

Supplementary Material for: The Co-evolution of RuBisCO, Photorespiration and Carbon Concentrating Mechanisms in Higher Plants (Peter L. Cummins, Department of Genome Sciences, John Curtin School of Medical Research, The Australian National University, Canberra ACT 0200, Australia.)

Table S1. Kinetic parameters V (s^{-1}), K (μM), S ($s^{-1}.mM^{-1}$) and $S_{C/O}$ for C_3 species.

| Species | V_c | K_c | S_c | V_o | K_o | S_o | $S_{C/O}$ |
|--------------------------------|-------|-------|-------|-------|-------|-------|-----------|
| <i>Limonium antonii</i> | 2.4 | 8.7 | 276 | 0.976 | 397 | 2.46 | 112 |
| <i>Limonium artruchium</i> | 3 | 9.4 | 319 | 0.909 | 321 | 2.83 | 113 |
| <i>Limonium balearicum</i> | 3.6 | 9.7 | 371 | 1.24 | 346 | 3.58 | 103 |
| <i>Limonium barceloi</i> | 3.4 | 9.3 | 366 | 1.19 | 346 | 3.44 | 106 |
| <i>Limonium biflorum</i> | 2.4 | 8 | 300 | 0.845 | 316 | 2.67 | 112 |
| <i>Limonium companyonis</i> | 3.3 | 8.9 | 371 | 1.42 | 429 | 3.31 | 112 |
| <i>Limonium echioides</i> | 3.9 | 10.7 | 364 | 1.46 | 427 | 3.42 | 106 |
| <i>Limonium ejulabilis</i> | 2 | 7.6 | 263 | 0.94 | 415 | 2.27 | 116 |
| <i>Limonium gibertii</i> | 2.5 | 9.1 | 275 | 1.06 | 431 | 2.46 | 111 |
| <i>Limonium grosii</i> | 2.9 | 8.1 | 358 | 1.04 | 328 | 3.17 | 113 |
| <i>Limonium gymnesicum</i> | 2.4 | 8.2 | 293 | 0.936 | 388 | 2.41 | 121 |
| <i>Limonium latebracteatum</i> | 2.7 | 8.8 | 307 | | 344 | | |
| <i>Limonium leonardi</i> | 2.8 | 8.8 | 318 | 1.27 | 438 | 2.90 | 110 |
| <i>Limonium magallufianum</i> | 2.6 | 7 | 371 | 0.997 | 297 | 3.36 | 109 |
| <i>Limonium retusum</i> | 2.1 | 7.1 | 296 | 0.968 | 396 | 2.44 | 121 |
| <i>Limonium stenophyllum</i> | 2.6 | 8.4 | 310 | 0.976 | 457 | 2.46 | |
| <i>Limonium virgatum</i> | 2.4 | 8.5 | 282 | 0.909 | 381 | 2.83 | |
| Mean | 2.76 | 8.61 | 320 | 1.09 | 380 | 2.92 | 112 |
| Standard Error | 0.13 | 0.23 | 9 | 0.05 | 12 | 0.13 | 1 |
| <i>Aegilops biuncialis</i> | 3.2 | 16.8 | 190 | 0.932 | 470 | 1.98 | 96.3 |
| <i>Aegilops comosa</i> | 2.86 | 13.5 | 212 | 0.722 | 360 | 2.01 | 106 |
| <i>Aegilops cylindrica</i> | 3.68 | 13.7 | 269 | 1.12 | 451 | 2.48 | 109 |
| <i>Aegilops juvenalis</i> | 3.25 | 20.6 | 158 | 0.86 | 492 | 1.75 | 90.4 |
| <i>Aegilops speltoides</i> | 3.24 | 16.5 | 196 | 0.86 | 447 | 1.92 | 102 |
| <i>Aegilops tauschii</i> | 2.86 | 14.9 | 192 | 0.892 | 495 | 1.80 | 107 |
| <i>Aegilops triuncialis</i> | 2.62 | 12.8 | 205 | 0.754 | 380 | 1.98 | 103 |
| <i>Aegilops uniaristata</i> | 2.7 | 13.8 | 196 | 0.865 | 450 | 1.92 | 102 |
| <i>Aegilops vavilovii</i> | 3.32 | 13.3 | 250 | 0.835 | 363 | 2.30 | 109 |
| Mean | 3.08 | 15.1 | 207 | 0.87 | 434 | 2.01 | 103 |
| Standard Error | 0.11 | 0.8 | 11 | 0.04 | 18 | 0.08 | 2 |
| <i>Oryza barthii</i> | 2.5 | 14 | 179 | 0.798 | 479 | 1.67 | 107 |
| <i>Oryza eichingeri</i> | 2.5 | 14.1 | 177 | 1.01 | 612 | 1.66 | 107 |
| <i>Oryza glaberrima</i> | 2.7 | 14.9 | 181 | 1.01 | 586 | 1.73 | 105 |
| <i>Oryza glumaepatula</i> | 2.4 | 15.2 | 158 | 0.693 | 484 | 1.45 | 109 |
| <i>Oryza longistaminata</i> | 2.2 | 15.1 | 146 | 1.02 | 757 | 1.35 | 108 |
| <i>Oryza meridionalis</i> | 2.6 | 14.6 | 178 | 0.636 | 382 | 1.66 | 107 |
| <i>Oryza nivara</i> | 2.7 | 15.6 | 173 | 0.991 | 611 | 1.62 | 107 |
| <i>Oryza punctata</i> | 2.7 | 14.9 | 181 | 0.571 | 342 | 1.87 | 97 |
| <i>Oryza sativa</i> | 2.4 | 8.9 | 265 | 0.941 | 369 | 2.65 | 100 |
| Mean | 2.52 | 14.1 | 182 | 0.85 | 514 | 1.74 | 105 |
| Standard Error | 0.06 | 0.7 | 11 | 0.06 | 46 | 0.12 | 1 |
| <i>Puccinellia distans</i> | 5.4 | 22.2 | 243 | 1.14 | 488 | 2.34 | 104 |
| <i>Puccinellia lemmonii</i> | 5.2 | 28.1 | 185 | 2.06 | 1120 | 1.81 | 102 |
| <i>Puccinellia maritima</i> | 5.4 | 20.8 | 260 | 1.65 | 676 | 2.45 | 106 |
| <i>Puccinellia nuttalliana</i> | 4 | 25.2 | 159 | 1.08 | 717 | 1.51 | 105 |
| Mean | 5.00 | 24.0 | 212 | 1.48 | 750 | 2.03 | 104 |
| Standard Error | 0.34 | 1.6 | 24 | 0.23 | 133 | 0.22 | 1 |
| <i>Agriophyllum squarrosum</i> | 2.8 | 15.4 | 182 | 0.656 | 339 | 1.94 | 93.9 |

