University of Groningen

Kinetic mechanism of phenylacetone monooxygenase from *Thermobifida fusca*

Torres Pazmiño, Daniel E.; Baas, Bert-Jan; Janssen, Dick B.; Fraaije, Marco W.; Gonzalo, Gonzalo de; Ottolina, Gianluca; Carrea, Giacomo

Published in:
Biochemistry

DOI:
[10.1021/bi702296k](https://doi.org/10.1021/bi702296k)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Torres Pazmiño, D. E., Baas, B.-J., Janssen, D. B., Fraaije, M. W., Gonzalo, G. D., Ottolina, G., & Carrea, G. (2008). Kinetic mechanism of phenylacetone monooxygenase from *Thermobifida fusca*. *Biochemistry*, 47(13), 4082-4093. <https://doi.org/10.1021/bi702296k>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

The kinetic mechanism of phenylacetone monooxygenase from *Thermobifida fusca*[†]

Daniel E. Torres Pazmiño, Bert-Jan Baas, Dick B. Janssen, Marco W. Fraaije*

Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute

University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

[†] We thank CERC3 for funding. COST D25/0005/03 is gratefully acknowledged.

* To whom correspondence should be addressed. Phone: (31) 50-3634345; fax: (31) 50-3634165; email: M.W.Fraaije@rug.nl.

Running title: Kinetic mechanism of PAMO

Reduction of PAMO using (R)-NADPD

The figures below show the data concerning the reductive half-reaction of PAMO with (R)-NADPD.

Figure 1. Diode array analysis

Figure 1. (A) Some selected flavin absorbance spectra observed during anaerobic reduction of 8 μ M wild-type PAMO by 50 μ M NADPD (50 mM Tris-HCl, pH 7.5 at 25 °C). Of all 1600 recorded spectra, only spectra recorded at the indicated times are shown. (B) Spectra of the deconvoluted enzyme species. By means of numerical integration (Pro-K software, Applied Photophysics Ltd.), the spectra were fitted using a two-step kinetic model ($A \rightleftharpoons B \rightarrow C$).

Figure 2. Reduction of PAMO by NADPD monitored by coenzyme fluorescence

Figure 2. Kinetic analysis of the fluorescence traces obtained after anaerobic mixing of wild-type PAMO and (R)-NADPD (50 mM Tris-HCl, pH 7.5 at 25 °C). Figure 2A shows the observed fluorescence trace upon mixing 0.12 μ M PAMO with 1.13 μ M NADPD. The solid line shows the fit using a double exponential equation, yielding k_{obs1} and k_{obs2} . Figure 2B: the solid dots (●) and the solid line (—) show the observed rates (k_{obs}) of the first step at various $[\text{NADPH}]$ and the corresponding linear fit ($k_{off} = 35 \text{ s}^{-1}$, $k_{on} = 47 \mu\text{M}^{-1} \cdot \text{s}^{-1}$). The open dots (○) and the dashed line (--) show the observed rates of the second step at various $[\text{NADPH}]$ and the corresponding fit. The maximum rate (k_{red}) was determined to be 2.0 s^{-1} .

Equations used according to the determinant method

We used the determinant method (1) for obtaining the steady-state rate equations of the proposed mechanism (Scheme 1). From these rate equations the steady-state kinetic parameters were calculated.

Scheme 1. Proposed kinetic scheme of phenylacetone monooxygenase (PAMO). The Baeyer-Villiger substrate and product are referred to as PA (phenylacetone) and BA (benzylacetate)

To solve the steady-state rate equation for the proposed mechanism, the steady-state concentrations of all enzyme species need to be solved. Because of the rapid equilibria, the following enzyme species are lumped;

(i) E_{ox} , $E_{ox}-NADPH$ and $E_{ox}-NADP^+$ = $E_{ox, total}$, and

(ii) $E_{per}-NADP^+$ and $E_{per}-NADP^+-PA$ = $E_{per, total}$.

This yields:

$$\frac{d(E_{\text{ox,total}})}{dt} = -k_{\text{r-red}} - \frac{[E_{\text{ox,total}}] \cdot k_{\text{red}} \cdot [\text{NADPH}]}{K_{\text{d,app}} + [\text{NADPH}]} + [E_{\text{ox}} - \text{NADP}^+] \cdot k_{\text{c}},$$

$$\text{in which } K_{\text{d,app}} = K_{\text{d,NADPH}} \left(1 + \frac{[\text{NADP}^+]}{K_{\text{d,NADP}^+}} \right),$$

$$\text{and } \frac{d(E_{\text{per,total}})}{dt} = -\frac{[E_{\text{per,total}}] \cdot k_{\text{BV}} \cdot [\text{PA}]}{K_{\text{d,PA}} + [\text{PA}]} + [E_{\text{red}} - \text{NADP}^+] \cdot k_{\text{ox}} \cdot [\text{O}_2]$$

