanism of decarboxylation which was first proposed by Metzler (1954) ,involves schiff base formation between coenzyme and substrate. PLP is linked to the ε-amino group of a specific lysyl residue by a labile covalent bond. Dunathan (1966) suggested that the limited rotation around the substrate α-carbon atom due to specific orientation of the groups and histidine residue, which precedes the PLP-lysyl residue (Boeker, 1972) in many decarboxylases with known amino acid sequence, must play roles in the specificity of these enzymes.

In bacteria, decarboxylation reactions a) control intracellular CO_2 level, b) have biosynthetic functions, c) participate in energy producing sequences.

It is clear from the schiff base mechanism of decarboxylation that carbonyl reagents must inhibit the reaction. Papers investigating the inhibition of methyl hydrazine (Harik, 1973), p-chloroacetic acid (Hamfelt, 1967), aminooxyacetate (John, 1978), α-halomethyl amino acids (Bey, 1979), hydroxyquinone (Mazelis, 1962), allenic amino acids (Castelhano, 1984) and phosphoric analogs of tyrosine (Iron, 1981, 1986) on PLP dependent enzymes have appeared. Yuan and Chang (1987) reported in vivo induction of DDT on tyrosine decarboxylase in the American cockroach.

In the present study, some kinetic properties of L-tyrosine decarboxylase were determined and the effect of glycine on the enzyme activity was investigated. The glycine is a common and important amino acid and is readily found in bacteria and biological samples. By comparison with the above mentioned acetic acid derivatives, the glycine seemed to be a possible candidate as an inhibitor of the enzyme.

TDAE decarboxylates L-tyrosine according to the following reaction

$$\text{Tyrosine} \xrightarrow[\text{PLP}]{\text{TDAE}} \text{Tyramine} + CO_2$$

Therefore, reaction rates were followed by measuring the produced CO_2 by a pCO_2 membrane electrode. Dynamic properties of this electrode are very suitable for kinetic measurements (Guilbault, 1972; Jensen, 1979).

EXPERIMENTAL

Apparatus: Diffusion type CO_2 electrode and milivoltmeter Type TS 80 were purchased from Tacussel Electronics. Measurements were carried out in a small double jacketed double neck cell, thermostated at 37 $\pm$ 0.1 °C by means of a Julabo constant temperature bath and circulator. UV spectra were taken with a Hitachi Model 200 double beam spectrophotometer.

Reagents: All chemicals used were reagent grade. L-tyrosine decarboxylase apoenzyme was purchased from Sigma Chemical Co. L-tyrosine solution ($3.2\ 10^{-2}$ M) was prepared in 10^{-2} M HCl. Pyridoxal 5′-phosphate stock solution (10^{-3} M) was prepared in triple distilled water, kept in dark, refrigerated and renewed every week. Necesssary

dilutions were made daily. L-tyrosine decarboxylase epoanzyme was prepared by dissolving ~12mg of the enzyme (1.35 U/mg) in proper amounts of 0.1 M citrate buffer of pH 5.8 to give a 1 U/0.2 mL solution. Enzyme solutions were kept at 5 °C and renewed every other day.

Procedure: In the experiments 0.2 mL (1U) enzyme solution, 0.2 mL PLP and calculated amount of 0.1 M citrate buffer, pH 5.8, are mixed in the cell. pCO_2 electrode with a rubber stopper is splaced above the solution (~2 mm from the surface) and the mixture is incubated while stirring with a magnetic stirrer for 20 minutes at 37 °C (Fig. 1). During this period, holoenzyme complex is formed and a steady potential is reached. At the end of incubation period a certain volume ranging from 50 to 500 μL of L-tyrosine solution is injected from the side arm. Final volume is always fixed at 3 mL. Potential drops due to CO_2 formation are recorded. Potential (mV) versus time (min). curves are plotted and initial reaction rates for different conditions are determined from the rate portion of these curves.

