PORPHYRIN BIOSYNTHESIS IN
RHODOPSEUDOMonas PALUSTRis—IX.
PBG-DEAMINASE. KINETIC STUDIES

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Abstract—1. PBG-Deaminase obtained from Rp. palustris exhibited classical Michaelis-Menten kinetics
 in the absence or presence of different ions.
2. Detailed kinetic studies were carried out in the presence of ammonium, phosphate and magnesium
 ions.
3. It has been found that the different effects observed are dependent on both the substrate and the
 ion concentration.

INTRODUCTION

The conversion of porphobilinogen (PBG) into uro-
porphyrinogen III is enzymically catalysed by the
porphobilinogenase system (PBG-ase) (Lockwood
and Rimington, 1957). The formation of uro-
porphyrinogen I from PBG is brought about by uroporphyrinogen I synthetase (URO-S, PBG-
Deaminase, EC 4.3.1.8). PBG-ase is a combination of
two separate enzymes, PBG-deaminase, a heat stable
protein and uroporphyrinogen III cosynthetase
(isomerase, EC 4.2.1.75), a heat labile protein.

PBG-Deaminase has been purified from several
sources and the properties of the protein have been
described (Jordan and Sbemin, 1973; Higuchi and
Bogorad, 1975; Miyagi et al., 1979; Rossetti
et al., 1980; Anderson and Desnick, 1980; Williams et al.,
1981; Battersby et al., 1983; Hart et al., 1984; Will-
liams, 1984; Fumagalli et al., 1985).

PBG-Deaminase from Rhodopseudomonas palu-
stris, the most common non sulfur bacteria, has also
been purified by Kotler et al. (1986). The present
work describes kinetic studies conducted on PBG-
Deaminase in the absence and presence of different
ions.

MATERIALS AND METHODS

Porphobilinogen was biosynthetically obtained (Sanco-
vich et al., 1970) and estimated as described by Moore and
Labbe (1964).

The standard incubation system contained the enzyme
preparation (50 pl) together with 0.05 M sodium phosphate
buffer (pH 7.6-7.8) and PBG (at the concentrations indi-
cated) with or without the addition of other reagents, in a
final volume of 1.5 ml. Incubations were carried out aero-
bically in the dark with mechanical shaking at 37°C for
30 min.

Blanks were always run with PBG and without enzyme.

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Dedicated to Professor Claude Rimington FRS on the
occasion of his 85th birthday.

After incubation, TCA was added to precipitate the protein
(final concentration 5% w/v), the mixture was then exposed
to white light for 20 min to oxidize porphyrinogens, the
protein precipitate filtered off and total porphyrins and
remaining PBG estimated in the resulting solution (Rim-
ington, 1960; Moore and Labbe, 1964).

In all experiments reaction velocity was measured on the
basis of Uroporphyrinogen formation and substrate con-
sumption during 30 min or 1 hr.

The purification procedure for PBG-Deaminase, as well
as all other methods and materials not specified here, were
those described by Kotler et al. (1986).

RESULTS

Saturation curves (Fig. 1)

Plots of velocity measured as nmol of uro-
porphyrinogen formed against PBG concentration
and their reciprocals followed classical Michaelis-Menten kinetics.

When velocity was measured in terms of PBG
consumed, the saturation curve was linear and the
double reciprocal plots also showed a normal kinetic
pattern.

Michaelis constants and maximum velocities were
calculated from Lineweaver-Burk plots and the num-
ber of probable substrate binding sites (n) were
determined by the empirical Hill equation (Fig. 1,
inset).

In Euglena gracilis Williams et al. (1981) demon-
strated that PBG-deaminase combined four mole-
cules of substrate to produce one molecule of uro-
porphyrinogen I with the elimination of four
molecules of ammonia, this stoichiometry was ob-
served at different pH values (range 5.5–8.5).

In our laboratory, working with PBG-ase from
soya-bean callus (Llambias and Batlle, 1971), Eu-
glena gracilis (Rossetti, 1978) and Rp. palustris (Juk-
nat, 1983) we have shown that uroporphyrinogen
formation based on substrate consumption was ex-
tremely low, indicating a great deviation from sto-
ichiometric values. When PBG-deaminase from Rp.
palustris was studied, an excess of PBG consumption
of 5 times at low substrate concentration (8–26 μM) was found, increasing this value up to 50 times at 350 μM PBG (Table 1). Battersby et al. (1983a) reported an inhibitory effect of hydroxymethylbilane on PBG-Deaminase activity from *E. gracilis*, a fact that could be an explanation for these findings.

