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

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FIGURE LEGENDS

Fig. 1. Cross-linking and neutralisation of primary amino groups associated with the hydroxylase. The hydroxylase was reacted with the cross-linker (BS$_3^3$) or the lysine-neutralising reagent (sulfo-NHS-acetate) and excess reagent was removed by ultrafiltration. Samples from the cross-linking experiment were analysed by means of (A) SDS-7.5% PAGE and (B) nondenaturing, native PAGE. Samples from the lysine neutralisation experiment were analysed by using nondenaturing PAGE (C). Samples analysed before measurement of propene oxygenation activity: lanes 1 and 2, unreacted and reacted hydroxylase, respectively. Samples analysed after measurement of propene oxygenation activity in the sMMO complex: lane 3, unreacted hydroxylase; lane 4, control in which the probe was removed by ultrafiltration before addition of the hydroxylase; lane 5, reacted hydroxylase. Samples analysed after measurement of propene oxygenation activity via the peroxide shunt: lanes 6 and 7, unreacted and reacted hydroxylase, respectively. The proteins were visualised with Coomassie blue; molecular masses of standards in lane M of part A are indicated in kDa. The cross-linked species observed by means of SDS-PAGE (A) have been tentatively assigned based on the known molecular masses of the $\alpha$, $\beta$ and $\gamma$ subunits of the hydroxylase.

Fig. 2. Surface plasmon resonance analysis of the binding between the GST-protein B fusion protein and (A) native, (B) primary amine-modified and (C) arginine-modified hydroxylase. The points at which the hydroxylase was added to and removed from the buffer flowing across the sensor chip are indicated.
Fig. 3. Distribution of charged groups on the surface of the hydroxylase, based on the X-ray crystal structure (9). Nitrogen atoms of primary amino and arginyl side-chain guanidinium groups are shown in dark and light blue, respectively. Side-chain carboxylate oxygen atoms are shown in red. The uncharged parts of helices αE and αF of the α subunit, proposed to form the hydrophobic binding site for protein B, are coloured light green.
Fig. 1.

A

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<th>2</th>
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<th>4</th>
<th>5</th>
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<td>β (40 kDa)</td>
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</table>

B

1  2  3  4  5  6  7

native hydroxylase

cross-linked hydroxylase

C

1  2  3  4  5  6  7

native hydroxylase

modified hydroxylase

Before enzyme assay  After whole-complex assay  After peroxide shunt assay
Fig. 2.

A

Native hydroxylase added

Native hydroxylase removed

B

Amine-blocked hydroxylase added

Amine-blocked hydroxylase removed

C

Arginine-blocked hydroxylase added

Arginine-blocked hydroxylase removed
Fig. 3.