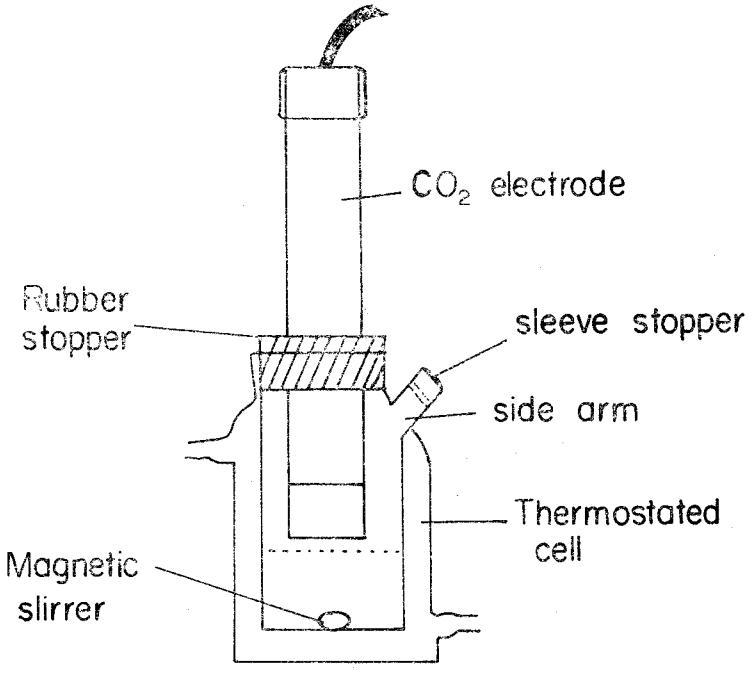


Figure 1. Schematic representation of reaction cell.

Inhibition experiments are performed in the same manner with a difference that in addition to PLP a certain amount of glycine solution is also added to the reaction mixture.

RESULTS AND DISCUSSION

In the literature, it was reported that (Sunderesan, 1970) TDAE shows maximum activity in acetate buffer. However, we could not obtain a steady potential with acetate buffer because acetate gives interference with diffusion type CO_2 membrane. So we used citrate buffer in our experiments.

A typical potential (mV)-time(min) plot is shown in Fig. 2. The optimal substrate (L-tyrosine) concentration required to saturate 1U enzyme in the presence of $6.6\ 10^{-5}$ M PLP in a total volume of 3 mL in citrate buffer at 37°C was found to be $5.3\ 10^{-3}$ M (Fig. 3). From the Lineweaver-Bruk plot (Fig. 4. curve a) Km value was found to be $1.5\ 10^{-3}$ M which agrees well with the values $1.6\ 10^{-3}$ (Sundaresan, 1970) and $5\ 10^{-4}$ (Hassan, 1981). From a different set of experiments performed in 0.1 M citrate buffer of pH 5, Km value was obtained as $2.6\ 10^{-3}$ M. (Fig. 5).

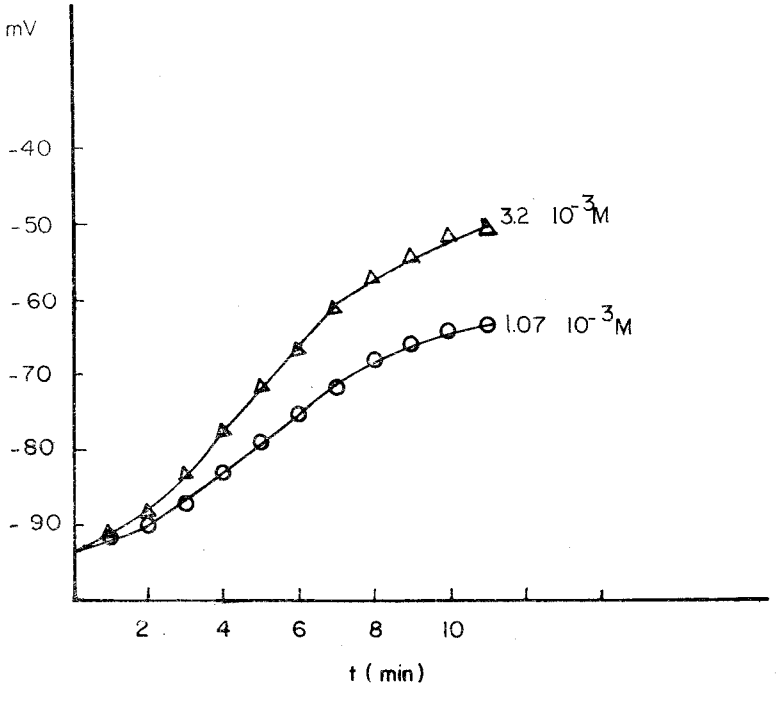


Figure 2. Time versus CO_2 electrode response curve for two different L-tyrosine concentration.

