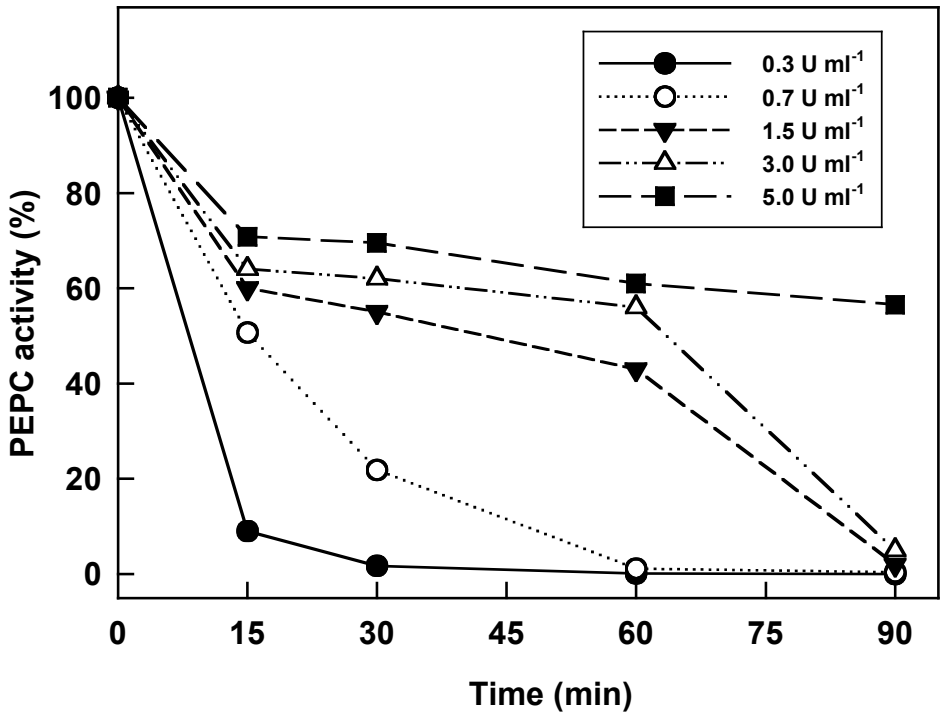
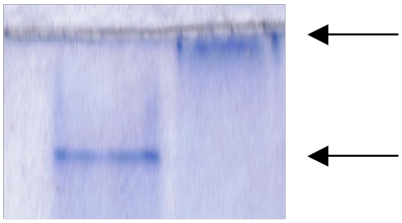
Supplemental Figures S1-S6

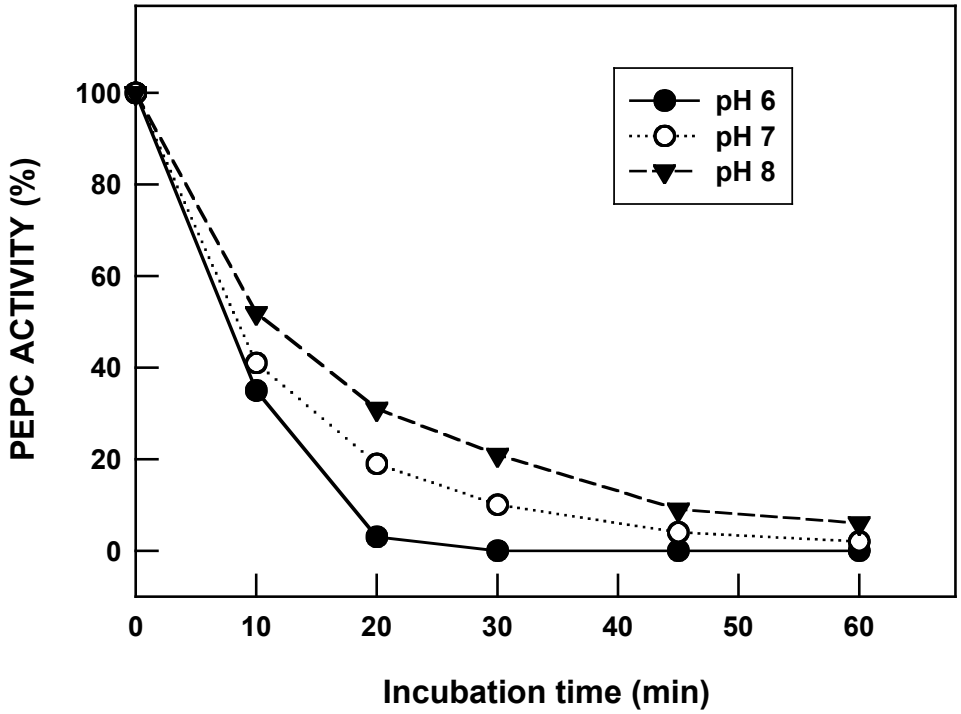


Supplemental Figure S1. Effect of PEPC concentration on the inhibition of its activity by PA (C8:0). Increasing amounts of purified sorghum C_4 PEPC (35 U mg^{-1} protein) were incubated at 30ºC in Tris-HCl 0.1 M, pH 8.0, containing 0.1 mM PA. PEPC activity was measured in aliquots of the incubation mixture at the indicated times.

