BJ www.biochemj.org

Biochem. J. (2011) **433**, 65–74 (Printed in Great Britain) doi:10.1042/BJ20101361

SUPPLEMENTARY ONLINE DATA

Phosphorylation of bacterial-type phosphoenolpyruvate carboxylase at Ser^{425} provides a further tier of enzyme control in developing castor oil seeds

Brendan O'LEARY*, Srinath K. RAO* and William C. PLAXTON*†[1]

*Department of Biology, Queen's University, Kingston, ON, Canada K7L 3N6, and †Department of Biochemistry, Queen's University, Kingston, ON, Canada K7L 3N6

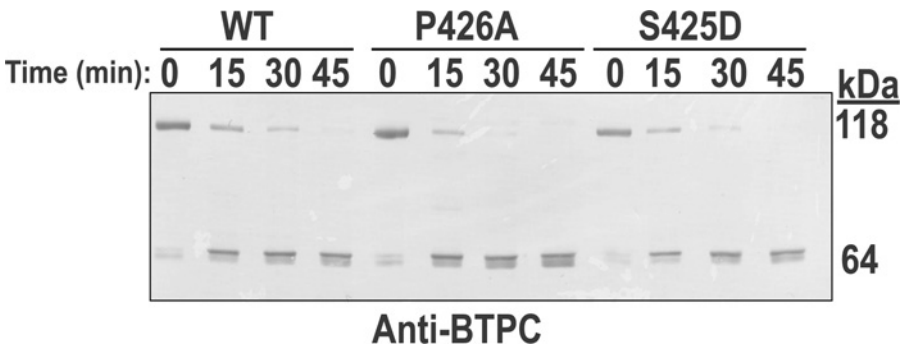

Figure S1 Sensitivity of recombinant COS BTPC mutants towards endogenous COS proteases

Purified recombinant Class-2 PEPCs (15 μg) containing wild-type PTPC (AtPPC3) and wild-type and mutant forms of COS BTPC (RcPPC4) were incubated at 30 °C with 50 μl of clarified stage VII COS endosperm extract (containing approx. 1 mg of protein) to observe the rate and extent of BTPC proteolysis. Aliquots were removed at various time points, boiled in SDS sample buffer, and subjected to SDS/PAGE followed by immunoblotting with anti-BTPC antibody. Each lane contained approx. 70 ng of BTPC.

Table S1 Primers used for site-directed mutagenesis

The base pair alterations leading to the missense mutation are highlighted in **bold**. Base pair alterations that introduced a new restriction site are underlined.

Target	Sequence	New site
AtPpc3 R644A	Forward: 5′-GTGGTGG**T**AC**C**GTCGGA**GC**AGGAGGTGGTCCTACTCATC-3′ Reverse: 5′-GATGAGTAGGACCACCTCCT**GC**TCCGAC**GGTA**CCACCAC-3′	KpnI
RcPpc4 S425D	Forward: 5′-GCTAATTCTAGTGGA**GA**TCCGCGGGCATCTTTC-3′ Reverse: 5′- GAAAGATGCCCGCGGA**TC**TCCACTAGAATTAGC-3′	None
RcPpc4 S425A	Forward: 5′-GCTAATTCTTCTGGAGCTCCTCGAGCATC-3′ Reverse: 5′-GATGCTCGAGGAGCTCCAGAAGAATTAGC-3′	None
RcPpc4 P426A	Forward: 5′-CTAATTCTAGTGGATCT**G**CGCGGGCATCTTTCAG-3′ Reverse: 5′-CTGAAAGATGCCCGCG**C**AGATCCACTAGAATTAG-3′	None
RcPpc4 R760A	Forward: 5′-CGTGGAGG**ATCC**ATTGGT**GC**TGGTGGTGGCCCCACATA-3′ Reverse: 5′-TATGTGGGGCCACCACCAGCACCAAT**GGATC**CTCCACG-3′	BamHI

[1] To whom correspondence should be addressed (email plaxton@queensu.ca).

Table S2 Purification of recombinant Class-2 PEPC from combined extracts originating from 5 g of AtPPC3_R644A- and 10 g of RcPPC4-expressing *E. coli*

Step	Activity (units)	Protein (mg)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Combined extracts	50	3000	0.016	1	100
Ni^{2+}-affinity FPLC	43	162	0.27	16	86
Superdex-200 FPLC	29	6.5	4.5	281	58
Superose-6 FPLC	12	1.8	6.8	425	24

Table S3 Purification of recombinant Class-2 PEPC from combined extracts originating from 5 g of AtPPC3- and 11 g of RcPPC4_R670A-expressing *E. coli*

Step	Activity (units)	Protein (mg)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Combined extracts	71	2196	0.033	1	100
Ni^{2+}-affinity FPLC	70	26.5	2.6	81	98
Superdex-200 FPLC	34	8.9	3.9	117	49
Superose-6 FPLC	7.6	1.6	4.8	145	11

Table S4 Purification of recombinant Class-2 PEPC from combined extracts originating from 5 g of AtPPC3_R644A- and 15 g of RcPPC4_S425D-expressing *E. coli*

Step	Activity (units)	Protein (mg)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Combined extracts	172	2198	0.079	1	100
Ni^{2+}-affinity FPLC	130	80	1.6	20	75
Superdex-200 FPLC	52	9.6	5.5	70	31
Superose-6 FPLC	40	4.2	9.5	120	25

Table S5 Purification of recombinant Class-2 PEPC from combined extracts originating from 5 g of AtPPC3_R644A- and 15 g of RcPPC4_S425A-expressing *E. coli*

Step	Activity (units)	Protein (mg)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Combined extracts	37	4800	0.008	1	100
Ni^{2+}-affinity FPLC	4.3	21.2	0.2	27	12
Superose-6 FPLC*	3.7	0.8	4.6	575	10

*Owing to low total activity, a Superose-6 16/50 prep grade column was used in place of the two gel filtration columns that were normally used.

Table S6 Purification of recombinant Class-2 PEPC from combined extracts originating from 5 g of AtPPC3_R644A- and 15 g of RcPPC4_P426A-expressing *E. coli*

Step	Activity (units)	Protein (mg)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Combined extracts	114	2850	0.04	1	100
Ni^{2+}-affinity FPLC	84	88	0.95	24	74
Superdex-200 FPLC	38	8.1	4.7	118	34
Superose-6 FPLC	11	1.4	8.4	210	10

Received 24 August 2010/12 October 2010; accepted 15 October 2010
Published as BJ Immediate Publication 15 October 2010, doi:10.1042/BJ20101361