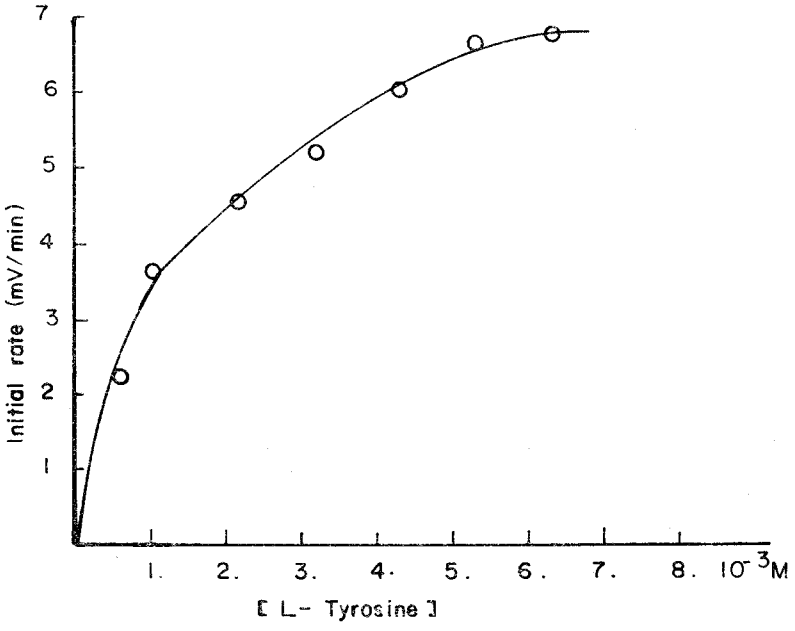


Figure 3. Effect of L-tyrosine concentration on the initial reaction rate.

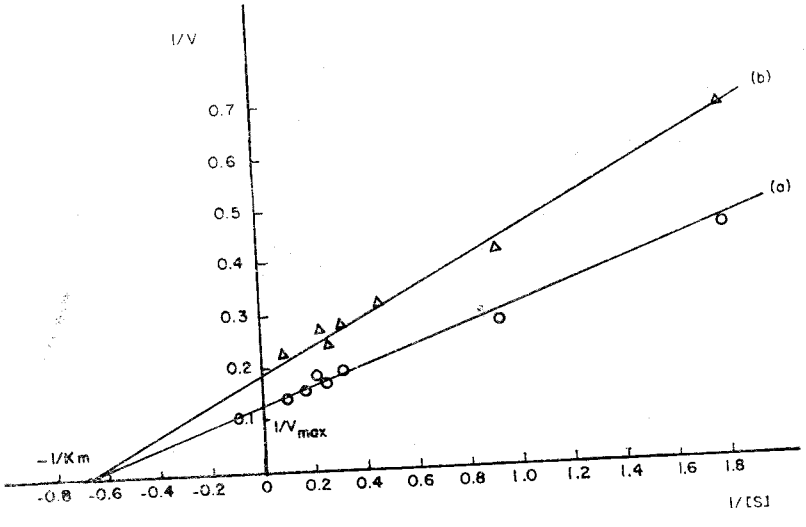


Figure 4. Lineweaver-Burk plots for L-tyrosine decarboxylase. Curve a, without inhibitor. Curve b, with inhibitor.

