SUPPLEMENTARY ONLINE DATA

In vivo regulatory phosphorylation of the phosphoenolpyruvate carboxylase AtPPC1 in phosphate-starved Arabidopsis thaliana

Allison L. GREGORY*1, Brenden A. HURLEY†, Hue T. TRAN†, Alexander J. VALENTINE‡, Yi-Min SHE§, Vicki L. KNOWLES† and William C. PLAXTON*†3

*Department of Biochemistry, Queen’s University, Kingston, ON, Canada, K7L 3N6, †Department of Biology, Queen’s University, Kingston, ON, Canada, K7L 3N6, ‡Plant Metabolomics Group, Department of Biotechnology, Faculty of Science, University of the Western Cape, Bellville 7535, South Africa, and §Department of Chemistry, Queen’s University, Kingston, ON, Canada, K7L 3N6

Figure S1 Anti-pSer-11 antibody specificity

Clarified extracts from 7-day-old -P, suspension cells (A) or roots and shoots of 21-day-old -P, seedlings (B) were incubated for 1 h with λ-phosphatase with or without protein phosphatase inhibitors as described in the Experimental section in the main article, subjected to SDS/PAGE and electroblotted on to a PVDF membrane. Immunoblots were probed with anti-pSer-11 IgG in the presence of 10 μg·ml⁻¹ of the corresponding dephosphopeptide or blocking phosphopeptide (P-peptide) [28] as indicated. AtPPC1 and RnPPC3 denote homogeneous native Class 1 PEPC preparations isolated from the -P Arabidopsis suspension cells (see Table 1 and Figure 2 in the main article) or the endosperm of developing castor-oil seeds respectively [28]. deP-peptide, dephosphopeptide; O, origin; P-peptide, phosphopeptide; TD, tracking-dye front.

1 Present address: Department of Immunology, University of Toronto, Toronto, ON, Canada, M5S 1A8.
2 Present address: Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, U.S.A.
3 To whom correspondence should be addressed (email plaxton@queensu.ca).

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Figure S2  Native molecular-mass estimation for PEPC from −P, Arabidopsis suspension cells

Native molecular-mass estimation was performed during FPLC on a calibrated Superdex-200 HR 16/60 gel-filtration column as described in the Experimental section in the main article. r², correlation coefficient.

Table S1  MALDI–QqTOF-MS identified tryptic peptides (derived from purified native PEPC from −P, Arabidopsis suspension cells) matching the deduced amino-acid sequence of AtPPC1

| Position | mz (Meas.) | [MH+1] (Calc.) | Delta | Peptide sequence |
|----------|------------|----------------|-------|-----------------|
| 734–739 | 860.431 | 860.430 | +0.001 | FVEYFR |
| 494–503 | 1129.628 | 1129.636 | −0.008 | RPLFGSDLPK |
| 153–162 | 1148.587 | 1148.583 | +0.004 | LATPELEYGR |
| 564–576 | 1267.686 | 1267.700 | −0.014 | LADLEAAPAAR |
| 553–563 | 1325.785 | 1325.794 | −0.009 | QPLRVVPLFEK |
| 482–493 | 1445.761 | 1445.774 | −0.013 | ROEWLSELGSK |
| 770–781 | 1489.783 | 1489.795 | −0.009 | DAGRLSAWQVYK |
| 782–795 | 1599.872 | 1599.879 | −0.007 | FHLPVVLFGSAIR |
| 711–725 | 1709.835 | 1709.841 | −0.006 | ALLDEMNAVTEYER |
| 725–739 | 1802.916 | 1802.922 | −0.006 | SVVFQEPFRVEYFR |
| 360–375 | 1815.965 | 1815.996 | −0.026 | SIPTTEPRVILGDVR |
| 398–412 | 1852.968 | 1852.968 | −0.004 | FTAATLHGRNNPISPK |
| 163–179 | 1878.976 | 1878.976 | 0.000 | NOTVDLVTAHPTQSVR |
| 908–923 | 1919.028 | 1919.061 | −0.033 | IRDPSYHTLRPHISK |
| 734–749 | 1989.989 | 1990.006 | −0.017 | FVEYFRLATPELEYGR |
| 163–180 | 2035.091 | 2035.104 | −0.013 | NOTVDLVTAHPTQSVR |
| 360–377 | 2059.102 | 2059.118 | −0.016 | SIPTTEPRVILGDVRD |
| 889–906 | 2159.085 | 2159.116 | −0.031 | LRDSYTTNLVCQYTLK |
| 644–665 | 2201.132 | 2201.132 | −0.035 | GGGFTHAILQDPPDINGSLR |
| 476–493 | 2262.100 | 2262.114 | −0.014 | EWSERRQEWLSELGSK |
| 97–116 | 2290.134 | 2290.164 | −0.030 | AFSHMLNLANLAEDVAYAR |
| 97–116 | 2306.159 | 2306.159 | 0.000 | AFSHMLNLANLAEDVAYAR |
| 666–685 | 2364.100 | 2364.128 | −0.028 | VTVQQGEVIEQSFGEEHLCFR |
| 690–710 | 2421.215 | 2421.249 | −0.034 | FRAATEGHMRPISPKPEWR |
| 97–117 | 2446.239 | 2446.266 | −0.027 | AFSHMLNLANLAEDVAYAR |
| 97–117 | 2462.225 | 2462.260 | −0.035 | AFSHMLNLANLAEDVAYAR |
| 207–227 | 2503.256 | 2503.278 | −0.022 | QELDEALOREMQAFRTDEIK |
| 227–249 | 2577.202 | 2577.230 | −0.028 | VPYNAPLQQSWMGDGGPVR |
| 229–250 | 2622.243 | 2622.211 | +0.032 | TPTPPQDEMAMGSSYTFEIKW |
| 711–733 | 2623.307 | 2623.333 | −0.026 | ALLDEMNAVTAEVEYRSVVFQEPR |
| 934–967 | 2903.323 | 2903.384 | −0.061 | EUELQPCTYAYPRSDTULM |
| 228–250 | 2778.299 | 2778.312 | −0.013 | RTPPTPPQDEMAMGSSYTFEIKW |
| 30–54 | 2984.489 | 2984.572 | −0.083 | LVEYDALLRFLDILQDLHGEDLR |
| 302–330 | 3626.669 | 3626.562 | +0.107 | DVCCLARMAAATMYQNIGQEDLFMSWVR |
Table S2 MALDI QqTOF MS identified Asp-N peptides (derived from purified native PEPC from −P, Arabidopsis suspension cells) matching AtPPC1