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|----------------------------------|------|------|-----|-------|-----|------|------|
| <i>Agrostis scabra</i> | 3.6 | 22 | 164 | 1.04 | 653 | 1.59 | 103 |
| <i>Agrostis stolonifera</i> | 5.2 | 25.3 | 206 | 1.57 | 802 | 1.96 | 105 |
| <i>Amphicarpaea bracteata</i> | 4 | 29 | 138 | 0.978 | 693 | 1.41 | 97.5 |
| <i>Arabidopsis thaliana</i> | 3.61 | 9.9 | 364 | | 333 | | |
| <i>Arctagrostis latifolia</i> | 5.8 | 21 | 276 | 1.31 | 497 | 2.63 | 105 |
| <i>Artemisia myriantha</i> | 3.1 | 26.4 | 117 | 0.903 | 844 | 1.07 | 110 |
| <i>Artemisia vulgaris</i> L. | 3.9 | 31.9 | 122 | 0.73 | 626 | 1.16 | 105 |
| <i>Atriplex glabriuscula</i> | | 27 | | | 328 | | |
| <i>Avena sativa</i> | 2.3 | 10.8 | 213 | | | 2.13 | 99.9 |
| <i>B. distachyon</i> | 2.05 | 11.9 | 172 | 0.613 | 396 | 1.55 | 111 |
| <i>Beta maritima</i> | | | | | | | 94.6 |
| <i>Beta vulgaris</i> | 2.9 | 13.9 | 209 | 0.916 | 401 | 2.10 | 99.4 |
| <i>Brassica oleracea</i> | 2.1 | 11.8 | 178 | | | 1.85 | 96.2 |
| <i>Bromus anomalus</i> | 2.9 | 16.9 | 172 | 0.833 | 494 | 1.70 | 101 |
| <i>Calamagrostis arundinacea</i> | 4.1 | 22.7 | 181 | 1.07 | 614 | 1.74 | 104 |
| <i>Calamagrostis canescens</i> | 2.5 | 15.2 | 164 | 0.992 | 594 | 1.67 | 98.5 |
| <i>Calamagrostis foliosa</i> | 3.5 | 20.9 | 167 | 0.527 | 330 | 1.59 | 105 |
| <i>Calamagrostis inexpansa</i> | 3.3 | 18.8 | 176 | 0.971 | 608 | 1.58 | 111 |
| <i>Calamagrostis nutkaensis</i> | 3.1 | 20.1 | 154 | 0.853 | 601 | 1.41 | 109 |
| <i>Capsicum annuum</i> | 1.9 | 9.6 | 198 | | | 2.06 | 96 |
| <i>Chenopodium alba</i> | 2.91 | 11.2 | 260 | 1.37 | 415 | 3.30 | 78.7 |
| <i>Chenopodium murale</i> | 4.4 | 23.8 | 185 | 0.6 | 354 | 1.70 | 109 |
| <i>Chenopodium petiolare</i> | 4.4 | 25.6 | 172 | 1.03 | 589 | 1.74 | 98.5 |
| <i>Chenopodium rubrum</i> | 4.1 | 14.5 | 283 | 0.998 | 346 | 2.89 | 97.8 |
| <i>Citrullus ecirrhosus</i> | 3.1 | 18.9 | 164 | 0.882 | 544 | 1.64 | 99.9 |
| <i>Citrullus lanatus</i> | 2.5 | 19.4 | 129 | 0.616 | 510 | 1.20 | 107 |
