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) 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 meaurement 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 α, β and γ 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.

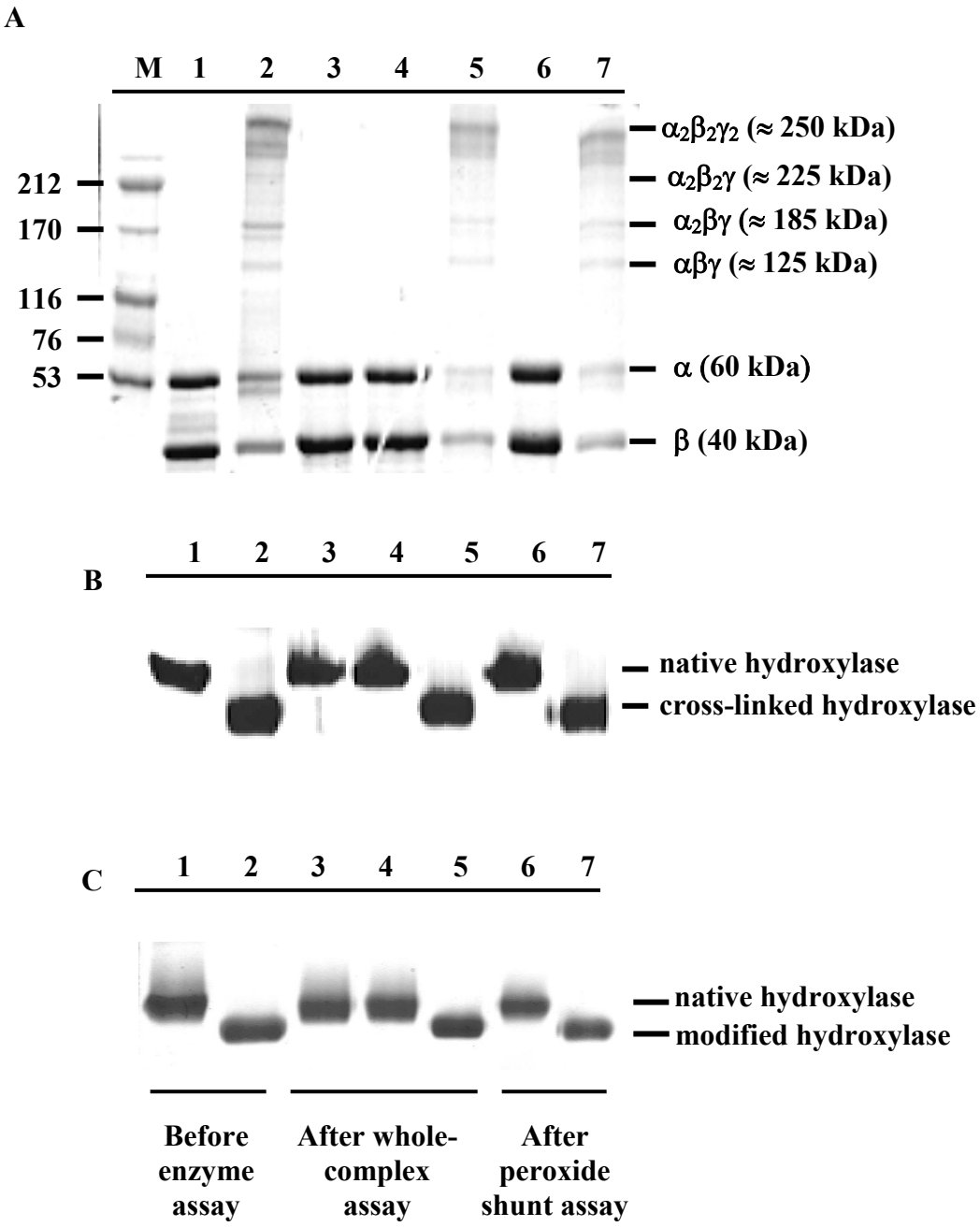
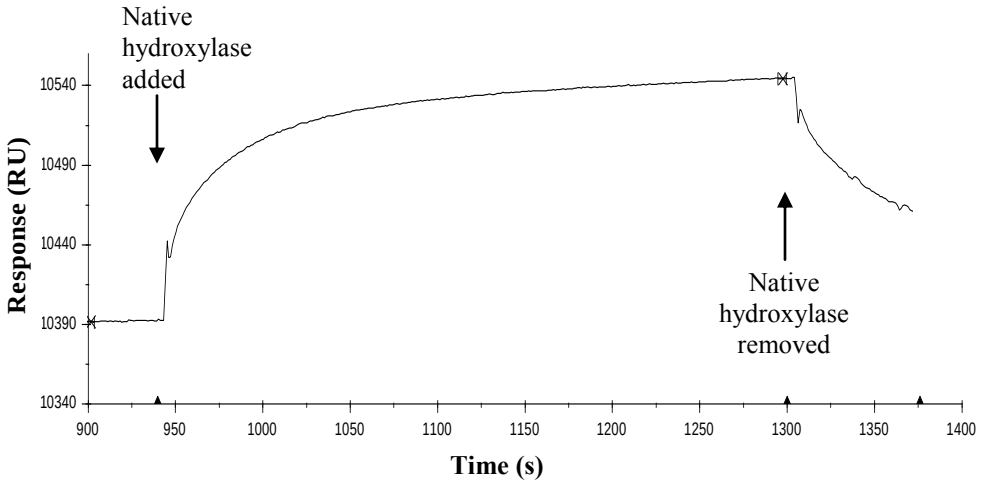
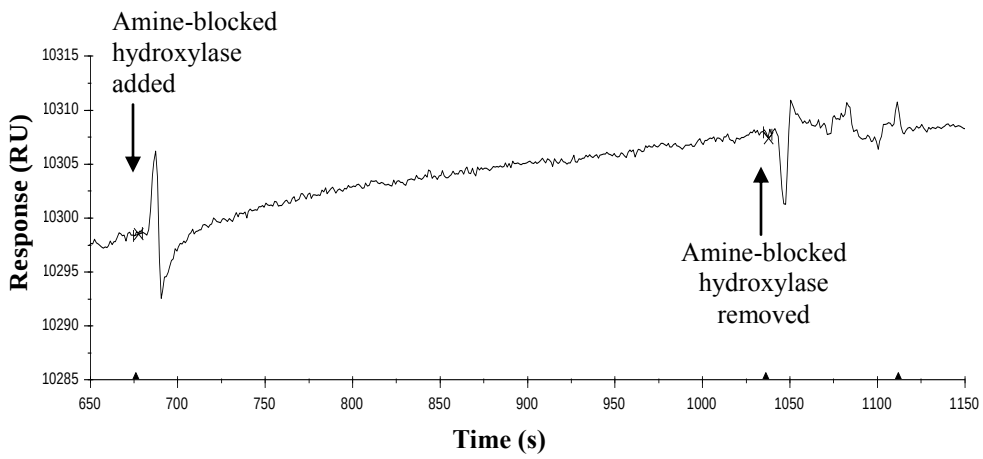