MS/MS-sequenced peptides are in bold font, with those unique to AtPPC1 italicized. Calc., calculated; meas., measured.

| Position | m/z (Meas.) | [MH+\(^{+}\)] (Calc.) | Delta | Peptide sequence |
|----------|-------------|------------------------|-------|-----------------|
| 333–339  | 830.466     | 830.447                | −0.019| DELRARA         |
| 500–508  | 1015.546    | 1015.530               | +0.016| DLPKTEEA        |
| 340–348  | 1055.581    | 1055.522               | +0.059| DEVHANSRK       |
| 470–478  | 1112.521    | 1112.500               | +0.021| DIGSYREWS       |
| 801–809  | 1125.581    | 1125.583               | +0.018| DVRLHMLG        |
| 149–158  | 1191.589    | 1191.589               | +0.032| DLKSPPEEF       |
| 512–522  | 1212.625    | 1212.625               | +0.017| DTFHAVAELPA     |
| 52–61    | 1265.637    | 1265.637               | +0.023| DIRETVQELY      |
| 138–148  | 1278.706    | 1278.694               | +0.012| DLEETFKXLV     |
| 224–234  | 1281.679    | 1281.679               | +0.011| DEIKRTPPTPQ     |
| 2–12     | 1302.719    | 1302.719               | +0.019| ANRKLKMAAI      |
| 293–301  | 1340.691    | 1340.691               | +0.018| DGPRVTPEYITR    |
| 880–890  | 1457.869    | 1457.869               | +0.015| DPKLQKLR       |
| 566–580  | 1529.832    | 1529.832               | +0.019| DLEAAPAVARLFSV |
| 13–26    | 1576.880    | 1576.880               | +0.013| DVHLQILPVGKVSE |
| 210–223  | 1647.844    | 1647.844               | +0.013| DEALKREIOQAFTRT|
| 13–27    | 1691.907    | 1691.907               | −0.019| DVHLQILPVGKVSED|
| 810–822  | 1739.836    | 1739.836               | +0.025| CMYQKHPFFRT     |
| 434–449  | 1851.048    | 1851.048               | +0.016| DFLRQVSTFGSLVRL|
| 659–713  | 2114.132    | 2114.132               | 0.000 | EHGMRPPISPKPWEAIL|
| 581–598  | 2201.080    | 2201.080               | +0.009| DwykNorinGKoEvGyS|
| 376–394  | 2262.148    | 2262.148               | +0.014| DKLHYTRERAHOLLNSGH|
| 891–909  | 2315.217    | 2315.217               | +0.015| DSYITTLNVDAYTLKIR|
| 138–158  | 2451.265    | 2451.265               | −0.007| DLEETFKXLVGLNKSPEEI|
| 479–499  | 2517.320    | 2517.320               | +0.011| EEERQDEWLLSelsGKRPLFGS|
| 167–190  | 2781.607    | 2781.607               | +0.005| DLVLAHPTDSVRSLLOKHSSIR|
| 349–372  | 2836.283    | 2836.283               | +0.186| DAAKHYEFKSIPTTEPYVRVLG|
| 235–259  | 3054.581    | 3054.581               | +0.030| DEMRAGMSYHETIWKGVPKFLRRV|
| 260–287  | 3106.566    | 3106.566               | −0.013| DTALKNGIIEEPYVNPAPIQFSSWIMGG|
| 470–499  | 3610.803    | 3610.803               | +0.004| DIGSYREWSEERRQDEWLLSelsGKRPLFGS|