HHS Public Access

Author manuscript

Biochem Biophys Res Commun. Author manuscript; available in PMC 2017 August 05.

Published in final edited form as:

Biochem Biophys Res Commun. 2016 August 5; 476(4): 457–461. doi:10.1016/j.bbrc.2016.05.144.

Author Manuscript

The Regulatory Domain of Human Tryptophan Hydroxylase 1 Forms a Stable Dimer

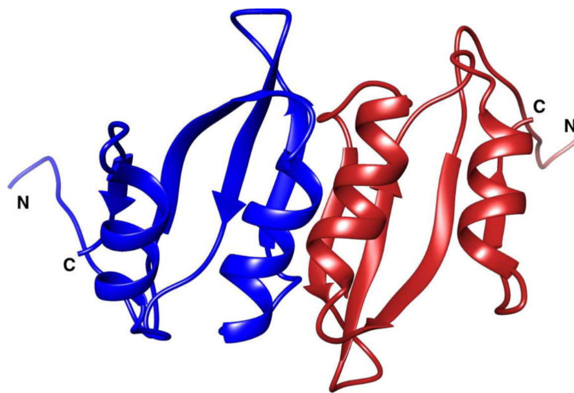
Shengnan Zhang, **Cynthia S. Hinck**, and **Paul F. Fitzpatrick**[*]

Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78229, USA

Abstract

The three eukaryotic aromatic amino acid hydroxylases phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase have essentially identical catalytic domains and discrete regulatory domains. The regulatory domains of phenylalanine hydroxylase form ACT domain dimers when phenylalanine is bound to an allosteric site. In contrast the regulatory domains of tyrosine hydroxylase form a stable ACT dimer that does not bind the amino acid substrate. The regulatory domain of isoform 1 of human tryptophan hydroxylase was expressed and purified; mutagenesis of Cys64 was required to prevent formation of disulfide-linked dimers. The resulting protein behaved as a dimer upon gel filtration and in analytical ultracentrifugation. The s_w value of the protein was unchanged from 2.7–35 μM, a concentration range over which the regulatory domain of phenylalanine hydroxylase forms both monomers and dimers, consistent with the regulatory domain of tryptophan hydroxylase 1 forming a stable dimer stable that does not undergo a monomer-dimer equilibrium. Addition of phenylalanine, a good substrate for the enzyme, had no effect on the s_w value, consistent with there being no allosteric site for the amino acid substrate.

Graphical Abstract



[*]Author to whom correspondence should be addressed. fitzpatrickp@uthscsa.edu; phone: 210-567-8264.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Keywords

Allostery; protein regulation; pterin

Tryptophan hydroxylase (TPH)[1] belongs to the family of the aromatic amino acid hydroxylases, together with tyrosine hydroxylase (TyrH) and phenylalanine hydroxylase (PheH)[1]. They each catalyze the hydroxylation of the aromatic ring of their respective aromatic amino acid substrate, using oxygen and tetrahydrobiopterin as the other substrates. TPH converts tryptophan to 5-hydroxytryptophan in the biosynthesis of the neurotransmitter serotonin. TPH is also the first enzyme in the synthesis of melatonin in the pineal gland [2]. Thus, dysfunction of TPH can result in decreases in serotonin and melatonin, leading to depression and other disorders[3, 4]. Two distinct isoforms of TPH (TPH1 and TPH2) have been identified in humans and other mammals, with an amino acid sequence identity of ~71%[5]. TPH1 is expressed mainly in the gut and pineal gland, while TPH2 is expressed almost exclusively in the brain[6].

All three hydroxylases are homotetramers, with three functional domains: a divergent N-terminal regulatory domain, a homologous catalytic domain, and a C-terminal tetramerization domain[1]. The catalytic domains have three-dimensional structures that are essentially identical[7–9]. The regulatory domains show a much lower sequence identity and vary in length (Fig. 1), suggesting that these enzymes have distinct regulatory mechanisms. Indeed, the regulatory mechanisms of TyrH and PheH have been established to be different[10]; however, the regulatory mechanism of TPH is poorly understood due largely to difficulties in obtaining stable recombinant enzyme for study.

PheH is activated by its substrate phenylalanine and by phosphorylation at Ser16 and inhibited by tetrahydrobiopterin[11]. The resting form of PheH is inactive, with the N-terminus of the regulatory domain partially occluding the active site[12]. Phenylalanine binding to an allosteric site in the regulatory domain[13, 14] causes a significant conformational change that opens up the active site[15, 16]. Key to this conformational change is formation of an ACT domain dimer by regulatory domains from two subunits[17, 18]. This model is supported by recent studies of the isolated regulatory domain[14, 19, 20] and of the intact protein[13, 18]. TyrH is activated by phosphorylation of Ser40 in its N-terminal regulatory domain and inhibited by catecholamines[10, 21]. While there is no available structure of TyrH with both the regulatory and catalytic domains, several studies support a model in which the protein exists in two conformations, one stabilized by catecholamine binding in which the N-terminus extends across the active site and prevents substrate access in a similar fashion to PheH, and one stabilized by phosphorylation in which the active site is accessible[22–24]. Studies of the isolated regulatory domain of TyrH have established that it forms a stable ACT domain dimer in solution even in the absence of tyrosine[25]. This supports a model in which TyrH has a quaternary structure similar to the active form of PheH, with two regulatory domains forming a side-by-side ACT domain dimer, but the N-terminus of the regulatory domain still extending across the active site[10].