| <i>Coffee arabica</i> | 2.1 | 11 | 191 | | | 1.93 | 98.7 |
| <i>Crithmum maritimum</i> | 3.4 | 8.7 | 391 | | 183 | | |
| <i>Cucurbita maxima</i> | 2.2 | 9 | 244 | | | 2.48 | 98.4 |
| <i>Dactylis glomerata</i> | 3.2 | 10.7 | 299 | | 453 | | |
| <i>Deschampsia danthonioides</i> | 4.5 | 22.3 | 202 | 1.09 | 580 | 1.87 | 108 |
| <i>Desmodium cinereum</i> | 3 | 12.8 | 234 | 0.97 | 403 | 2.40 | 97.5 |
| <i>Desmodium intortum</i> | 3.3 | 14.2 | 232 | 0.927 | 394 | 2.35 | 98.7 |
| <i>Desmodium psilocarpum</i> | 3.6 | 15.6 | 231 | 1.09 | 452 | 2.41 | 95.9 |
| <i>Diplotaxis ibicensis</i> | | | | | | | 95.6 |
| <i>Elymus farctus</i> | 3.3 | 19.5 | 169 | 0.476 | 300 | 1.60 | 106 |
| <i>Erythrina flabelliformis</i> | 3.6 | 18.4 | 196 | 1.36 | 665 | 2.03 | 96.4 |
| <i>Espeletia schultzei</i> | | 23.3 | | | | | |
| <i>Eucalyptus moorei</i> | 3.2 | 10 | 320 | | 285 | | |
| <i>Eucalyptus neglecta</i> | 2.5 | 7.9 | 316 | | 230 | | |
| <i>Euphorbia helioscopia</i> | 1.9 | 11.5 | 165 | 0.77 | 453 | 1.71 | 96.8 |
| <i>Euphorbia microsphaera</i> | 4.5 | 25.8 | 174 | 0.954 | 546 | 1.75 | 99.7 |
| <i>Festuca gigantea</i> | 5.1 | 31.2 | 163 | 0.902 | 595 | 1.51 | 108 |
| <i>Festuca pratensis</i> | 5.1 | 23.1 | 221 | 1.43 | 686 | 2.08 | 106 |
| <i>Flueggea suffruticosa</i> | 3.4 | 19.2 | 177 | 0.96 | 547 | 1.75 | 101 |
| <i>Foeniculum vulgare</i> | 4.4 | 20.7 | 213 | 1.14 | 512 | 2.25 | 94.3 |
| <i>Glycine canescens</i> | 2.6 | 17.2 | 151 | 0.914 | 587 | 1.56 | 97.1 |
| <i>Glycine max</i> | 2 | 10.3 | 195 | 1.25 | 475 | 2.10 | 92.9 |
| <i>H. vulgare</i> | 3.99 | 15.2 | 263 | 1.2 | 465 | 2.57 | 102 |
| <i>Helianthus annuus</i> | | | | | | | 73.6 |
| <i>Helianthus maximus</i> | | 10 | | | | | 77 |
| <i>Hordeum brachyantherum</i> | 2.9 | 16.2 | 179 | 0.656 | 371 | 1.77 | 101 |
| <i>Hordeum murinum</i> | 4.2 | 21.5 | 195 | 0.993 | 511 | 1.95 | 100 |
| <i>Hordeum vulgare</i> | 2.4 | 9 | 267 | | | 2.93 | 91.0 |
| <i>Hypericum balearicum</i> | | | | | | | 93.6 |
| <i>Ipomoea batatas</i> | 2.5 | 12 | 208 | | | 2.12 | 98.5 |