Concentrations of the various enzyme species can be obtained from the following matrix:

	$[E_{\text{ox, total}}]$	$[E_{\text{red}} - \text{NADP}^+]$	$[E_{\text{per, total}}]$	$[E'_{\text{ox}} - \text{NADP}^+]$
$\frac{d[E_{\text{ox,total}}]}{dt}$	$-k_{\text{r-red}} - \frac{k_{\text{red}}[\text{NADPH}]}{K_{\text{d,app}} + [\text{NADPH}]}$	0	0	k_{c}
$\frac{d[E_{\text{red}} - \text{NADP}^+]}{dt}$	$k_{\text{r-red}} + \frac{k_{\text{red}}[\text{NADPH}]}{K_{\text{d,app}} + [\text{NADPH}]}$	$-k_{\text{ox}}[\text{O}_2]$	0	0
$\frac{d[E_{\text{per, total}}]}{dt}$	0	$k_{\text{ox}}[\text{O}_2]$	$-\frac{k_{\text{BV}}[\text{PA}]}{K_{\text{d,PA}} + [\text{PA}]}$	0
$\frac{d[E'_{\text{ox}} - \text{NADP}^+]}{dt}$	0	0	$\frac{k_{\text{BV}}[\text{PA}]}{K_{\text{d,PA}} + [\text{PA}]}$	$-k_{\text{c}}$

From this matrix the concentration for $E_{\text{ox, total}}$ is obtained by deleting the first row and first column and then calculating the determinant of the remaining smaller matrix. In a similar way the concentrations of the remaining enzyme species can be obtained. These values can then be used to write down the steady-state rate equation in the form of a Michaelis-Menten equation:

$$V = \frac{k_{\text{c}}[E'_{\text{ox}} - \text{NADP}^+]}{[E_{\text{ox,total}}] + [E_{\text{red}} - \text{NADP}^+] + [E_{\text{per,total}}] + [E'_{\text{ox}} - \text{NADP}^+]}$$

$$V = \frac{k_{ox}k_{BV}k_c[O_2][PA]}{(k_{BV}k_c[PA] + k_{ox}k_{BV}[O_2][PA] + k_{ox}k_c[O_2](K_{d,PA} + [PA])) + \frac{k_{ox}k_{BV}k_c[O_2][PA](K_{d,app} + [NADPH])}{k_{red}[NADPH]}}$$

From this rate equation the kinetic parameters k_{cat} , $K_{M,NADPH}$, $K_{M,oxygen}$ and $K_{M,PA}$ can be derived. As no $NADP^+$ is initially present, $K_{d,app}$ can be simply replaced by $K_{d, NADPH}$. Under saturating conditions ($[NADPH] > K_{d, NADPH}$ and $[PA] > K_{d, PA}$), these kinetic parameters are:

$$k_{cat} = \frac{k_{red}k_{BV}k_c k_{ox}[O_2]}{(k_{BV}k_c + k_{red}k_c + k_{red}k_{BV})k_{ox}[O_2] + k_{red}k_{BV}k_c}$$

$$K_{M,NADPH} = \frac{k_{BV}k_c K_{d,NADPH} k_{ox}[O_2]}{(k_{BV}k_c + k_{red}k_c + k_{red}k_{BV})k_{ox}[O_2] + k_{red}k_{BV}k_c}$$

$$K_{M,O_2} = \frac{k_{red}k_{BV}k_c}{k_{BV}k_c k_{ox} + k_{red}k_c k_{ox} + k_{red}k_{BV}k_{ox}}$$

$$K_{M,PA} = \frac{k_{red}k_c K_{d,PA} k_{ox}[O_2]}{(k_{BV}k_c + k_{red}k_c + k_{red}k_{BV})k_{ox}[O_2] + k_{red}k_{BV}k_c}$$

These equations could be simplified by calculating the catalytic efficiency of the enzyme for substrates NADPH, oxygen and phenylacetone:

$$\frac{k_{cat}}{K_{M,NADPH}} = \frac{k_{red}k_{on}}{k_{off}}$$

$$\frac{k_{cat}}{K_{M,O_2}} = \frac{k_{ox}^2 (k_c k_{red} + k_{BV}k_c + k_{BV}k_{red})[O_2]}{k_{BV}k_c k_{red} + k_{ox} (k_c k_{red} + k_{BV}k_c + k_{BV}k_{red})[O_2]}$$

$$\frac{k_{cat}}{K_{M,PA}} = \frac{k_{BV}}{K_{d,PA}}$$

Supporting Information References

1. Huang, C. Y. (1983) Derivation of initial velocity and isotope exchange rate equations, in *Contemporary Enzyme Kinetics and Mechanism* (Purich, D., Ed.) 2nd ed., pp 54-84, Academic Press, Inc., New York.