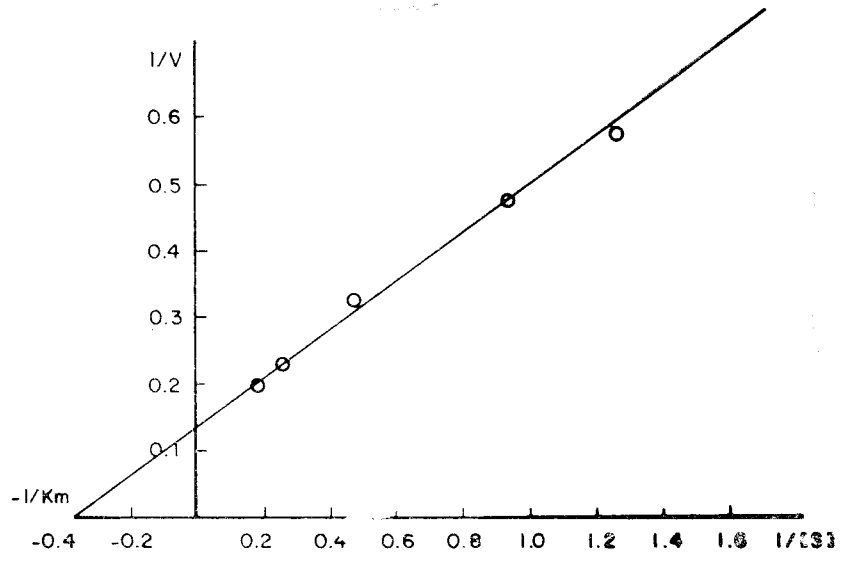


Figure 5. Lineweaver-Burk plot drawn for pH 5.

Km value of the enzyme for pyridoxal 5′-phosphate was also determined by changing the PLP concentrations for fixed substrate and enzyme quantities. It was found to be $8.9\ 10^{-8}$ M (Fig. 6), which is rather close to the value, $3.0\ 10^{-7}$, given for the acetate buffer by Sundaresan (1970).

Effect of glycine ($1.7\ 10^{-4}$ M) on TDAE activity was measured for different concentrations of L-tyrosine in the presence of $6.6\ 10^{-5}$ M PLP .The results are plotted in curve b of Fig. 4. The appearence of this curve suggest a noncompetitive inhibition. Hence, by employing the noncompetitive inhibition double reciprocal equation

$$\frac{1}{V} = \frac{Km}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

K_i value is calculated from the intercept which is equal to $\frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$. From the figure, $1/V_{max}$ and intercept values are

read as 0.13 min mV^{-1} and 0.18 min mV^{-1}, respectively. For [I] = 1.7 10^{-4} M, substitution of these values in the intercept expression yields K_i = 4.42 10^{-4} M.

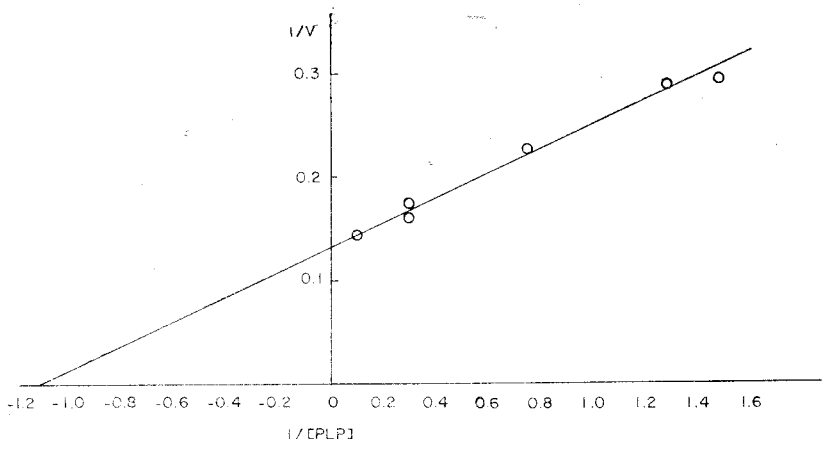


Figure 6. Double reciprocal curve plotted for diffcrent PLP concentrations.

Activation energy of the enzymatic decarboxylation of L-tyrosine was calculated from the experiments performed at 25, 30, and 37 °C's. (Fig. 7 curve a). The average value calculated from two sets of experiments is ~3.2 kcal mol^{-1}. In the presence of glycine (1.7 10^{-4} M) activation energy of the reaction becomes 7.2 kcal mol^{-1}. (Fig. 7. curve b).

Effects of different factors (such as substrate and PLP concentrations, pH and temperature) on percent inhibition are given in Table 1. From the data in this table, it is clear that substrate concentrations don't effect inhibition, which is characteristic for noncompetitive inhibition. On the other hand pH, temperature and coenzyme concentrations are effective on percent inhibition.