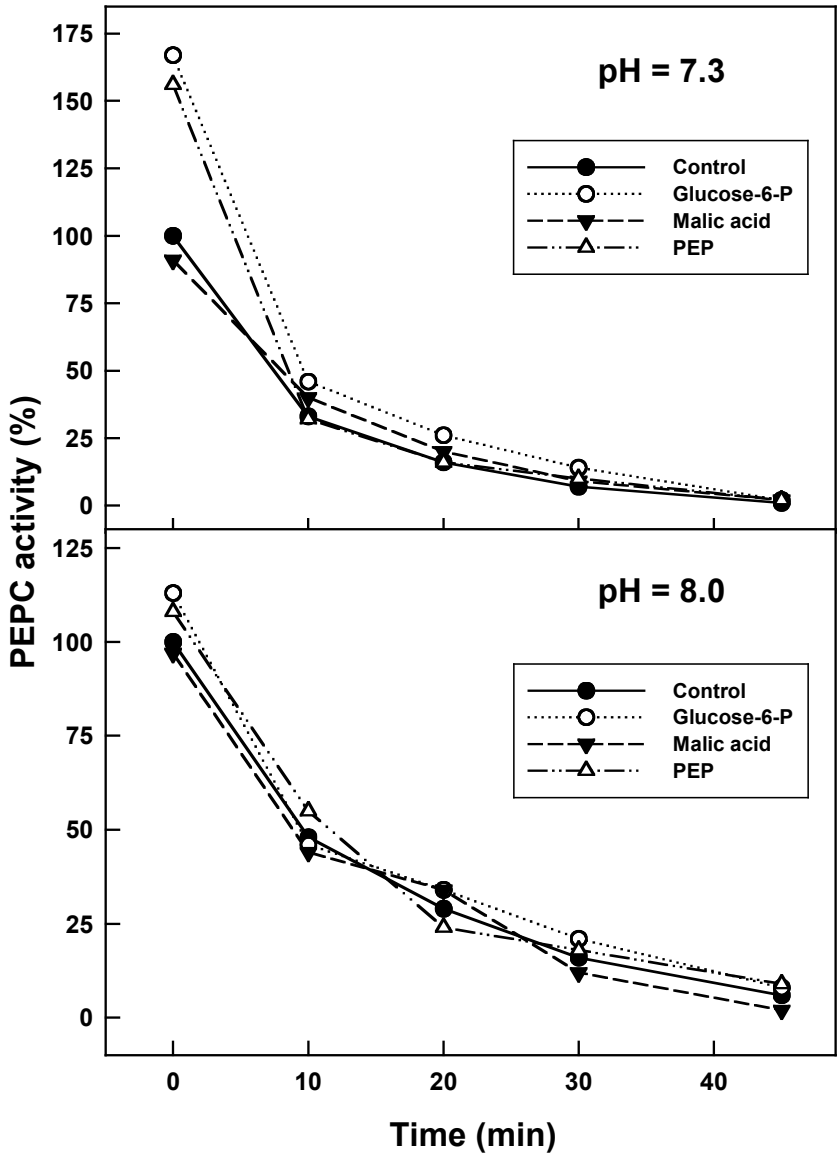


PA (mM)	0	1
PEPC activity (U ml^{-1})	3.2	0.0

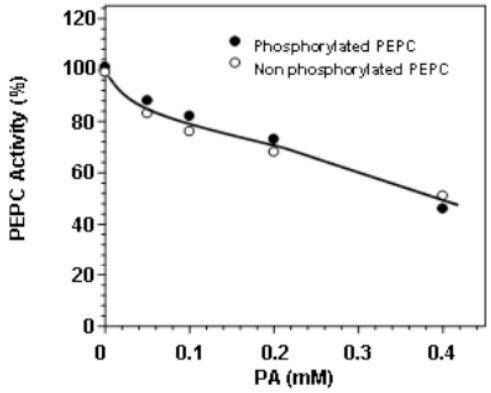
Supplemental figure S2. Electrophoretic mobility shift of sorghum purified PEPC. Purified sorghum C_4 PEPC (35 U mg-1 protein) was incubated for 30 min at 30ºC in 0.1 M Tris, pH 8.0, in the presence or absence of 1 mM (C8:0) PA. PEPC activity was measured in aliquots of the incubation mixture and proteins were resolved on native PAGE (7% acrylamide) electrophoresis and stained with Coomassie Brilliant Blue. The lower arrow indicates the PEPC tetrameric complex, the higher arrow the PA-induced higher MW complex.