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|--|------|------|-----|-------|-----|------|------|
| <i>Iris douglasiana</i> | 3.5 | 9.7 | 361 | | 413 | | |
| <i>Kundmannia sicula</i> | | | | | | | 89.2 |
| <i>Lablab purpureus</i> | 5.3 | 21.7 | 244 | 1.49 | 556 | 2.68 | 91.1 |
| <i>Lactuca sativa</i> | 2.2 | 11.1 | 198 | | | 2.11 | 94 |
| <i>Lepidium campestre</i> | 3.4 | 15.8 | 215 | 0.778 | 336 | 2.32 | 92.8 |
| <i>Lolium multiflorum</i> | 4.5 | 29.1 | 155 | 1.14 | 740 | 1.55 | 99.9 |
| <i>Lolium perenne</i> | | 16 | | | 500 | | 80 |
| <i>Lolium rigidum</i> | 4.7 | 25 | 188 | 0.973 | 520 | 1.88 | 100 |
| <i>Lycopersicon esculentum</i> | | 8.2 | | | | | 82 |
| <i>Lysimachia minoricensis</i> | | | | | | | 93.8 |
| <i>Macrotyloma uniflorum</i> | 4.4 | 25.2 | 175 | 0.897 | 519 | 1.73 | 101 |
| <i>Manihot esculenta</i> | 1.9 | 10.6 | 180 | | | 1.75 | 103 |
| <i>Medicago sativa</i> | 1.7 | 12.7 | 134 | | | 1.55 | 86.3 |
| <i>Mentha aquatica</i> | | | | | | | 97.2 |
| <i>Mercurialis annua</i> | 3.4 | 17 | 200 | 0.87 | 417 | 2.09 | 95.7 |
| <i>Musa velutina</i> | 3.2 | 19 | 168 | 0.852 | 564 | 1.52 | 111 |
| <i>Nicotiana glauca</i> Grah. | | | | | | | 73.7 |
| <i>Nicotiana tabacum</i> | 3.37 | 9.96 | 338 | 1.19 | 291 | 3.91 | 86.5 |
| <i>Pallenis maritima</i> | 2.7 | 6.4 | 422 | | 321 | | |
| <i>Petroselinum crispum</i> | | 11.6 | | | | | 77 |
| <i>Phaseolus carteri</i> | 3.2 | 14.2 | 225 | 1.13 | 422 | 2.67 | 84.5 |
| <i>Phaseolus coccineus</i> | 3.9 | 15.6 | 250 | 1.18 | 491 | 2.40 | 104 |
| <i>Phaseolus lunatus</i> | 3.2 | 17.3 | 185 | 0.981 | 537 | 1.83 | 101 |
| <i>Phaseolus vulgaris</i> | 2.6 | 14 | 186 | 0.761 | 463 | 1.81 | 102 |
| <i>Pistacia lentiscus</i> | | | | | | | 97.2 |
| <i>Pisum sativum</i> | | | | | | | 90.2 |
| <i>Plantago lanceolata</i> L. | | | | | | | 77.3 |
| <i>Poa palustris</i> | 4.2 | 19.2 | 219 | 1.1 | 562 | 1.95 | 112 |
| <i>Pueraria montana</i> | 2.7 | 20.9 | 129 | 0.871 | 679 | 1.28 | 101 |
| <i>Rhamnus alaternus</i> | | | | | | | 94.7 |
| <i>Rhamnus ludovici-salvatoris</i> | | | | | | | 94.4 |
| <i>S. cereale</i> | 3.23 | 20.2 | 160 | 0.826 | 472 | 1.75 | 91.5 |
| <i>Sideritis cretica</i> subsp. <i>spicata</i> | 2 | 7.8 | 256 | | 328 | | |
| <i>Solanum lycopersicum</i> | 2.3 | 9.7 | 237 | | | 2.57 | 92.4 |
| <i>Solanum tuberosum</i> | 2 | 9.6 | 208 | | | 2.18 | 95.4 |
| <i>Sphenostylis stenocarpa</i> | 2.8 | 17.4 | 161 | 0.981 | 574 | 1.72 | 93.6 |
| <i>Spinacia oleracea</i> | 2.76 | 12.1 | 228 | 1.39 | 461 | 2.53 | 90.5 |
| <i>Steinchisma laxa</i> | 2.3 | 7.7 | 299 | 1.35 | 419 | 3.27 | 91.4 |
| <i>T. aestivum</i> | 3.45 | 16.8 | 205 | 0.917 | 429 | 2.15 | 95.7 |
| <i>T. dicoccon</i> 1 | 3.51 | 16.1 | 218 | 1.02 | 434 | 2.33 | 93.5 |
| <i>T. monococcum</i> | 3.18 | 14 | 227 | 0.877 | 401 | 2.18 | 104 |
| <i>T. timonovum</i> | 3.48 | 16.8 | 207 | 1.02 | 495 | 2.05 | 101 |
| <i>T. timopheevii</i> | 3.43 | 16.2 | 212 | 0.893 | 429 | 2.08 | 102 |
| <i>Tephrosia candida</i> | 2.2 | 15.9 | 138 | 0.667 | 481 | 1.41 | 97.8 |
| <i>Tephrosia purpurea</i> | 2.2 | 12.4 | 177 | 0.575 | 333 | 1.72 | 103 |
| <i>Tephrosia rhodesica</i> | 2.2 | 13.7 | 161 | 0.988 | 565 | 1.75 | 91.6 |
| <i>Tetragonium expansa</i> | | 13 | | | 600 | | 81 |
| <i>Teucrium heterophyllum</i> | 2.7 | 6.7 | 403 | | 359 | | |
| <i>Trachycarpus fortunei</i> | 2.8 | 9 | 311 | | 364 | | |
| <i>Trifolium repens</i> | | 13.1 | | | 565 | | |
| <i>Triticale</i> | 3.48 | 15.7 | 222 | 0.905 | 396 | 2.28 | 97.2 |
| <i>Triticum aestivum</i> | 3.18 | 13.1 | 243 | 1.39 | 543 | 2.55 | 95.4 |
| <i>Triticum baeticum</i> | 3.8 | 19.7 | 193 | 1.8 | 905 | 2.01 | 96 |
| <i>Urtica atrovirens</i> | | | | | | | 90.2 |
| <i>Urtica membranacea</i> | | | | | | | 102 |

