Supporting Information

Phenylalanine Binding Is Linked to Dimerization of the Regulatory Domain of Phenylalanine Hydroxylase

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Protein expression and purification

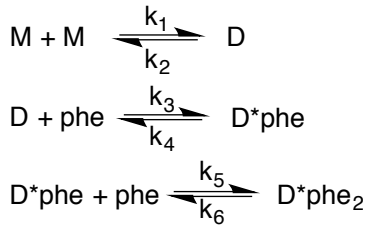
The expression and purification of RDPheH from rat were modified from the method described previously (1) in that a gel filtration step (Superdex 200 10/300 GL) was added after the HisTrap FF column (5 ml, GE Healthcare Life Science, Piscataway, NJ). The expression vector for the N-terminal 24 residue deletion mutant $RDPheH_{25\text{-}117}$ was produced from the plasmid used to express RDPheH using the QuikChange Mutagenesis protocol (Agilent Technologies). The oligonucleotides used as primers were 5′-ccgcgcggcagccatattgaagacaactcca-3′ and 5′-tggagttgtcttcaatatggctgccgcgcgg-3′. $RDPheH_{25\text{-}117}$ was expressed in *E. coli* BL21 (DE3) and purified using the same protocol as for RDPheH. The purities of all protein preparations were greater than 95% based on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Protein kinase A was purified from beef heart using the protocol of Flockhart and Corbin (*2*). Phosphorylation of RDPheH by protein kinase A and purification of the phosphorylated protein were performed as previously described (*3*).

Analytical ultracentrifugation

The sedimentation velocity experiments were performed in the Center for Macromolecular Interactions at the University of Texas Health Science Center in San Antonio. A Beckman Coulter XL-I ultracentrifuge (Beckman Instruments, CA, USA) with an An60Ti 4-hole rotor was used at 60, 000 rpm at 20 ^{o}C. All AUC samples were prepared in 50 mM phosphate, 100 mM NaCl, at pH 8.0. Protein concentrations were monitored at 280 or 230 nm. Ultrascan III (*4*) was used for van Holde-Weischet analyses of the AUC data (*5*).The standard c(s) model of SEDFIT was used to generate c(s) distributions of each SV experiment. The values for the weighted-average sedimentation coefficient (s_w) were determined by integration of the c(s) distribution between 1 and 3 S.

KinTek Explorer analysis

KinTek Explorer (*6*) (KinTek Corp, Austin, TX) was used for global analysis of the effects of phenylalanine binding on dimerization and to evaluate different binding models. Several variations on the model in Scheme S1 were considered. The expected s_w values were calculated using eq S1, where s_1 and s_2 are the sedimentation coefficients of the monomer and dimer, respectively, and M and D are the molar concentrations of the monomer and the dimer, respectively. The calculated values for s_w were treated as equilibrium values reached after reaction times from 5 - 60 s. Each forward rate constant was fixed at an arbitrarily high value sufficient that the equilibrium constant was determined only by the value of the respective reverse rate constant. As a result, fitting the dissociation rate constants (k_{-1}, k_{-2} and k_{-3}) also yielded the corresponding equilibrium constants (K_n), including the relative errors, from k_{-n}/k_n; the values for s_1 and s_2 were also allowed to float in these calculations. The statistical confidences for the best-fit values for the equilibrium constants were determined using the FitSpace module of KinTek Explorer (*7*). Reported confidence intervals represent values for each parameter for which the best fit has a sum square error 30% greater than the minimum global error.



Scheme S1

$$s_w = \frac{s_1 * M + s_2 * 2 * \sum(DPhe_n)}{M + 2 * \sum(DPhe_n)} \qquad (2)$$

References

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