Structural and Mechanistic Studies with Pterin-4a-carbinolamine Dehydratase from *Pseudomonas aeruginosa.* Three-Dimensional Structure and Comparison with the Human Enzyme.

Spacing: 3x *12° (point) steps*

S. Köster, H.-Ch. Curtius, S. Ghisla
Department of Molecular Biology, University of Konstanz, Germany
R. Ficner
Institute of Molecular Biology and Tumor Research, University of Marburg, Germany
G. Stier, D. Suck
EMBL, Structural Biology Program, Heidelberg, Germany
U.H. Sauer
Umeå Institute of Molecular Pathogenesis UCMP, Umeå University, Sweden

Spacing: 3x 12° (point) steps

Introduction

Mammalian pterin-4a-carbinolamine dehydratase (PCD/DCoH) is a bifunctional protein that catalyzes the dehydration of 4a-OH-tetrahydrobiopterin (4a-carbinol-amine) to quinonoid dihydrobiopterin (1). This reaction is a part of the regeneration cycle of tetrahydrobiopterin, the essential cofactor of the aromatic amino acid hydroxylases. PCD/DCoH has a second function as a dimerization cofactor for the transcriptional protein hepatocyte nuclear factor 1-α (HNF1-α) (2). PCD from *Pseudomonas aeruginosa* (PCD/PhhB) also has been proposed to be a bifunctional protein, since it appears to regulate the expression of phenylalanine hydroxylase (PAH) (3). The genes for these proteins are placed next to each other and are controlled by the same operon. Human PCD/DCoH and PCD/PhhB have ~30 % identity and ~60 % similarity.

Spacing: 3x 12° (point) steps

Materials and Methods

PCD/PhhB and the mutant enzymes were expressed in *E.coli* BL21(DE3) and purified to homogeneity by affinity chromatography over Ni-nitriloacetic acid-agarose column as detailed in (4). It should be noted that the enzyme contains no His-tag; it never-theless binds to the column due to the presence of five closely located histidines.

Spacing: 3x 12° (point) steps

Results

Three-dimensional structure of PCD/PhhB

The structure was refined at a resolution of 1.75 Å. The enzyme crystallizes as a homodimer, which appears to be the functional form also in solution. One monomer consists of three α-helices which are packed against a four-stranded anti-parallel β-sheet. The dimer is built by interaction of the α-helices a2 and the β-sheets b3 of

two monomers. In Fig.1 the dimer of PCD/PhhB (right hand side) and a dimer of human PCD/DCoH (left), which normally build a tetramer, are shown side by side for comparison.

Figure 1: Ribbon rendering of the dimers of PCD/PhhB (left) and PCD/DCoH (right).