**Effect of magnesium ions**

The effect of varying Mg²⁺ ion concentrations on PBG-Deaminase activity was studied at different PBG concentrations. Plots of rate of urogen I formation against PBG concentration, both in the absence and the presence of Mg²⁺ ions up to 25 mM, showed classical Michaelis–Menten kinetic and the reciprocal plots were linear (Fig. 2). Mg²⁺ ions at concentrations between 5 and 25 mM behaved as a non-competitive inhibitor. The Michaelis constant, $K_m = 45$ μM (Inset, Fig. 2), was found to be essentially dependent of Mg²⁺ concentration, increasing the affinity for PBG. At the same time there was a decrease in $V_{max}$ tending to 2 nmol/hr. Inhibition of PBG-Deaminase activity by high substrate concentration at 50 and 100 mM Mg²⁺ was also found.

It has also been observed that at 25, 50 and 100 μM PBG, low concentration of Mg²⁺ activated the enzyme (Fig. 3). Increasing Mg²⁺ concentrations however produced a great inhibition, reaching 100% for 100 mM Mg²⁺ and 500 μM PBG.

Mg²⁺ ions had no effect on PBG consumption.

**Effect of phosphate ions**

Activity plots of PBG-deaminase vs PBG concentration at different PO₄³⁻ concentrations, showed an hyperbolic pattern. Reciprocal plots (Fig. 4) and Eadie curves were also linear. From the reciprocal plots, it can be seen that at 1 and 10 mM, phosphate behaved as an non-competitive inhibitor. When velocity of PBG-deaminase against PO₄³⁻ concentrations was plotted, typical inhibition curves were obtained (Fig. 5).

### Table 1. Stoichiometry of PBG-deaminase reaction

| µg | pmol | μM | µg | pmol | Theoretic | Real | R² |
|----|------|----|----|------|-----------|------|----|
| 3  | 13.27| 8.85| 2.55| 11.28| 2.82      | 0.58 | 4.86|
| 9  | 39.82| 26.55| 4.96| 21.95| 5.49      | 1.08 | 5.08|
| 15 | 66.37| 44.25| 11.53| 51.02| 12.76     | 1.27 | 10.05|
| 30 | 132.72| 88.50| 17.55| 77.65| 19.41     | 1.54 | 12.60|
| 45 | 199.12| 132.74| 29.34| 129.82| 32.45    | 1.80 | 18.03|
| 60 | 265.49| 177.00| 37.02| 163.81| 40.95    | 1.87 | 21.90|
| 90 | 398.23| 260.49| 65.47| 289.69| 72.42    | 2.04 | 35.50|
| 120| 530.97| 353.98| 92.35| 408.63| 102.16   | 2.00 | 31.08|

The experiments were performed as described in the text.

*Calculated on the basis of the PBG uptake.

*R²: nmol theoretical porphyrins/nmol porphyrins really formed.
Porphyrin biosynthesis in *Rp. palustris*

Fig. 3. Effect of different concentrations of Mg$^{2+}$ on PBG-deaminase activity, measured in terms of porphyrin formation, at varying concentrations of PBG: (O) 25 μM; (●) 50 μM; (□) 100 μM; (■) 250 μM; (△) 500 μM. Activity of a control without Mg$^{2+}$ and measured under the standard incubation conditions was taken as 0 level so changes stimulating or inhibiting were referred to this value as such.

Experimental conditions are indicated in the text.

It is interesting to note that if PBG consumption was taken as a measure of enzyme activity, velocity plots of PBG-deaminase against PBG concentration showed normal kinetic pattern at all PO$_4^{3-}$ concentrations studied (Fig. 6). It was also found that in this case PO$_4^{3-}$ acted as a non-competitive inhibitor.

**Effect of ammonium ions**

Direct and reciprocal plots of reaction velocity against PBG concentration, both in the absence and the presence of NH$_4^+$ ions, showed classical Michaelis-Menten kinetics (Fig. 7). Eadie plots were also linear.