The order of addition of the inhibitor also effects the results. When glycine is added to the reaction mixture with PLP prior to preincubation with enzyme (Fig. 8. curve a), potential drop is less pronounced compared to the potential drop in case of glycine addition at the end of incubation period. (Fig. 8 curve b).

Table 1. Effect of different factors on percent inhibition.

[L-Tyr]	[PLP]	[I]	Temp C	pH	%Inhibition
1 10^{-3} M	1 10^{-4} M	1.7 10^{-4}M	37	5.8	33
2 10^{-3}	"	"	"	"	31
5 10^{-3}	"	"	"	"	34
"	6.6 10^{-5}	"	"	"	24
"	3.3 10^{-7}	"	"	"	5.7
"	6.6 10^{-5}	"	30	"	61
"	1 10^{-4}	2.3 10^{-4}	37	5.0	16

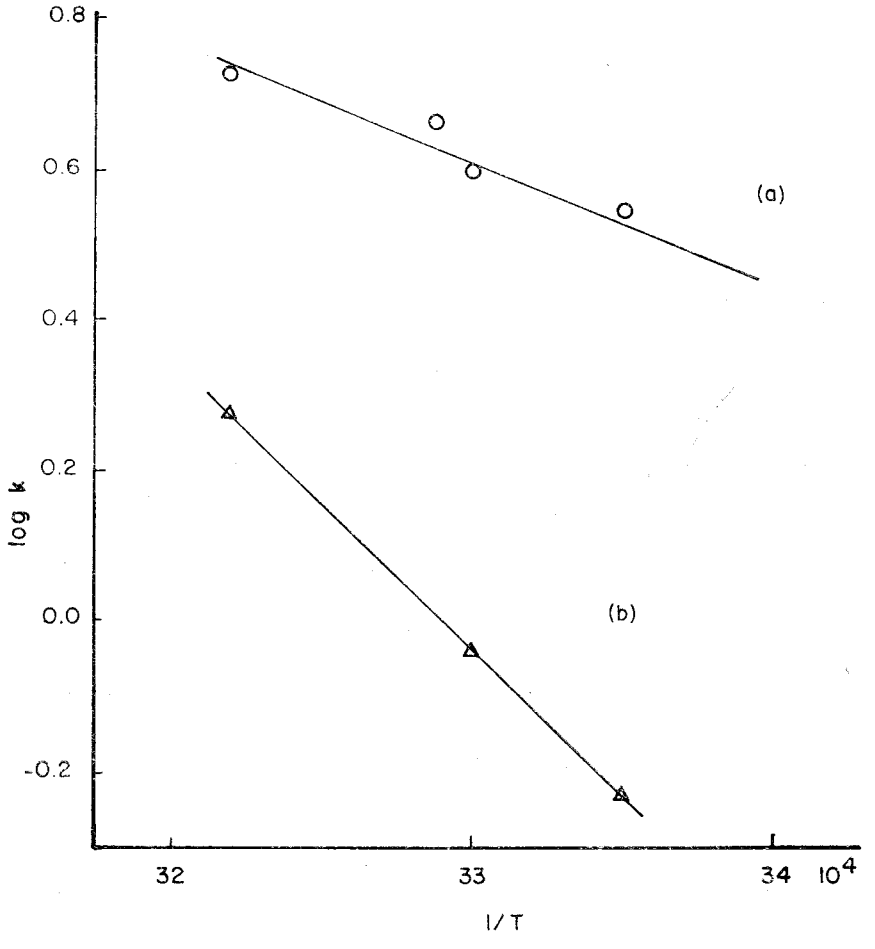


Figure 7. Effect of temperature on the decarboxylation rate. Curve a, in the absence of inhibitor. Curve b in the presence of inhibitor.