Table S2. Kinetic parameters V (s^{-1}), K (μM), S ($s^{-1} \cdot mM^{-1}$) and $S_{C/O}$ for C_3 , transitional and C_4 species.

| Species | | V_c | K_c | S_c | V_o | K_o | S_o | $S_{C/O}$ |
|--------------------------------|---------------|-------|-------|-------|-------|-------|-------|-----------|
| <i>Flaveria cronquistii</i> | C_3 | 3.04 | 10.3 | 297 | 2.34 | 431 | 3.51 | 84.6 |
| <i>Flaveria pringlei</i> | C_3 | 2.80 | 12.2 | 229 | 1.61 | 321 | 2.63 | 86.9 |
| <i>Flaveria angustifolia</i> | C_3 - C_4 | 2.82 | 12.8 | 220 | | | 2.53 | 86.8 |
| <i>Flaveria anomala</i> | C_3 - C_4 | 3.8 | 10.7 | 355 | 2.75 | 605 | 4.56 | 77.9 |
| <i>Flaveria chloraefolia</i> | C_3 - C_4 | 3.35 | 12.4 | 270 | 2.46 | 740 | 3.31 | 81.6 |
| <i>Flaveria floridana</i> | C_3 - C_4 | 3.22 | 13.2 | 244 | 1.55 | 530 | 2.92 | 83.6 |
| <i>Flaveria linearis</i> | C_3 - C_4 | 3.43 | 12.5 | 274 | 1.46 | 415 | 3.51 | 78.1 |
| <i>Flaveria ramosissima</i> | C_3 - C_4 | 2.77 | 12 | 231 | 2.09 | 722 | 2.89 | 79.8 |
| <i>Flaveria sonorensis</i> | C_3 - C_4 | 2.69 | 10.2 | 264 | 2.46 | 785 | 3.13 | 84.3 |
| <i>Flaveria brownii</i> | C_4 like | 2.58 | 12.8 | 202 | 0.907 | 378 | 2.41 | 83.8 |
| <i>Flaveria palmeri</i> | C_4 like | 3.54 | 13.5 | 262 | 0.603 | 193 | 3.13 | 83.8 |
| <i>Flaveria vaginata</i> | C_4 like | 3.78 | 21.4 | 177 | 1.97 | 880 | 2.24 | 78.7 |
| <i>Flaveria australasica</i> | C_4 | 3.84 | 22 | 175 | 0.697 | 309 | 2.62 | 77.2 |
| <i>Flaveria bidentis</i> | C_4 | 4.13 | 20.0 | 206 | 1.48 | 530 | 2.61 | 78.8 |
| <i>Flaveria kochiana</i> | C_4 | 3.68 | 22.7 | 162 | | 150 | 2.11 | 77 |
| <i>Flaveria trinervia</i> | C_4 | 3.85 | 18.2 | 212 | 2.15 | 671 | 2.73 | 77.7 |
| Mean | | 3.33 | 14.8 | 236 | 1.72 | 521 | 2.91 | 81.3 |
| Standard Error | | 0.12 | 1.1 | 13 | 0.17 | 58 | 0.16 | 0.9 |
| <i>Panicum bisulcatum</i> | C_3 | 2.6 | 7.8 | 333 | 1.57 | 416 | 3.80 | 87.7 |
| <i>Panicum milioides</i> | C_3 - C_4 | 2.2 | 7.4 | 297 | 1.24 | 387 | 3.22 | 92.3 |
| <i>Panicum amarum</i> | C_4 | 3.2 | 33.1 | 97 | 0.86 | 800 | 1.08 | 89.5 |
| <i>Panicum antidotale</i> | C_4 | 3.9 | | | | | | 74.5 |