A
M 1 2 3 4 5 6 7
212
170
116
76
53
α2β2γ2 (≈ 250 kDa)
α2β2γ (≈ 225 kDa)
α2βγ (≈ 185 kDa)
αβγ (≈ 125 kDa)
α (60 kDa)
β (40 kDa)
B
1 2 3 4 5 6 7
native hydroxylase
cross-linked hydroxylase
C
1 2 3 4 5 6 7
native hydroxylase
modified hydroxylase
Before enzyme assay
After whole-complex assay
After peroxide shunt assay

Fig. 2.

A


Native
hydroxylase
added
Native
hydroxylase
removed
Response (RU)
Time (s)
10340
10390
10440
10490
10540
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400

B


Amine-blocked
hydroxylase
added
Amine-blocked
hydroxylase
removed
Response (RU)
Time (s)
10285
10290
10295
10300
10305
10310
10315
650
700
750
800
850
900
950
1000
1050
1100
1150

C

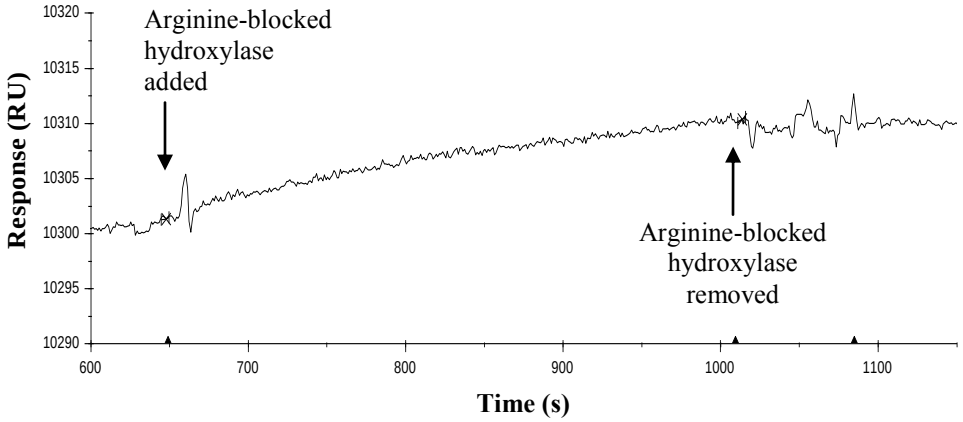

Arginine-blocked
hydroxylase
added
Arginine-blocked
hydroxylase
removed
Response (RU)
Time (s)
10290
10295
10300
10305
10310
10315
10320
600
700
800
900
1000
1100

Fig. 3.