[1]Abbreviations: TPH, tryptophan hydroxylase; PheH, phenylalanine hydroxylase; TyrH, tyrosine hydroxylase; RDTPH, regulatory domain of tryptophan hydroxylase; AUC analytical ultracentrifugation; s_W, weight average sedimentation coefficient.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

TyrH contains a flexible N-terminal tail of ~ 70 residues[25], much longer than that in PheH, allowing it to accommodate the increased distance to the active site when the regulatory domain dimerizes. Thus, while regulation of TyrH and PheH both involve an N-terminal extension from an ACT domain regulatory domain hindering access to the active site, they differ in whether the regulatory domain forms a dimer reversibly.

PheH and TyrH provide discrete models for the arrangement of regulatory domains in TPH, with clear implications for regulation of that enzyme. To better understand the regulation of TPH, we have expressed the isolated regulatory domain of human TPH1 (RDTPH1) and analyzed the oligomerization state of the protein. The results suggest that the regulatory domain of TPH1 forms a dimer in the resting form of the enzyme similar to that in TyrH despite the greater sequence similarity to PheH.

Experimental Procedures

Materials

Dithiothreitol was from Inalco, S.p.A. (Milan, Italy). Leupeptin and pepstatin A were from Peptide Institute, Inc (Osaka, Japan). Restriction and DNA modification enzymes were purchased from New England Biolabs (Ipswich, MA). All the other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO). The plasmid pGro7 for co-expression of GroEL and GroES was from TaKaRa Bio Inc.

Protein Expression and Purification

A synthetic gene for human TPH1 optimized for expression in *Escherichia coli* was obtained from DNA2.0 (Menlo Park, CA). The gene, which contains a histidine tag at the N-terminus, was extracted from the commercial vector and cloned into pET23d using the restriction enzymes NcoI and BamHI. The plasmid for expressing only the regulatory domain of TPH1 (RDTPH1, residues 1–101) was constructed by introducing a stop codon into the gene for TPH1 in pET23d. Cys64 in RDTPH1 was mutated to serine using the QuikChange Mutagenesis protocol (Agilent Technologies). Sequences of the resulting plasmids were verified by DNA sequencing (Genscript, NJ). Both plasmids were used to transform BL21 (DE3) cells containing pGro7.

For protein expression and purification, a single colony from an agar plate was used to inoculate LB media containing 150 μg/L ampicillin, 50 μg/L chloramphenicol, and 0.5 mg/ml arabinose. After overnight growth 14 ml were transferred to 1 l of the same media. When the absorbance at 600 nm reached 0.3, the temperature was reduced to 18 °C. Expression was induced with 0.25 mM isopropyl β-D-1-thiogalactopyranoside when the absorbance at 600 nm reached 0.6; cells were harvested after 20 h. The cell pellets were suspended in lysis buffer (300 mM NaCl, 10 mM imidazole, 1 μM pepstatin A, 1 μM leupeptin, 50 mM phosphate, pH 8.0) plus 100 μg/ml phenylmethylsufonyl fluoride, and lysed by sonication. The resulting cell suspension was centrifuged at 30,000g for 30 min at 4 °C. The supernatant was loaded onto a 5 mL HisTrap HP column (GE Healthcare) equilibrated with lysis buffer. The column was washed with 50 ml lysis buffer; the protein was then eluted with a 100 ml gradient of lysis buffer containing 0–0.5 M imidazole. The

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

fractions showing a band with an apparent molecular weight of ~13 kDa in a SDS-polyacrylamide gel were pooled and concentrated using an Amicon Ultra-15 10K centrifugal filter (10K molecular weight cutoff, Millipore). The concentrated sample was then loaded to a HiPrep 16/60 Superdex 200 pre grade (GE Healthcare life science, Piscataway, NJ) gel filtration column equilibrated with 50 mM phosphate, 100 mM NaCl, pH 8.0. Fractions containing pure protein were pooled and stored at −80 °C. The protein concentration was calculated using an e_{230} of 23.2 $mM^{-1}cm^{-1}$, determined from the results of amino acid analysis of a purified sample at the Protein Chemistry Laboratory at Texas A&M University. The purity of all enzyme preparations was greater than 95% based on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. For molecular weight determinations, the Superdex column was standardized with aprotinin, ribonuclease A, carbonic anhydrase, ovalbumin, and conalbumin.

Analytical Ultracentrifugation

Analytical ultracentrifugation (AUC) sedimentation velocity analyses were performed using a Beckman Coulter XL-I ultracentrifuge (Beckman Instruments, CA, USA) with an An60Ti 4-hole rotor at 20 °C at 5000 rpm and monitored at 230 nm. All AUC samples were prepared in a buffer of 100 mM NaCl, 50 mM phosphate, pH 8.0. Ultrascan III[26] was used for van Holde-Weischet analyses of the AUC data. The standard c(s) model of SEDFIT[27] version 14.1 was used to generate c(s) distributions. The values for the weighted-average sedimentation coefficient (s_w) were determined by integration of the c(s) distribution between 1 and 3 S.

Results and Discussion

Expression of the regulatory domain of human TPH1

Based on previous successful efforts to obtain the regulatory domains of PheH and TyrH[19, 25], an expression vector was constructed for a protein containing the N-terminal residues of human TPH1 through Gly101, just before the conserved VPWFPR sequence of the catalytic domain (Fig. 1). This allowed expression of the isolated regulatory domain in *E. coli* BL21(DE3) cells. Co-expression of the chaperones GroEL and GroES resulted in a significant amount of soluble protein, suggesting that RDTPH1 is properly folded. However, upon purification, RDTPH1 showed two protein bands around 13 kDa and 26 kDa in SDS-polyacrylamide gel electrophoresis in the absence of reductant, but only one band of 13 kDa in the presence of dithiothreitol (data not shown). This suggested that RDTPH1 forms a disulfide bond in solution. To avoid this, the single cysteine residue in the regulatory domain, Cys64, was mutated to serine to yield RDTPH1 C64S. Expression of RDTPH1 C64S in *E. coli* BL21(DE3) cells containing pGro7 also resulted in a significant amount of soluble protein, and only one band of 13 kDa in SDS-polyacrylamide gel electrophoresis in the absence or presence of reductant. Kuhn et al.[28] previously reported that the regulatory domain of TPH2 forms disulfide-linked dimers and that this involves Cys110, the analogous residue to Cys64 in TPH1.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Oligomerization state of the regulatory domain of TPH1