| <i>Panicum coloratum</i> | C_4 | 3.4 | 11.1 | 306 | 1.59 | 445 | 3.61 | 84.8 |
| <i>Panicum deustum</i> | C_4 | 5 | 15.4 | 325 | 1.17 | 306 | 3.83 | 84.8 |
| <i>Panicum dichotomiflorum</i> | C_4 | 3.1 | 36.3 | 85 | 1.41 | 154 | 0.92 | 92.6 |
| <i>Panicum milliaceum</i> | C_4 | 2.1 | 7.2 | 292 | 1.13 | 313 | 3.65 | 79.9 |
| <i>Panicum monticola</i> | C_4 | 5.3 | 18.2 | 291 | 1.97 | 543 | 3.67 | 79.4 |
| <i>Panicum phragmitoides</i> | C_4 | 2.8 | 25.1 | 112 | 0.707 | 687 | 1.04 | 107 |
| <i>Panicum virgatum</i> | C_4 | 3.3 | 12.7 | 260 | 0.854 | 271 | 3.15 | 82.6 |
| Mean | | 3.35 | 17.4 | 240 | 1.25 | 571 | 2.80 | 86.6 |
| Standard Error | | 0.31 | 3.38 | 32 | 0.12 | 120 | 0.40 | 2.9 |
| <i>Amaranthus edulis</i> | C_4 | 4.14 | 18.2 | 227 | 0.847 | 289 | 2.94 | 77.5 |
| <i>Amaranthus hybridus</i> | C_4 | 3.8 | 16 | 238 | 1.85 | 640 | 2.97 | 80 |
| <i>C. dactylon</i> | C_4 | | 21 | | | 402 | | 89.2 |
| <i>Cenchrus ciliaris</i> | C_4 | 6 | 19 | 316 | 2.1 | 470 | 4.52 | 69.9 |
| <i>Chrysanthellum indicum</i> | C_4 | 4.7 | 28.1 | 167 | 1.21 | 598 | 2.03 | 82.4 |
| <i>Echinochloa crus-galli</i> | C_4 | | 18.4 | | | | | 83 |
| <i>Eragrostis tef</i> | C_4 | 7.1 | 34.9 | 203 | 1.46 | 640 | 2.29 | 89 |
| <i>Megathyrsus maximus</i> | C_4 | 5.3 | 13.9 | 381 | 1.25 | 265 | 4.75 | 80.3 |
| <i>P. dilatatum</i> | C_4 | | 19.9 | | | 415 | | 88 |
| <i>Potulaca oleracea</i> | C_4 | 5.9 | 13.6 | 434 | | | 5.56 | 78 |
| <i>Saccharum officinarum</i> | C_4 | 3.9 | 26.3 | 148 | | | 1.80 | 82.2 |
| <i>Setaria italica</i> | C_4 | | 32.1 | | | | | 58 |
| <i>Setaria viridis</i> | C_4 | 5.67 | 18.1 | 313 | 2.77 | 619 | 4.31 | 72.7 |
| <i>Sorghum bicolor</i> | C_4 | 5.4 | 29.9 | 181 | | | 2.58 | 70 |
| <i>Urochloa mosambicensis</i> | C_4 | 5.7 | 14.8 | 385 | 2.14 | 464 | 4.67 | 82.5 |
| <i>Urochloa panicoides</i> | C_4 | 5.6 | 15.4 | 364 | 2.04 | 444 | 4.64 | 78.3 |
| <i>Z. japonica</i> | C_4 | | 18.5 | | | 403 | | 84.1 |
| <i>Zea mays</i> | C_4 | 4.19 | 30.6 | 137 | 0.920 | 596 | 1.68 | 81.6 |
| Mean | | 5.18 | 21.6 | 269 | 1.66 | 480 | 3.44 | 79.0 |
| Standard Error | | 0.27 | 1.6 | 28 | 0.20 | 36 | 0.37 | 1.8 |