Spacing: 3x 12° (point) steps

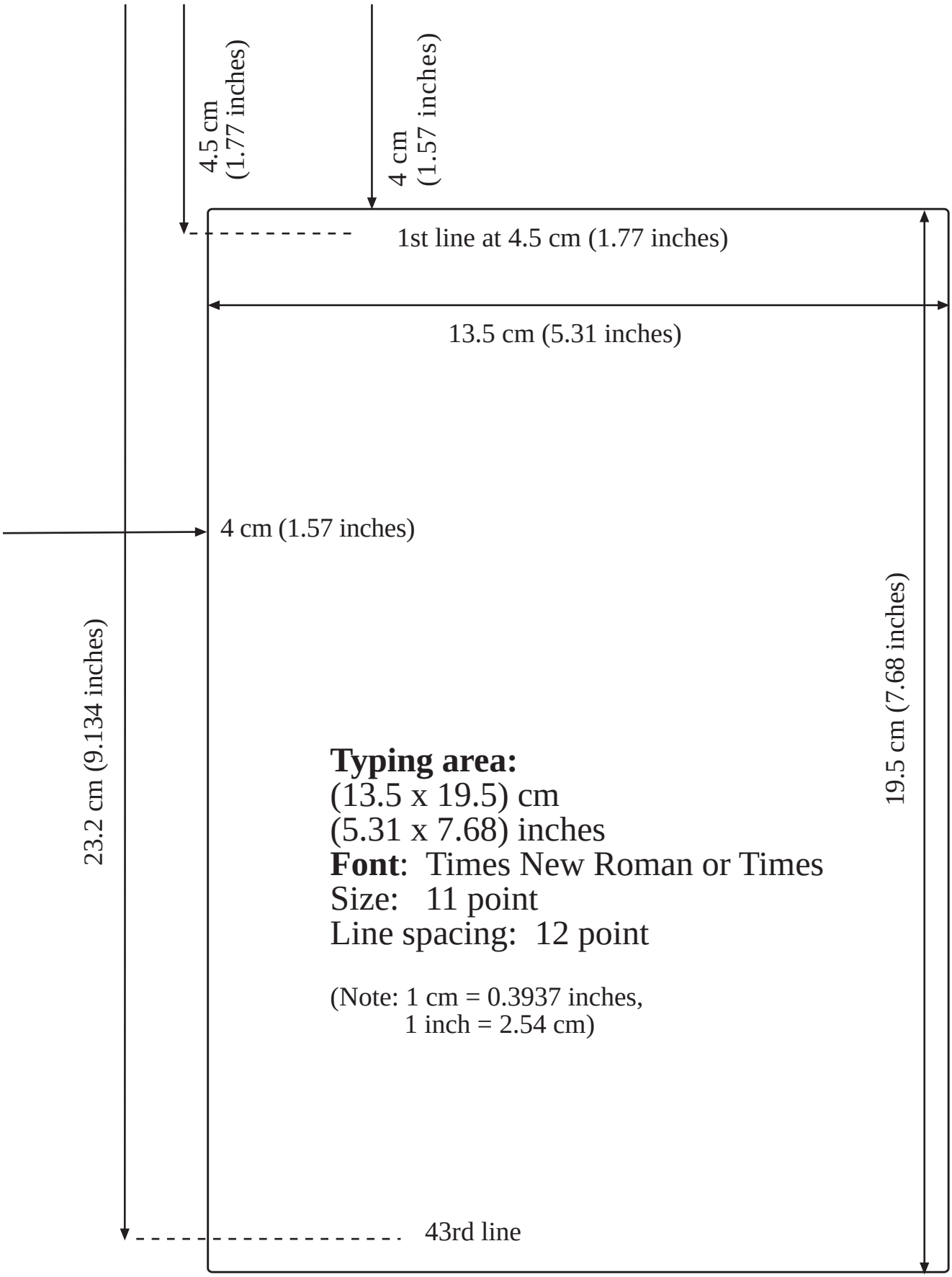
Catalytic properties of PCD/PhhB and some mutants
The enzymatic activity of PCD/PhhB observed in the direct assay using the synthetic substrate 6-MeCA is ~40 % of that found with human PCD/DCoH (Table 1), whereas in the enzyme coupled assay it has approximately the same value (90 %) (4).

Spacing: 3x 12° (point) steps

Discussion
The homodimeric enzyme PCD/PhhB shows a high similarity to the dimer of PCD/DCoH, although the amino acid sequence is only 30 % identical.

Spacing: 3x 12° (point) steps

References
1. Kaufman S. (1970): A protein that stimulates rat liver phenylalanine hydroxylase. J. Biol.Chem. **245**, 4751–4759.
Spacing: 1x 12° (point) step
2. Cronk J.D., Endrizzi J.A., Alber T. (1996): High resolution structures of the bifunctional enzyme and transcriptional coactivator DCoH and its complex with a product analogue. Protein Science **5**, 1963–1972.
Spacing: 1x 12° (point) step
3. Köster S., Stier G., Ficner R., et al (1996): Location of the active side and proposed catalytic mechanism of pteridin-4a-carbinolamine dehydratase. Eur. J. Biochem. **241**, 858–864.


4.5 cm
(1.77 inches)
4 cm
(1.57 inches)
1st line at 4.5 cm (1.77 inches)
13.5 cm (5.31 inches)
4 cm (1.57 inches)
23.2 cm (9.134 inches)
19.5 cm (7.68 inches)
Typing area:
(13.5 x 19.5) cm
(5.31 x 7.68) inches
Font: Times New Roman or Times
Size: 11 point
Line spacing: 12 point
(Note: 1 cm = 0.3937 inches,
1 inch = 2.54 cm)
43rd line