The oligomerization state of purified RDTPH1 C64S was initially analyzed using gel filtration. During purification the protein eluted as two individual peaks with retention volumes consistent with molecular weights of 22 kDa and 41 kDa, respectively (data not shown). The calculated molecular weight for monomeric RDTPH1 C64S is 12.7 kDa, suggesting that the regulatory domain of TPH1 forms dimers and tetramers in solution. No evidence was seen for a monomeric species. When the dimeric species was collected and rerun over the gel filtration column, a single predominant peak was seen that eluted with a calculated molecular weight of 23 kDa (Fig. 2). A 2D ^{1}H-^{15}N HSQC NMR spectrum of the purified protein at concentrations of 100 μM only showed crosspeaks around 7.5–8.5 ppm and above (data not shown). This suggested that the dimer of RDTPH1 C64S readily aggregates to form higher oligomers in solution, consistent with the tetramer seen in the gel filtration studies. Similar aggregation was previously observed for the regulatory domains of TyrH and PheH using NMR spectroscopy; however, for those proteins this occurred over several days at higher protein concentrations (0.4–1 mM). It has previously been reported that removal of the regulatory domain is required for expression of TPH in recombinant systems[29]; this is consistent with this region of the protein tending to aggregate even in the context of the intact protein.

The oligomerization state of RDTPH1 C64S as a function of protein concentrations was analyzed further by sedimentation velocity analytical ultracentrifugation. Detection was at 230 nm, since this protein lacks tryptophan and tyrosine residues. The protein concentration ranged from 2.7 to 35 μM. The results are shown in Table 1. Over this concentration range the weight-average sedimentation coefficient was independent of the protein concentration, with an average value of 1.84 S (Table 1). The lack of any dependence of the s_w value on the protein concentration establishes that there is no meaningful equilibrium between different oligomeric states of RDTPH1 C64S with a K_{eq} between ~1 and 100 μM. In contrast, the K_{eq} for dimerization of the regulatory domain of PheH is 34 μM[20], and the s_w value is strongly dependent on the protein concentration over the concentration range used here. Thus, TPH1 resembles TyrH, where the regulatory domain forms a stable dimer, rather than PheH in the effect of concentration on its quaternary structure.

To determine if the oligomeric status of RDTPH1 is sensitive to the presence of its substrate, an AUC experiment was carried out with 15 μM RDTPH1 C64S in the presence and absence of 1 mM phenylalanine. Phenylalanine rather than tryptophan was used to detect the interaction because of the strong UV absorbance of tryptophan. Phenylalanine is an effective substrate of TPH1, with a k_{cat}/K_m value close to that for tryptophan[30, 31]. The van Holde-Weischet distribution for RDTPH1 C64S is unchanged in the presence of phenylalanine (Fig. 2), suggesting that this amino does not have a significant interaction with the regulatory domain of TPH1. Tryptophan has been reported to have no effect on the stability of the isolated regulatory domain of TPH1[14], suggesting that the natural substrate does not bind the protein either. The allosteric site for phenylalanine binding to the regulatory domain of PheH has been identified. While most of the residues that interact with phenylalanine in that protein are conserved in TPH1 and 2, almost all the interactions are with backbone carbonyls and amides rather than amino acid side chains. The one exception is Glu43 in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

PheH, which has one carboxylate oxygen 2.6 Å from the amino group of phenylalanine; this residue is an asparagine in both TPH1 and TPH2 and thus cannot form a similar ionic interaction. Thus, TPH1 resembles TyrH more than PheH in lacking an allosteric site for its amino acid substrate in the regulatory domain[10].

The s_w values for the monomer and dimer forms of $RDPheH_{25-117}$ are 1.2 and 2.1[20], respectively, compared to the value of 1.8 (Table 1) for RDTPH1 C64S, although the molecular weight for the $RDPheH_{25-117}$ monomer is 12.8 kDa, very close to that of RDTPH1 C64S. The gel filtration results (Figure 2) support a dimer for the quaternary structure of RDTPH1 C64S, and the s_w value for RDTPH1 C64S is significantly closer to that of the $RDPheH_{25-117}$ dimer, so that the present data are most consistent with the quaternary structure of RDTPH1 C64S being dimeric. While there is no structure for a regulatory domain of TPH, this domain is readily identified as containing an ACT domain from its sequence[32]. The sequences of the cores of the regulatory domains of TPH1 (residues 17–101) and TPH2 (residues 63–147) are very similar to the sequence of the ACT domain core of PheH (residues 35–117) (Figure 1). Although the sequences of the regulatory domains of TyrH and PheH are quite different, their three-dimensional structures are similar: core ACT domains with flexible N-terminal tails[12, 25]. [13, 14, 25] Since the regulatory domains of both PheH and TyrH form side-by-side ACT dimers[13, 14, 25], it is reasonable to conclude that the regulatory domain of TPH1 also does so. The lower s_w value for RDTPH1 C64S can be attributed to either an increased frictional coefficient or a decreased partial specific volume, suggesting that the TPH regulatory dimer is somewhat more open that those of PheH and TyrH.