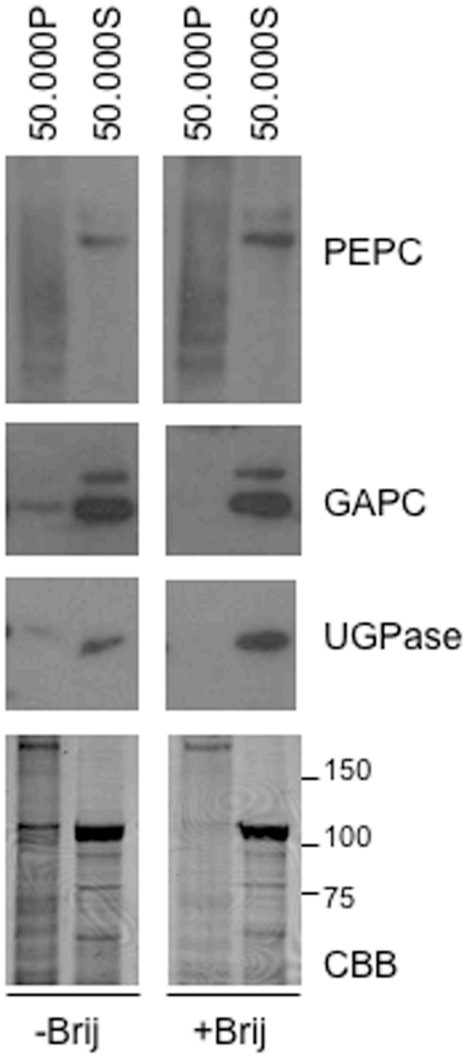
Supplemental figure S3. Effect of pH on the inhibition of PEPC activity by PA. Aliquots of purified sorghum C_4 PEPC (70 U mg-1 protein) were incubated at 30ºC in MES 0.1 M pH 6.0, or in Tris-HCl 0.1 M, pH 7.0 or 8.0 in the presence of 50 μM (C8:0) PA. PEPC activity was measured at pH 8.0 in aliquots of the incubation mixture at the indicated times. 100% of PEPC activity corresponded to 2.0, 2.9 and 2.9 μmol min^{-1} ml^{-1} for pH 6.0, 7 .0 and 8.0, respectively.



Supplemental figure S4. Effect glucose-6-P, malic acid and PEP on the inhibition of PEPC activity by PA. Aliquots of purified sorghum C_4 PEPC (70 U mg-1 protein) were pre-incubated for 15 min at 30ºC in in Tris-HCl 0.1 M, pH 8.0 or pH 7.3, in the presence or in the abscense of 5 mM glucose-6-P, 1 mM malic acid or 5 mM PEP. PEPC activity was measured at pH 8.0 in aliquots (5 µl) of the incubation mixture, and 50 µM (C8:0) PA was added (t=0). 100% of PEPC activity corresponded to 3.2 U ml^{-1}.



Supplemental figure S5. No effect of phosphorylation of PEPC on its sensitivity to PA. Purified sorghum PEPC (20 U ml^{-1}) was incubated for 1h at 30ºC with (black circles) or without (open circles) 5 U of PKA in 0.1 M Tris pH 8.0, 0.5 mM EGTA, 5 mM $MgCl_2$, 5 mM DTT, 2 mM ATP and proteinase inhibitor cocktail. The apparent phosphorylation state of PEPC was estimated by the malate test as described in the Materials and Methods section. Different concentrations of PA were added, and PEPC activity was measured after 30 min at 30ºC. PA (C18:1) was used here to avoid interferences from the Mg^{2+}included in the phosphorylation mixture. The specific activity of the PEPC preparation was 30 U mg^{-1} protein.



Supplemental figure S6. Brij58 treatment releases entrapped cytosolic proteins from the 50.000g pellet but does not decrease PEPC in this fraction. After sequential centrifugation at 1500g, 10.000g and 50.000g, the resulting supernatant (50.000S) and the 50.000g pellets (50.000P), washed twice with buffer with or without 0.1% Brij58 as indicated, were loaded on SDS-PAGE. Western analyses of PEPC, and GAPDH and UGP-ase subunits (cytosolic markers) were performed. Total protein for each fraction was analysed on CBB-stained gels.