Appendix

Derivation of the kinetic equations: The concentrations used in the kinetic equations are E : activated form of the enzyme; R : unbound RuBP; ER : RuBisCO...RuBP complex; ER^* : RuBisCO...enediolate of RuBP complex; C : free CO_2 ; O : free O_2 ; ERC : RuBisCO...carboxylated intermediate complex; ERO : RuBisCO...oxygenated intermediate complex; EP : RuBisCO...carboxylated product complex; EX : RuBisCO...oxygenated product complex; G : 3-phosphoglyceric acid; Q : 2-phospho-glycolate.

The mass balance equation for the kinetic mechanism (Fig. 1) is given by (E_t is the total activated enzyme concentration)

$$E + ER + ER^* + ERC + ERO + EP + EX - E_t = 0 \quad (\text{A1})$$

The steady state ordinary differential equations (ODEs) for this kinetic scheme are

$$d ER/dt = k_1 E \cdot R - (k_2 + k_3)ER + k_4 ER^* = 0 \quad (\text{A2})$$

$$d ER^*/dt = k_3 ER - (k_4 + k_5 C + k_{11} O)ER^* + k_6 ERC + k_{12} ERO = 0 \quad (\text{A3})$$

$$d ERC/dt = k_5 C \cdot ER^* - (k_6 + k_7)ERC + k_8 EP = 0 \quad (\text{A4})$$

$$d EP/dt = k_7 ERC - (k_8 + k_9)EP + k_{10} E \cdot G \cdot G = 0 \quad (\text{A5})$$

$$d ERO/dt = k_{11} O \cdot ER^* - (k_{12} + k_{13})ERO + k_{14} EX = 0 \quad (\text{A6})$$

$$d EX/dt = k_{13} ERO - (k_{14} + k_{15})EX + k_{16} E \cdot G \cdot Q = 0 \quad (\text{A7})$$

It is convenient to define the following constants:

$$\alpha_C = (k_9 + k_8)/k_7 \quad \alpha_O = (k_{15} + k_{14})/k_{13}$$

$$\beta_C = k_9 + k_6 \alpha_C \quad \beta_O = k_{15} + k_{12} \alpha_O$$

From the above steady state ODEs we can readily express the concentrations of free enzyme E and all reaction intermediates in terms of the product complexes (either EP or EX). For the carboxylation reaction (EP) we obtain (assuming only that product release is “irreversible” i.e. $k_{10} = k_{16} = 0$):

$$\text{Summing (A2) to (A7): } E = \frac{k_2 ER + k_9 EP + k_{15} EX}{k_1 R} \quad (\text{A8})$$

$$\text{From (A5): } ERC = \alpha_C EP \quad (\text{A9})$$

$$\text{From (A7): } ERO = \alpha_O EX \quad (\text{A10})$$

$$\text{From (A4) + (A5) and (A9): } ER^* = \frac{k_9 EP + k_6 ERC}{k_5 C} = \frac{\beta_C}{k_5 C} EP \quad (\text{A11})$$

$$\text{From (A6) + (A7), (A10) and (A11): } EX = \frac{k_{11} O \cdot ER^*}{\beta_O} = \frac{\beta_C k_{11} O}{\beta_O k_5 C} EP \quad (\text{A12})$$

$$\text{From (A10) and (A12): } ERO = \alpha_O EX = \frac{\alpha_O \beta_C k_{11} O}{\beta_O k_5 C} EP \quad (\text{A13})$$

From (A2), (A8), (A11) and (A12): $ER = \frac{k_9 EP + k_{15} EX + k_4 ER^*}{k_3}$

$$= \frac{k_9}{k_3} EP + \frac{\beta_C k_{11} k_{15} O}{\beta_O k_3 k_5 C} EP + \frac{k_4 \beta_C}{k_3 k_5 C} EP \quad (A14)$$

Substituting (A8)-(A14) into (A1) and factorizing we get the steady state equation in the form

$$\frac{E_t}{EP} = 1 + f_1^C + \frac{f_2^C}{R} + \frac{f_3^C}{C} + \frac{f_4^C}{RC} \quad (A15)$$

where the coefficients f_i^C are given by

$$f_1^C = \frac{k_9 + k_3 \alpha_C}{k_3} \quad (A16a)$$

$$f_2^C = \frac{k_9(k_2 + k_3)}{k_1 k_3} \quad (A16b)$$

$$f_3^C = \frac{\beta_C}{k_3 k_5} \left(k_3 + k_4 + \frac{(k_3 + k_{15} + k_3 \alpha_O) k_{11} O}{\beta_O} \right) \quad (A16c)$$

$$f_4^C = \frac{\beta_C}{k_1 k_3 k_5} \left(k_2 k_4 + \frac{(k_2 + k_3) k_{11} k_{15} O}{\beta_O} \right) \quad (A16d)$$

The rate of CO₂ consumption is given by

$$V_C = -\frac{dC}{dt} = k_5 C \cdot ER^* - k_6 ERC = (k_9 + k_6 \alpha_C) EP - k_6 \alpha_C EP = k_9 EP \quad (A17)$$