The present results predict that the quaternary structure of TPH1, and likely TPH2, resembles those of TyrH[25] and phenylalanine-activated PheH[10, 18]. Figure 4 shows a model for the structure of the dimer of the regulatory domain of tTPH1 based on these structures. The extended beta sheet formed in the dimer would form the interface with the catalytic domains, with the flexible N-termini extending over the catalytic domain. The flexible N-terminal tails in the regulatory domains of PheH and TyrH end at Ala34 and Val 79, respectively (Fig.1). The model in Fig. 4 predicts that TPH1 has a flexible N-terminal tail of 17 residues, while a similar structure for the regulatory domain of TPH2 would predict a flexible N-terminal tail of 63 residues. If so, the short N-terminal tail of TPH1 is likely to be too short to hinder access to the active site in the same way that the much longer of TyrH does, while the longer tail in TPH2 could do so. At present there is no evidence for TPH2 requiring activation in the fashion of PheH or dopamine-inhibited TyrH, and thus no evidence for a similar structural change being required to allow substrate access to the active site. While TPH2 is phosphorylated on Ser19 by protein kinase A both in vitro and in cells, this results in a modest effect on activity but a significant increase in affinity for some 14-3-3 proteins[31, 33]. TyrH similarly binds 14-3-3 proteins more tightly upon binding phosphorylation of Ser19[34], but there is only a small effect on activity, while there is no evidence that PheH binds 14-3-3 proteins.

In summary, the present results support a structure for the resting form of intact TPH1 that resembles TyrH and phenylalanine-activated PheH in having its regulatory domains as ACT dimers rather than one resembling nonactivated PheH. The short N-terminal tail resulting

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from formation of a side-by-side ACT dimer in TPH1 similar to that in the other two enzymes would appear to preclude a regulatory mechanism in which the N-terminal tail acts as an auto-inhibitory peptide[15]. In contrast, the longer N-terminal tail in TPH2 could allow regulatory mechanisms similar to those in TyrH to operate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Jinny Johnson from the Protein Chemistry Lab at Texas A&M University for the amino acid analyses. Support for the Center for Macromolecular Interactions from the Office of the Vice President for Research at UTHSCSA is gratefully acknowledged.

Funding

This work was supported by the National Institutes of Health [grant R01 GM098140].

References

1. Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. Ann. Rev. Biochem. 1999; 68:355–381. [PubMed: 10872454]
2. Lovenberg W, Jequier E, Sjoerdsma A. Tryptophan hydroxylation in mammalian systems. Advances in pharmacology. 1968; 6:21–36. [PubMed: 5664047]
3. Antypa N, Serretti A, Rujescu D. Serotonergic genes and suicide: A systematic review. Eur. Neuropsychopharmacol. 2013; 23:1125–1142. [PubMed: 23742855]
4. De Luca V, Likhodi O, Van Tol HH, Kennedy JL, Wong AH. Tryptophan hydroxylase 2 gene expression and promoter polymorphisms in bipolar disorder and schizophrenia. Psychopharmacol. 2005; 183:378–382.
5. Walther DJB, Michael. A unique central tryptophan hydroxylase isoform. Biochem. Pharmacol. 2003; 66:1673–1680. [PubMed: 14563478]
6. Sakowski SA, Geddes TJ, Thomas DM, Levi E, Hatfield JS, Kuhn DM. Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. Brain Res. 2006; 1085:11–18. [PubMed: 16581041]
7. Goodwill KE, Sabatier C, Marks C, Raag R, Fitzpatrick PF, Stevens RC. Crystal structure of tyrosine hydroxylase at 2.3 Å and its implications for inherited neurodegenerative diseases. Nat. Struct. Biol. 1997; 4:578–585. [PubMed: 9228951]
8. Andersen OA, Flatmark T, Hough E. High resolution crystal structures of the catalytic domain of human phenylalanine hydroxylase in its catalytically active Fe(II) form and binary complex with tetrahydrobiopterin. J. Mol. Biol. 2001; 314:279–291. [PubMed: 11718561]
9. Wang L, Erlandsen H, Haavik J, Knappskog PM, Stevens RC. Three-dimensional structure of human tryptophan hydroxylase and its implications for the biosynthesis of the neurotransmitters serotonin and melatonin. Biochemistry. 2002; 41:12569–12574. [PubMed: 12379098]
10. Fitzpatrick PF. Structural insights into the regulation of aromatic amino acid hydroxylation. Curr. Opin. Struct. Biol. 2015; 35:1–6. [PubMed: 26241318]
11. Fitzpatrick PF. Allosteric regulation of phenylalanine hydroxylase. Arch. Biochem. Biophys. 2012; 519:194–201. [PubMed: 22005392]
12. Kobe B, Jennings IG, House CM, Michell BJ, Goodwill KE, Santarsiero BD, Stevens RC, Cotton RGH, Kemp BE. Structural basis of autoregulation of phenylalanine hydroxylase. Nat. Struct. Biol. 1999; 6:442–448. [PubMed: 10331871]
13. Zhang S, Fitzpatrick PF. Identification of the allosteric site for phenylalanine in rat phenylalanine hydroxylase. J. Biol. Chem. 2016; 291:7418–7425. [PubMed: 26823465]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

