Supplementary Information

Table S1. Oligonucleotide sequences used to incorporate site-directed mutagenesis. Underlines indicate nucleotide changes.

Mutant	Forward oligonucleotide sequence	Reverse oligonucleotide sequence
E218Q	5'-gcccgccacgtccaaagccagatcc-3'	5'-gatctggctttggacgtggcgggc-3'
E218A	5'-cccgccacgtcgcaagccagatcct-3'	5'-aggatctggcttgcgacgtggcggg-3'
K245Q	5'-cgccgcaatcagcaagtcgtcgagcgc-3'	5'-gcgctcgacgacttgctgattgcggcg-3'
R301Q	5'-gaagtcaatccgcaaatccaggtc-3'	5'-gacctggatttgcggattgacttc-3'
R301K	5'-gaagtcaatccgaaaatccaggtcgag -3'	5'-ctcgacctggatttttcggattgacttc-3'
E305A	5'-cgcatccaggtcgcgcacacggtgacc-3'	5'-ggtcaccgtgtgcgcgacctggatgcg-3'
E305D	5'-gcatccaggtcgaccacacggtgaccg-3'	5'-cggtcaccgtgtggtcgacctggatgc-3'
E305Q	5'-cgcatccaggtccaacacacggtgacc -3'	5'-ggtcaccgtgtgttggacctggatgcg-3'
R353M	5'cacgccctgcagtgcatggtgacgacggaagat-3'	5'-atcttccgtcgtcaccatgcactgcaggggcgtg-3'
R353K	5'-cacgccctgcagtgcaaggtgacgacggaagat-3'	5'-atcttccgtcgtcaccttgcactgcaggggcgtg-3'

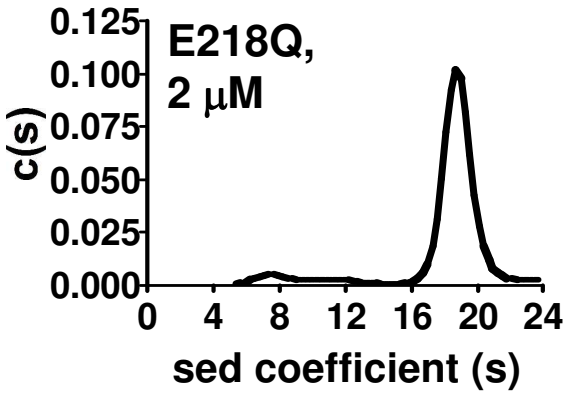
Sedimentation analysis of the enzymic quaternary structure of the E218Q mutant.

SV AUC was performed in 0.1 Tris-HCl (pH7.8), 20 mM $NaHCO_3$, 5 mM $MgCl_2$, 10 mM pyruvate, 0.1 mM acetyl CoA, and 1 mM DTE. The concentration of enzymes used in experiments, are shown in the Table. The proteins were visualized at 280 nm when the concentration was 2 μM and at 295 nm when the concentration was 10 μM.

Table S1

Enzyme	[Enzyme-monomer], (μM)	Monomers		Dimers		Tetramers	
		Sedimentation coefficient	%	Sedimentation coefficient	%	Sedimentation coefficient	%
E218Q	2	6.03 ± 1.16	7	10.69 ± 1.64	6	18.79 ± 0.60	87

Figure S1.


E218Q,
2 μM
c(s)
0.125
0.100
0.075
0.050
0.025
0.000
0
4
8
12
16
20
24
sed coefficient (s)

Detailed Assay Methods.

*Pyruvate Carboxylation Activity (Full forward reaction).*Pyruvate carboxylation activity was determined by monitoring the concomitant reduction of NADH to NAD^+ at 340 nm as oxaloacetate is converted to malate using malate dehydrogenase. Assays were performed at 25° C and initiated with the addition of PC (~5-500 µg). The $k_{cat}/K_{m\ MgATP}$ was determined at varying concentrations of MgATP (0.05-3.0 mM) and saturating concentrations of all other substrates. Concentrations of MgATP stock solutions were determined via end-point analysis using the glucose-6-phosphate/hexokinase coupled assay system. The 1 mL reactions contained 50 mM Bis-Tris, 25 mM Tricine and 25 mM glycine (100 mM total buffer, pH 7.5), 20 mM HCO_3^-, 12 mM pyruvate, 7.0 mM $MgCl_2$, 0.25 mM acetyl-CoA, 0.24 mM NADH and malate dehydrogenase (10 U). Data were fitted to either the Michaelis-Menten equation, or the substrate inhibition equation when substrate inhibition was observed. Errors reported are standard errors from fits to the respective equations.