Jenkins and D'ari (1966) have studied UV absorption spectra of PLP dependent glutamate transaminase enzyme system in detail and showed that the correct interpretation of subtle differences in the UV spectra of the coenzyme may provide valuable information about events at the active site. Therefore, we have attempted to take the UV absorption spectra of different combinations of enzyme-coenzyme-substrate and inhibitor. These spectra are shown in Fig. 9 a, b, c, and d for the holoenzyme complex, holoenzyme + substrate, holoenzyme + glycine (added prior to incubation); and holoenzyme + glycine (glycine added after incubation), respectively. When glycine is added before incubation, a peak appears at the same wavelength, 274 nm, given by substrate. But this peak does not appear when glycine is added at the end of enzyme coenzyme incubation period. These results suggest that the glycine is a weak inhibitor and once holoenzyme complex forms inhibition is not significant.

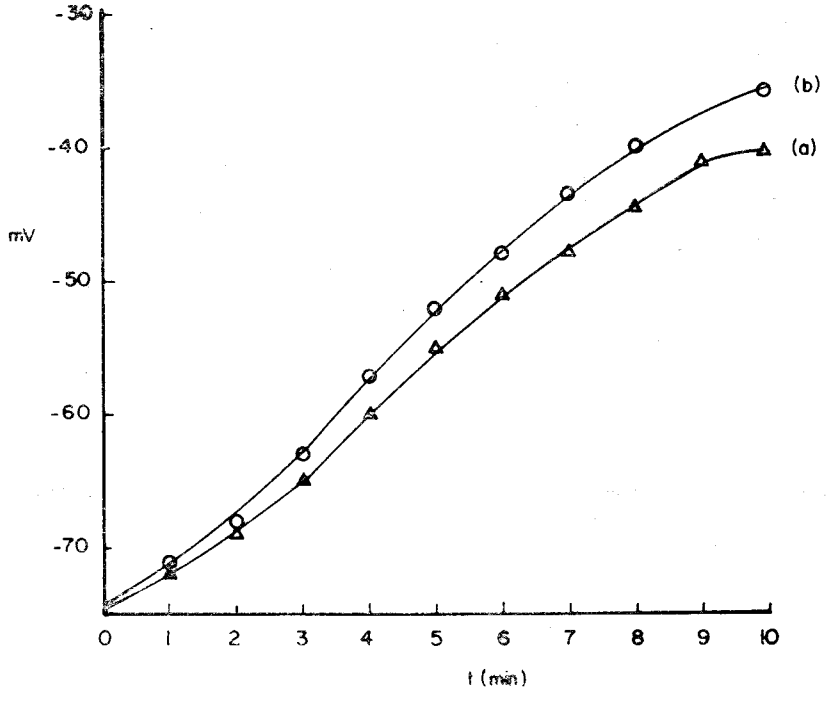


Figure 8. Effect of addition order of the inhibitor: a) glycine added before incubation of coenzyme-apoenzyme, b) glycine added after incubation period