14. Patel D, Kopec J, Fitzpatrick F, McCorvie TJ, Yue WW. Structural basis for ligand-dependent dimerization of phenylalanine hydroxylase regulatory domain. Sci. Rep. 2016; 6:23748. [PubMed: 27049649]
15. Jennings IG, Teh T, Kobe B. Essential role of the N-terminal autoregulatory sequence in the regulation of phenylalanine hydroxylase. FEBS Lett. 2001; 488:196–200. [PubMed: 11163771]
16. Roberts KM, Khan CA, Hinck CS, Fitzpatrick PF. Activation of phenylalanine hydroxylase by phenylalanine does not require binding in the active site. Biochemistry. 2014; 53:7846–7853. [PubMed: 25453233]
17. Jaffe EK, Stith L, Lawrence SH, Andrake M, Dunbrack RL Jr. A new model for allosteric regulation of phenylalanine hydroxylase: Implications for disease and therapeutics. Arch. Biochem. Biophys. 2013; 530:73–82. [PubMed: 23296088]
18. Meisburger SP, Taylor AB, Khan CA, Zhang S, Fitzpatrick PF, Ando N. Domain movements upon activation of phenylalanine hydroxylase characterized by crystallography and chromatography-coupled small-angle X-ray scattering. J. Am. Chem. Soc. 2016 in press.
19. Li J, Ilangovan U, Daubner SC, Hinck AP, Fitzpatrick PF. Direct evidence for a phenylalanine site in the regulatory domain of phenylalanine hydroxylase. Arch. Biochem. Biophys. 2011; 505:250–255. [PubMed: 20951114]
20. Zhang S, Roberts KM, Fitzpatrick PF. Phenylalanine binding is linked to dimerization of the regulatory domain of phenylalanine hydroxylase. Biochemistry. 2014; 53:6625–6627. [PubMed: 25299136]
21. Daubner SC, Le T, Wang S. Tyrosine hydroxylase and regulation of dopamine synthesis. Arch. Biochem. Biophys. 2011; 508:1–12. [PubMed: 21176768]
22. McCulloch RI, Fitzpatrick PF. Limited proteolysis of tyrosine hydroxylase identifies residues 33-50 as conformationally sensitive to phosphorylation state and dopamine binding. Arch. Biochem. Biophys. 1999; 367:143–145. [PubMed: 10375411]
23. Wang S, Sura GR, Dangott LJ, Fitzpatrick PF. Identification by hydrogen/deuterium exchange of structural changes in tyrosine hydroxylase associated with regulation. Biochemistry. 2009; 48:4972–4979. [PubMed: 19371093]
24. Wang S, Lasagna M, Daubner SC, Reinhart GD, Fitzpatrick PF. Fluorescence spectroscopy as a probe of the effect of phosphorylation at Ser40 of tyrosine hydroxylase on the conformation of its regulatory domain. Biochemistry. 2011; 50:2364–2370. [PubMed: 21302933]
25. Zhang S, Huang T, Ilangovan U, Hinck AP, Fitzpatrick PF. The solution structure of the regulatory domain of tyrosine hydroxylase. J. Mol. Biol. 2014; 426:1483–1497. [PubMed: 24361276]
26. Demeler, B. UltraScan-a comprehensive data analysis software package for analytical ultracentrifugation experiments. In: Scott, DJ.; Harding, SE.; Row, AJ., editors. Modern Analytical Ultracentrifugation: Techniques and Methods, Royal Society of Chemistry, London. 2005. p. 210-229.
27. Schuck P. Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling. Biophys. J. 2000; 78:1606–1619. [PubMed: 10692345]
28. Kuhn DM, Sykes CE, Geddes TJ, Jaunarajs KLE, Bishop C. Tryptophan hydroxylase 2 aggregates through disulfide cross-linking upon oxidation: possible link to serotonin deficits and non-motor symptoms in Parkinson's disease. J. Neurochem. 2011; 116:426–437. [PubMed: 21105877]
29. Moran GR, Daubner SC, Fitzpatrick PF. Expression and characterization of the catalytic core of tryptophan hydroxylase. J. Biol. Chem. 1998; 273:12259–12266. [PubMed: 9575176]
30. Moran GR, Derecskei-Kovacs A, Hillas PJ, Fitzpatrick PF. On the catalytic mechanism of tryptophan hydroxylase. J. Am. Chem. Soc. 2000; 122:4535–4541.
31. McKinney J, Knappskog PM, Haavik J. Different properties of the central and peripheral forms of human tryptophan hydroxylase. J. Neurochem. 2005; 92:311–320. [PubMed: 15663479]
32. Aravind L, Koonin EV. Gleaning non-trivial structural, functional and evolutionary information about proteins by iterative database searches. J. Mol. Biol. 1999; 287:1023–1040. [PubMed: 10222208]

33. Winge I, McKinney JA, Ying M, D'Santos CS, Kleppe R, Knappskog PM, Haavik J. Activation and stabilization of human tryptophan hydroxylase 2 by phosphorylation and 14-3-3 binding. Biochem. J. 2008; 410:195–204. [PubMed: 17973628]

34. Kleppe R, Rosati S, Jorge-Finnigan A, Alvira S, Ghorbani S, Haavik J, Valpuesta JM, Heck AJ, Martinez A. Phosphorylation dependence and stoichiometry of the complex formed by tyrosine hydroxylase and 14-3-3gamma. Mol. Cell. Proteomics. 2014; 13:2017–2030. [PubMed: 24947669]

35. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23:2947–2948. [PubMed: 17846036]

36. Robert X, Gouet P. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res. 2014; 42:W320–W324. [PubMed: 24753421]

37. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera-A visualization system for exploratory research and analysis. J. Comput. Chem. 2004; 25:1605–1612. [PubMed: 15264254]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

- The C64S variant regulatory domain of human tryptophan hydroxylase 1 was expressed.
- The results of gel filtration are consistent with formation of a dimer.
- Analytical ultracentrifugation shows the oligomeric state does not change with concentration.
- The regulatory domain does not bind amino acid substrates.
- The implications for regulation of tryptophan hydroxylase 1 are discussed.