Rewriting (A15) in terms of EP and substituting the result into (A17) gives

$$V_C = \frac{k_9 E_t RC}{f_4^C + f_3^C R + f_2^C C + (f_1^C + 1) RC} \quad (A18)$$

When both substrates, R and C , are saturating the maximum rate of CO₂ consumption, V_{\max}^C , is obtained as,

$$V_{\max}^C = \frac{k_9}{1 + f_1^C} E_t = k_{cat}^C E_t \quad (A19)$$

Substituting (A16a) into (A19) and rearranging we get for k_{cat}^C in terms of the rate constants k_i

$$k_{cat}^C = \frac{k_3 k_7 k_9}{k_3 k_7 + k_3 k_8 + k_3 k_9 + k_7 k_9}$$

Finally, rewriting (A18) in terms of V_{\max}^C gives the familiar (e.g. (Farquhar, 1979)) general form of the steady state rate equation,

$$V_C = \frac{V_{\max}^C R \cdot C}{K_3^C + K_2^C R + K_1^C C + R \cdot C} \quad (\text{A20})$$

$$\text{where } K_i^C = f_{i+1}^C / (1 + f_1^C). \quad (\text{A21})$$

It immediately follows that the rate of oxygen consumption by the enzyme can be written as

$$V_O = \frac{V_{\max}^O R \cdot O}{K_3^O + K_2^O R + K_1^O O + R \cdot O}$$

$$\text{where } V_{\max}^O = k_{cat}^O E_t \text{ and } k_{cat}^O = \frac{k_{15}}{1 + f_1^O} = \frac{k_3 k_{13} k_{15}}{k_3 k_{13} + k_3 k_{14} + k_3 k_{15} + k_{13} k_{15}}.$$

The Michaelis-Menten equation (A20) for the single substrate C when R is saturating becomes

$$V_C = \frac{V_{\max}^C C}{K_2^C + C}$$

From (A21), (A16a) and (A16c):

$$K_2^C = \frac{f_3^C}{1 + f_1^C} = \frac{\beta_C}{(1 + f_1^C) k_3 k_5} \left[k_3 + k_4 + \frac{(k_3 + k_{15} + k_3 \alpha_O) k_{11} O}{\beta_O} \right] \quad (\text{A22})$$

$$\text{Substituting } f_1^O = \frac{k_{15} + k_3 \alpha_O}{k_3}, K_R = \frac{k_3}{k_3 + k_4}, \gamma_C = \frac{\alpha_C}{1 + f_1^C} \text{ and } \gamma_O = \frac{\alpha_O}{1 + f_1^O}$$

into (A22) yields the Michaelis constant in the presence of the other (O) substrate

$$K_2^C = K_C \left(1 + \frac{O}{K_O} \right)$$

$$\text{where } K_C = \frac{k_9 + k_6 \alpha_C}{(1 + f_1^C) K_R k_5} = \frac{k_{cat}^C + \gamma_C k_6}{K_R k_5} \quad (\text{A23})$$

$$\text{and } K_O = \frac{k_{15} + k_{12} \alpha_O}{(1 + f_1^O) K_R k_{11}} = \frac{k_{cat}^O + \gamma_O k_{12}}{K_R k_{11}} \quad (\text{A24})$$

The specificities of each of the reactions are then

$$S_c = \frac{k_{cat}^c}{K_c} = \frac{k_{cat}^c K_R k_5}{k_{cat}^c + \gamma_c k_6} \quad \text{and} \quad S_o = \frac{k_{cat}^o}{K_o} = \frac{k_{cat}^o K_R k_{11}}{k_{cat}^o + \gamma_o k_{12}}$$

and so the specificity of carboxylation relative to oxygenation (relative specificity) is given by

$$S_{c/o} = \frac{S_c}{S_o} = \frac{k_5 k_{cat}^c (k_{cat}^o + \gamma_o k_{12})}{k_{11} k_{cat}^o (k_{cat}^c + \gamma_c k_6)} \quad (\text{A25})$$

In terms of the rate constants we find the coefficients of k_6 and k_{12} :

$$\gamma_c = \frac{\alpha_c}{1 + f_1^c} = \frac{(k_8 + k_9) k_{cat}^c}{k_7 k_9} = \frac{k_3 k_8 + k_3 k_9}{k_3 k_7 + k_3 k_8 + k_3 k_9 + k_7 k_9} \quad (\text{A26})$$

$$\gamma_o = \frac{\alpha_o}{1 + f_1^o} = \frac{(k_{14} + k_{15}) k_{cat}^o}{k_{13} k_{15}} = \frac{k_3 k_{14} + k_3 k_{15}}{k_3 k_{13} + k_3 k_{14} + k_3 k_{15} + k_{13} k_{15}} \quad (\text{A27})$$

Thus the range of γ is limited to [0,1].