Ammonium ion is a non-competitive inhibitor of porphyrin synthesis, resulting in lower values of $K_m$ and $V_{max}$. These results are in agreement with Sancovich et al. (1969) and Llambias and Batlle (1971b).

It was found that ammonium at concentrations up to 100 mM inhibited 57% urogen I formation at 310 μM PBG (Fig. 8).

However, it is interesting to add that NH$_4^+$ at concentrations that inhibited porphyrin biosynthesis, had no effect upon the rate of PBG consumption.

**DISCUSSION AND CONCLUSIONS**

The kinetic experiments showed in this report demonstrated that PBG-deaminase from *Rp. palustris* had a Michaelis kinetic behavior (Fig. 1).

The action of Mg$^{2+}$ on PBG-deaminase activity showed that this metal had two different effects, acting as activator or as a non-competitive inhibitor at low and high concentrations respectively. The existence and extent of these effects were found to be dependent on PBG concentration (Fig. 3).

It must also be noted here that Mg$^{2+}$ ions did not change normal kinetic pattern.

Studying the effect of Mg$^{2+}$ on PBG-ase activity of the same source it was observed that 50 mM Mg$^{2+}$ also produced activation when 24 μM PBG was

![Fig. 4. Double reciprocal plots of velocity against PBG concentration without additions (O) and in the presence of different concentrations of PO$_4^{3-}$: (●) 1 mM; (□) 10 mM; (■) 25 mM; (△) 50 mM. Experimental conditions are indicated in the text.](image-url)

![Fig. 5. Effect of different concentrations of PO$_4^{3-}$ on the reversal of PBG-deaminase activity measured in terms of porphyrin formation, at varying concentrations of PBG: (O) 51 μM; (●) 102 μM; (□) 205 μM and (■) 310 μM. Experimental conditions are indicated in the text.](image-url)
employed as substrate concentration (Juknat et al., 1986).

The activating effect of certain concentrations of Mg$^{2+}$ could be explained as if they were producing some association-dissociation phenomena, which gives the enzyme a definitive structural arrangement necessary for maximal activity (Batlle and Rossetti, 1977).

We proposed that magnesium could act by binding at different sites. At low concentration of Mg$^{2+}$, this ion will act producing the optimum structural arrangement, as already suggested. By increasing its concentration, Mg$^{2+}$ could inhibit blocking the tetrahydropyrole liberation on the PBG-deaminase.

Studies carried out on PBG-deaminase in the presence of phosphate ions, showed that PO$_4^{3-}$ was acting as an inhibitor of velocity in terms of porphyrin synthesis and PBG consumption, without changes in the kinetic pattern.

It was suggested (Batlle and Rossetti, 1977; Pollack and Russell, 1978) that certain groups (e-amines, imidazoles or guanidines) would be involved in the binding of PBG to PBG-deaminase. It is possible that phosphate could act partially or totally neutralizing these recognition sites. So, complexes like E-PBG-I and E-I between the enzyme (E), the substrate (PBG) and the inhibitor (I) could be formed. The non-competitive type of inhibition found in these experiments, could be explained by the formation of this kind of complex.

Taking into account these hypothesis, once produced these complexes, the polypyrrol chain could not reach the length of a tetrapyrrol. So, the PBG consumption diminished, without being reversed by increasing substrate concentration. As a consequence, the synthesis of porphyrins will also be inhibited.

Increasing PO$_4^{3-}$ concentration, a change in the type of inhibition for porphyrin synthesis can be observed. This effect may be due to the existence of an interaction phenomena between PO$_4^{3-}$ and porphyrins, which could modify negatively the rate of porphyrin liberation.

Results obtained studying the kinetics of PBG-deaminase in the presence and the absence of ammonium ions, showed classical Michaelis–Menten behaviour and $n$ was near 1. We also found that PBG consumption was not affected, while porphyrin synthesis was inhibited by ammonium ions. Taking into account these results, we suggested that ammonium has no binding site on the PBG-deaminase but produces a decrease in the amount of available tetrapyrroles. Basic derivates could be formed by direct reaction of NH$_4^+$ with di and tripyrrylmethanes (Davies and Neuberger, 1973). So, polypyrrole intermediates will be liberated and the tetrapyrrylmethane (TPM) concentration on the PBG-Deaminase will be in turn reduced.

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