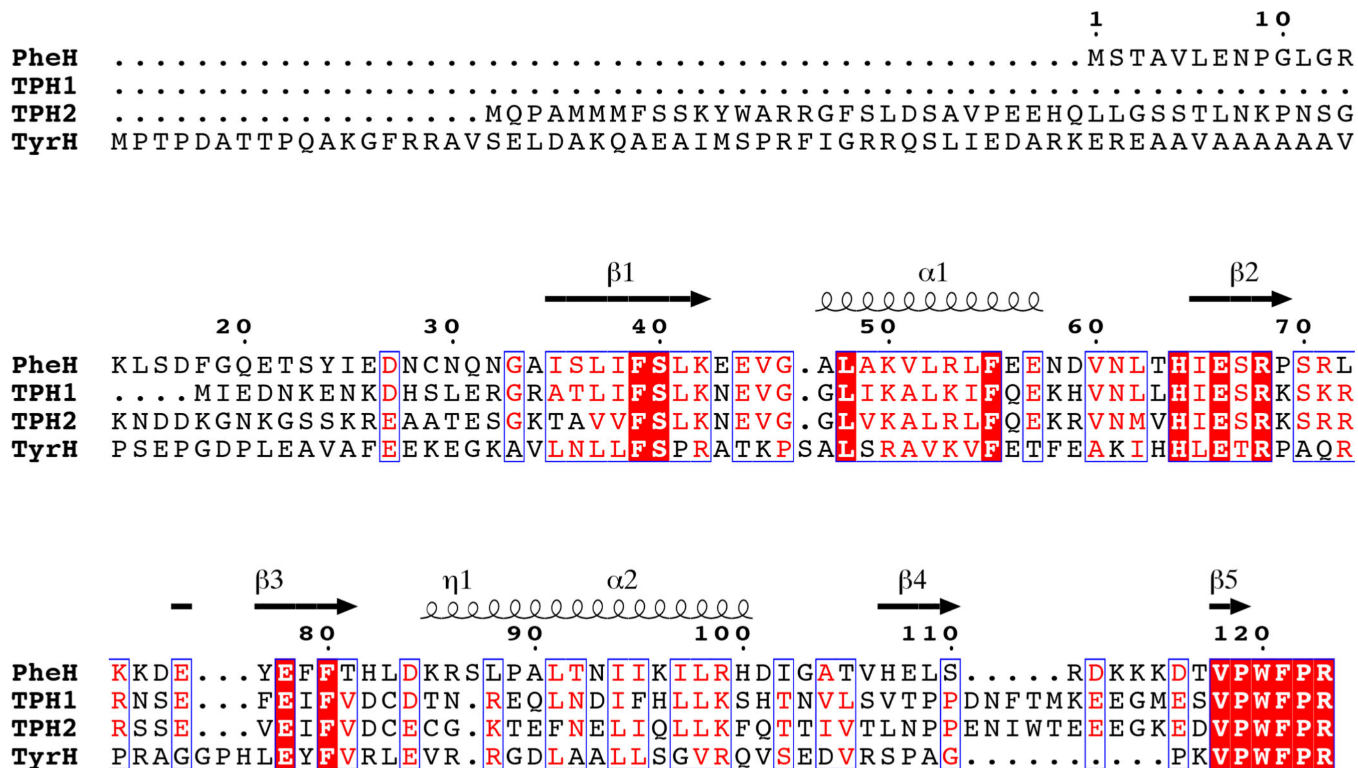


Figure 1.

Sequence alignment of the regulatory domains of the human aromatic amino acid hydroxylases. The secondary structure locations are from the crystal structure of rat PheH (2PHM). Residues that are identical in all four proteins are highlighted in red. The alignment was generated using CLUSTALW[35]. The figure was generated using ESPript[36].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

