



SHARPENS YOUR THINKING

## **Positively charged amino acids are essential for electron transfer and protein-protein interactions in the soluble methane monooxygenase complex from methylococcus capsulatus (Bath)**

BALENDRA, S., LESIEUR, C., SMITH, T. J. and DALTON, H.

Available from Sheffield Hallam University Research Archive (SHURA) at:

<http://shura.shu.ac.uk/368/>

---

This document is the author deposited version. You are advised to consult the publisher's version if you wish to cite from it.

### **Published version**

BALENDRA, S., LESIEUR, C., SMITH, T. J. and DALTON, H. (2001). Positively charged amino acids are essential for electron transfer and protein-protein interactions in the soluble methane monooxygenase complex from methylococcus capsulatus (Bath). *Biochemistry*, 41 (8), 2571-2579.

---

### **Repository use policy**

Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in SHURA to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

**Table 1. Effect of cross-linking and covalent modification reagents on activity of the hydroxylase.**

Reagent	Assay	Specific activity (nmol min <sup>-1</sup> [mg of hydroxylase] <sup>-1</sup> )
None	Whole complex	220 ± 8
	Peroxide shunt	98 ± 2
BS <sup>3</sup> (cross-linker)	Whole complex	0
	Peroxide shunt	97 ± 4
Sulfo-NHS-acetate (primary amine neutralizer)	Whole complex	0
	Peroxide shunt	93 ± 5
<i>p</i> -hydroxyphenylglyoxal (arginine modifier)	Whole-complex	0
	Peroxide shunt	97 ± 4

**Table 2. Effect of covalent modification of the hydroxylase on NADH oxidation activity.**

Assay components	Rate of NADH oxidation (nmol min <sup>-1</sup> [mg of hydroxylase] <sup>-1</sup> ).
Hydroxylase	0
Reductase	1.93
Hydroxylase + reductase	24.1
Hydroxylase + reductase + protein B	20.3
Hydroxylase + reductase + protein B + propene	29.4
Primary amine-blocked hydroxylase + reductase	1.44
Primary amine-blocked hydroxylase + reductase + protein B	2.41
Primary amine-blocked hydroxylase + reductase + protein B + propene	1.76
Arginine-blocked hydroxylase + reductase + protein B	6.59

**Table 3. Effect of covalent modification of the hydroxylase on inhibition of the peroxide shunt reaction by protein B.** Specific activity was measured at 1 mg.mL<sup>-1</sup> of hydroxylase and expressed in nmol of epoxypropane formed min<sup>-1</sup>.(mg of hydroxylase)<sup>-1</sup>.

Protein B <sup>a</sup>	Specific activity via the peroxide shunt (nmol min <sup>-1</sup> [mg of hydroxylase] <sup>-1</sup> )		
	Native hydroxylase	Primary-amine neutralised hydroxylase	Arginine modified hydroxylase
0	98 ± 2	93 ± 5	97 ± 4
5	30 ± 3	91 ± 7	93 ± 4

<sup>a</sup> Concentration of protein B expressed as moles per mole of hydroxylase αβγ monomer