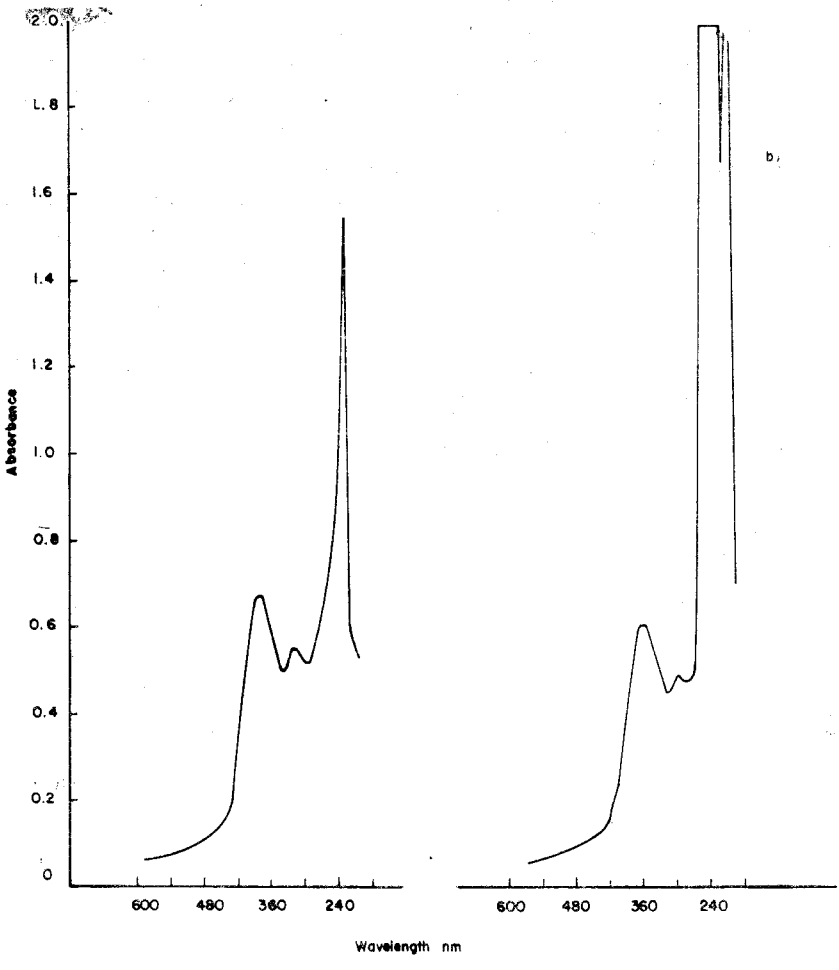
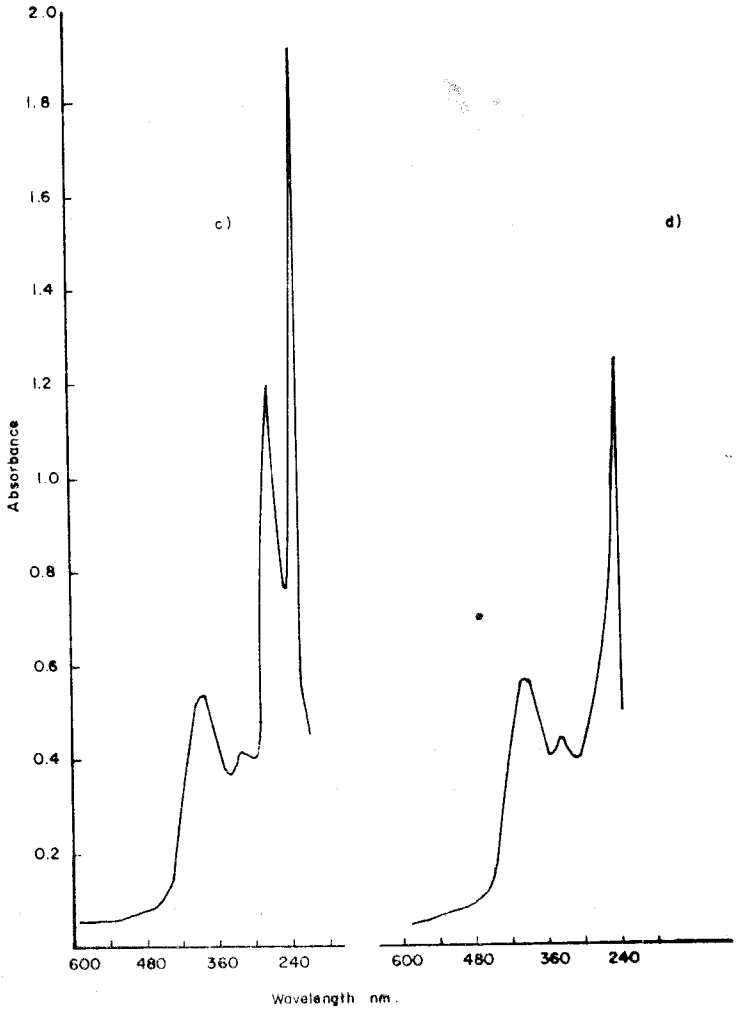


Figure 9. UV absorbance spectra of a) holoenzyme complex, b) holoenzyme + substrate,



c) holoenzyme + glycine (glycine added prior to incubation), d) holoenzyme + glycine (glycine added after incubation).

CONCLUSION

pCO_2 membrane electrode is a suitable tool to study kinetic properties. It is rapid and sensitive. Main disadvantages are a) when it is used above room temperature, the hydrophobic air gap membrane

becomes wet throughout due to the evaporation of water and its average life shortents; b) temperature difference between sample and internal electrolite solution causes drift and water transport across the membrane. Therefore, the membrane and the internal electrolite solution should be changed periodically to be able to get reproducible results..

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