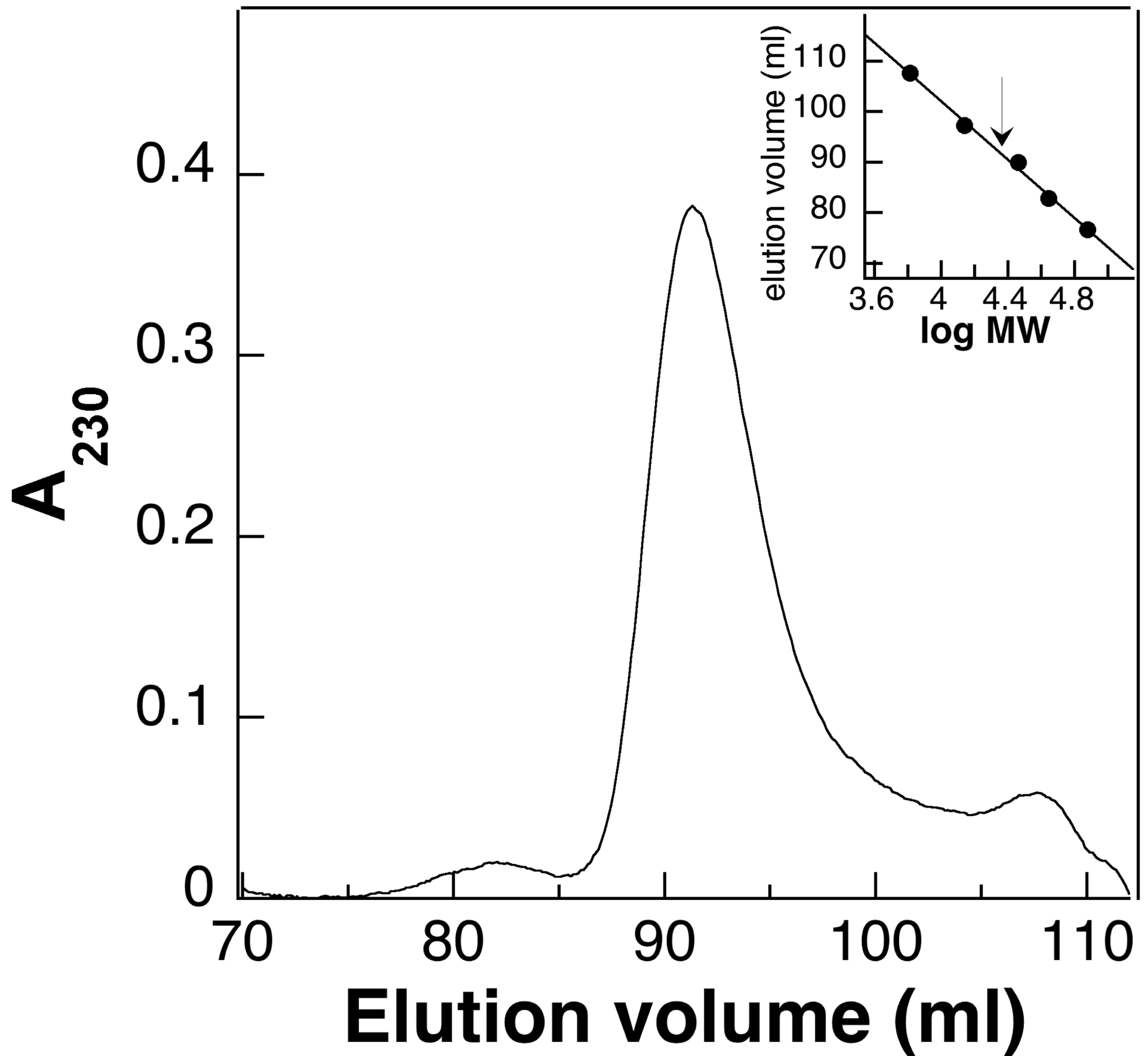


Figure 2.
Gel filtration of RDTPH1 C64S. Conditions: 100 mM NaCl, 1 μM pepstatin A, 1 μM leupeptin, 50 mM phosphate, pH 8.0, 23 °C. Inset: elution volumes of the standard proteins aprotinin (6,500 Da), ribonuclease A (13,700 Da), carbonic anhydrase (29,000 Da) ovalbumin (44,000 Da), conalbumin (75,000 Da). The arrow indicates the elution volume of the major peak in the chromatogram.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

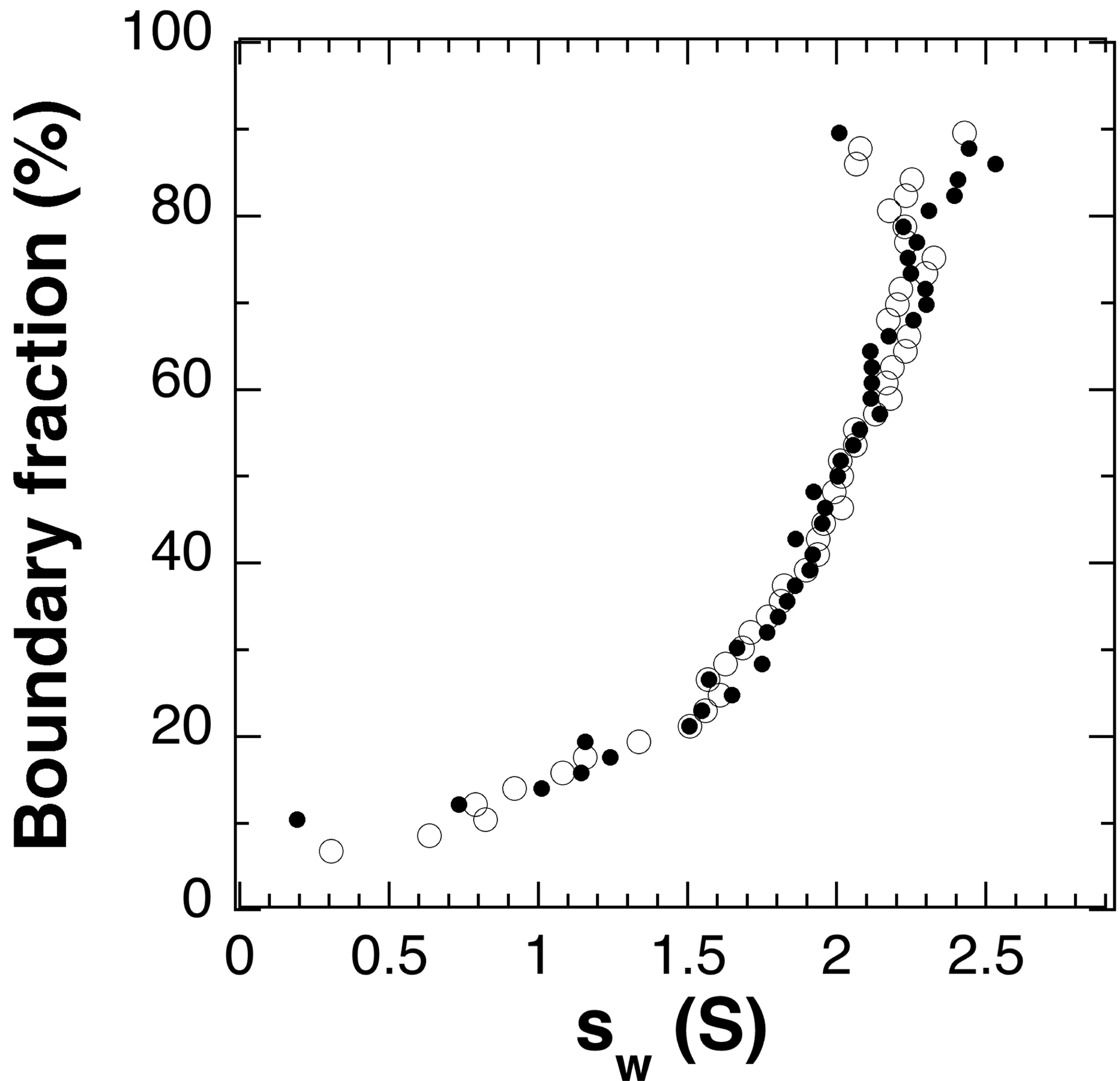


Figure 3.