Activities of the Full Reverse Reaction. The specific activities of oxaloacetate decarboxylation and subsequent formation of ATP was determined at 25º C at pH 7.5 (50 mM Bis-Tris, 25 mM Tricine, 25 mM glycine) by monitoring ATP production using the glucose-6-phosphate dehydrogenase/ hexokinase coupled assay system. The 3 mL reactions contained 7.5 mM $MgCl_2$, 3.0 mM MgADP, 2.5 mM phosphate, 0.95 mM oxaloacetate, 0.3 mM glucose, 0.25 mM acetyl-CoA, 0.24 mM NADP, glucose-6-phosphate dehydrogenase (5 U) and hexokinase (1 U). Errors reported are the standard deviations of three separate determinations.

*Oxamate-induced Decarboxylation of Oxaloacetate (Reverse reaction of the CT domain).*The specific activities for oxaloacetate decarboxylation in the presence of oxamate were determined by measuring the reduction of pyruvate to lactate using lactate dehydrogenase. All assays were performed at 25° C in a total volume of 3 mL. Specific activities were determined in triplicate at saturating concentrations of all substrates, and reactions were initiated with the addition of PC (300-900 µg). Reactions contained 50 mM Bis-tris, 25 mM Tricine and 25 mM glycine (pH 7.5), 0.95 mM oxaloacetate, 1.0 mM oxamate,

0.25 mM acetyl-CoA, 0.24 mM NADH, and lactate dehydrogenase (10 U). Errors reported are the standard deviations for three separate determinations.

*Phosphorylation of ADP using Carbamoyl or Acetyl Phosphate (Partial reverse reaction of the BC domain).*Carbamoyl phosphate and acetyl phosphate solutions were made just prior to use and kept on ice. In order to remove contaminating nucleotides, a 0.2 M solution of the lithium sodium acetyl phosphate salt (85% purity) was acidified (pH 2.0) and stirred with activated charcoal for 30 min. After filtering, the solution was lyophilized to dryness and the resulting acetyl phosphate was used for all subsequent experiments. Glucose-6-phosphate dehydrogenase/ hexokinase and phosphoenol pyruvate kinase/ lactate dehydrogenase coupled assay systems were used to confirm the absence of contaminating ATP or ADP in the purified acetyl phosphate solutions.

The phosphorylating ability of wild-type and mutant RePC was determined by measuring the initial rates of ATP production using the hexokinase/glucose-6-phosphate dehydrogenase coupled assay at various concentrations of either carbamoyl phosphate (1.0-20 mM) or acetyl phosphate (0.1-20 mM) in the presence or absence of 10 mM biotin. All assays were performed at 25º C and a total reaction volume of 3 mL. The k_{cat}/K_m for both carbamoyl phosphate and acetyl phosphate was determined from fits to either the Michaelis-Menten equation or to that for substrate inhibition and errors reported are determined from the overall fits to the equations. Reactions contained 50 mM Bis-Tris, 25 mM Tricine, 25 mM glycine (pH 7.5), 3.5 mM MgADP, 7.5 mM $MgCl_2$, 0.25 mM acetyl-CoA, 0.4 mM glucose, 0.36 mM NADP, glucose-6-phosphate dehydrogenase (5 U) and hexokinase (1U).

Bicarbonate-dependent ATPase Activities (Forward reaction of the BC domain). The relative activities for the bicarbonate-dependent ATPase reaction were determined by measuring the initial rate of $P_i$released using the glucose-6-phosphate dehydrogenase/phosphoglucomutase/phosphorylase coupled assay system. Due to the binding of MgATP to phosphorylase *a* (###), the $K_{m\ MgATP}$ values determined for the wild-type and mutant RePC catalyzed reactions under these conditionsshould be considered relative, rather than absolute, values. All assays were performed at 25° C in 3 mL total reaction volumes and were initiated by the addition of PC (300-990 μg).$k_{cat}/K_{m\ MgATP}$ were determined at

varying concentrations of MgATP (0.09-3.0 mM) and saturating concentrations of HCO_3^- (15 mM) and $MgCl_2$ (7.5 mM) in the presence and absence of 10 mM free biotin. Concentrations of MgATP stock solutions were determined via end-point analysis. Standard errors in the kinetic parameters were determined from fits of the data to either the Michaelis-Menten equation or substrate inhibition equation. Biotin inhibition of the R353M/K1119Q bicarbonate-dependent ATP cleavage activity was monitored at varying concentrations of MgATP (0.053-2.05 mM), fixed concentrations of biotin (0-10 mM) and saturating HCO_3^- (15 mM) and $MgCl_2$ (7.5 mM). All reactions contained 50 mM Bis-Tris, 25 mM Tricine, 25 mM glycine (pH 7.5), 0.50 mM NADP, 0.25 mM acetyl-CoA, 0.2 mM glycogen, 5μM α-D-glucose-1-phosphate, glucose-6-phosphate dehydrogenase (10 U), phosphorylase *a* (5 U), and phosphoglucomutase (10 U). The inhibition data were best described by the equation for linear, uncompetitive inhibition and the standard errors were determined from the global-fits of these data to the equation.

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