Van Holde-Weischet distribution plot for RDTPH1 C64S (15 μM total monomer) without (open circles) and with 1 mM phenylalanine (filled circles). Buffer conditions: 50 mM phosphate, 100 mM NaCl, pH 8.0, 20 °C.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

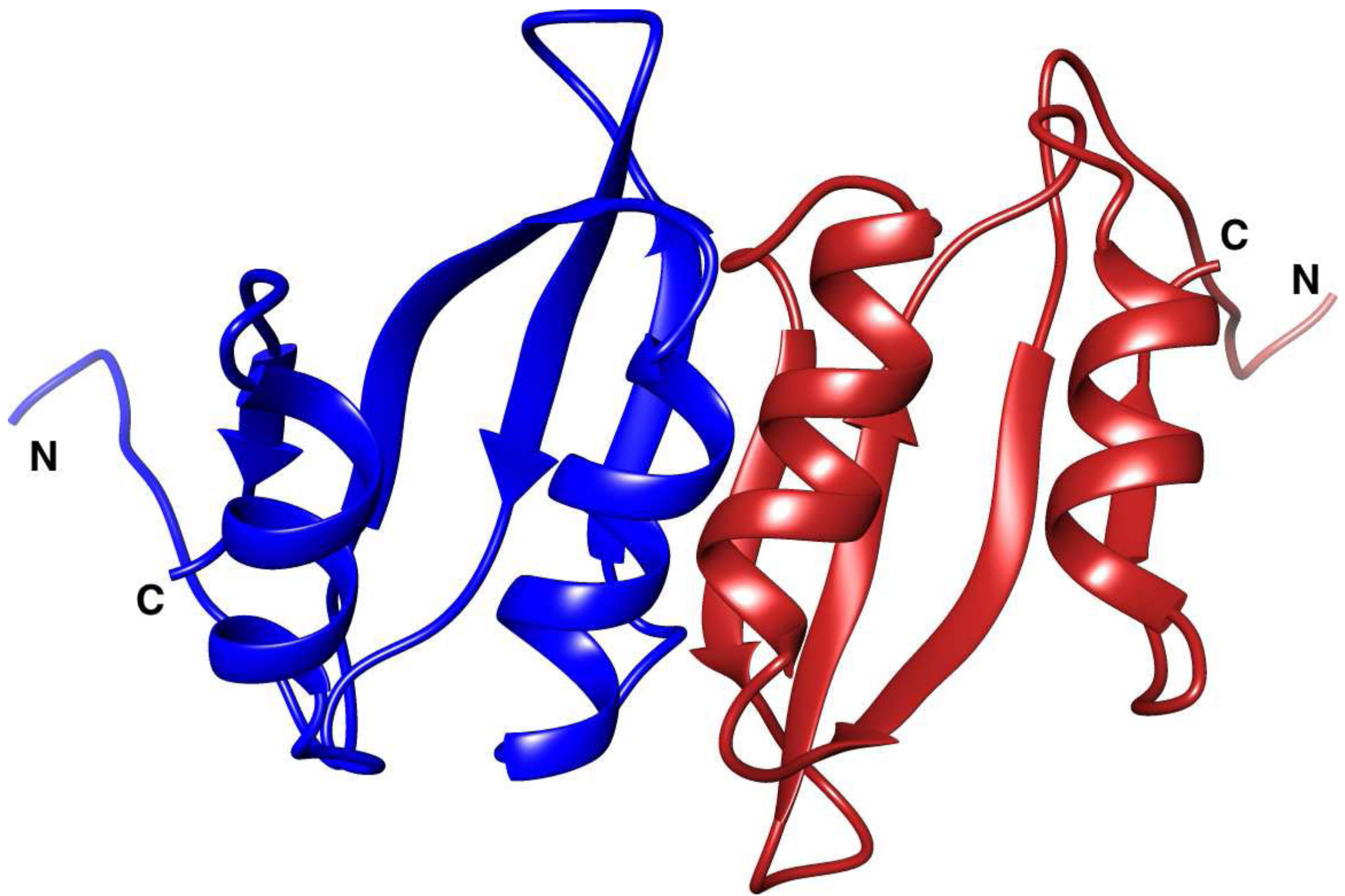


Figure 4.

Model of the structure of the dimer of the regulatory domain of human tryptophan hydroxylase 1. The model was constructed using Chimera[37] based on the structure of the dimers of the regulatory domains of tyrosine hydroxylase and phenylalanine hydroxylase.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Sedimentation coefficients for the regulatory domains of TPH1 and PheH.

protein	Concentration (μM)	s_w (S)	s_w (S) plus 1 mM phenylalanine
RDTPH1 C64S	2.7	1.84	1.87
	8.0	1.84	
	14.6	1.80	1.81
	16.5	1.83	
	24	1.89	
	35	1.85	
	average	1.84 ± 0.03	1.84 ± 0.04
$RDPheH_{25-117}$[a]	7.2	1.37	2.12
	Monomer	1.22	
	Dimer	2.11	2.11

[a] reference[20].