Protein Science (1992), *1*, 688–690. Cambridge University Press. Printed in the USA.

FOR THE RECORD

Human platelet-derived endothelial cell growth factor is homologous to *Escherichia coli* thymidine phosphorylase

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(RECEIVED December 12, 1991; REVISED MANUSCRIPT RECEIVED January 28, 1992)

Monomeric human platelet-derived endothelial cell growth factor (PD-ECGF) is a single-chain protein of relative molecular mass (M_r) ≈45 kDa, which stimulates the growth and chemotaxis of endothelial cells in vitro and possesses angiogenic activity in vivo (Miyazono et al., 1987; Ishikawa et al., 1989). As angiogenesis is central to the pathological conditions of tumor growth, rheumatoid arthritis, diabetic retinopathy, psoriasis, and hemangiomas, a detailed understanding of the molecular action of PD-ECGF would provide clues to therapeutic strategies for these disease states.

Here we report the striking similarity between the primary sequences of PD-ECGF and thymidine phosphorylase (TP) from *Escherichia coli* (Shwartz, 1978). Human TP catalyzes the reversible phosphorolysis of thymidine and other pyrimidine 2′-deoxyribosides, with the exception of 4-amino-substituted compounds and has nucleoside deoxyribosyl transferase activity. TP is one of two pyrimidine phosphorylases in the base and nucleoside salvage pathway. Under near-physiological conditions TP is a homodimer with a molecular mass of 110 kDa in mammals (Desgranges, 1981) and 90 kDa in *E. coli* (Shwartz, 1978). Specific inhibitors of TP are considered as potential chemotherapeutics either to reduce clearance of thymidine and other deoxyuridine analogues presently in use as antineoplastic and antiviral agents or by interfering with the salvage process (Desgranges et al., 1983). The design of such inhibitors may be aided by the 2.8-Å-resolution crystal structure of *E. coli* TP, which shows TP to consist of a small helical domain and a larger α/β domain both of which comprise two noncontinuous segments of polypeptide (residues 1–65, 163–193 and 80–154, 197–440, respectively) (Walter et al., 1990). Thymidine and phosphate moieties appear to be bound in a cleft between these two domains.

Figure 1 highlights the sequence similarity between the human PD-ECGF and *E. coli* TP sequences. The similarity extends over all but the N-terminal 32 and the C-terminal 4 residues of PD-ECGF. Human PD-ECGF apparently undergoes posttranslational maturation, whereby 10 and 4 amino acids are removed from the amino and carboxyl termini, respectively (Ishikawa et al., 1989). Mature PD-ECGF has a 22-amino acid N-terminal extension with respect to the *E. coli* TP sequence. The sequences show 40% identity calculated over the 438 common amino acid positions (see legend to Fig. 1) and are therefore likely to have diverged from a common genetic ancestor and share the same overall tertiary fold (Barton & Sternberg, 1987). Thus, the crystal structure of *E. coli* TP may be used as a scaffold on which to model the structure of human PD-ECGF. The amino acids of the putative phosphate binding sites of TP are conserved in PD-ECGF (indicated by P in Fig. 1), as are the thymidine-binding residues (Arg 171, Ser 186, and Lys 190, indicated by T in Fig. 1). The small helical domain of TP has 46% identity with PD-ECGF, whereas the larger domain has 37% identity. Further subdivision of these domains shows the segments containing binding residues for thymidine (163–193, helical domain) and phosphate (80–154, α/β domain) to be the most highly conserved regions of the molecules (74% and 60% identity, respectively). In contrast, the remainder of the small and large domains (1–65 and 197–440) show 32% and 30% identity, respectively. The large domain may be divided between helices 14 and 15 into two putative folding units (Walter et al., 1990), this division coincides with an eight-residue insertion in PD-ECGF relative to TP. The C-terminal unit (330–440) is the least conserved region of the proteins (21% identity), whereas the N-terminal unit (197–320) shows 38% identity with

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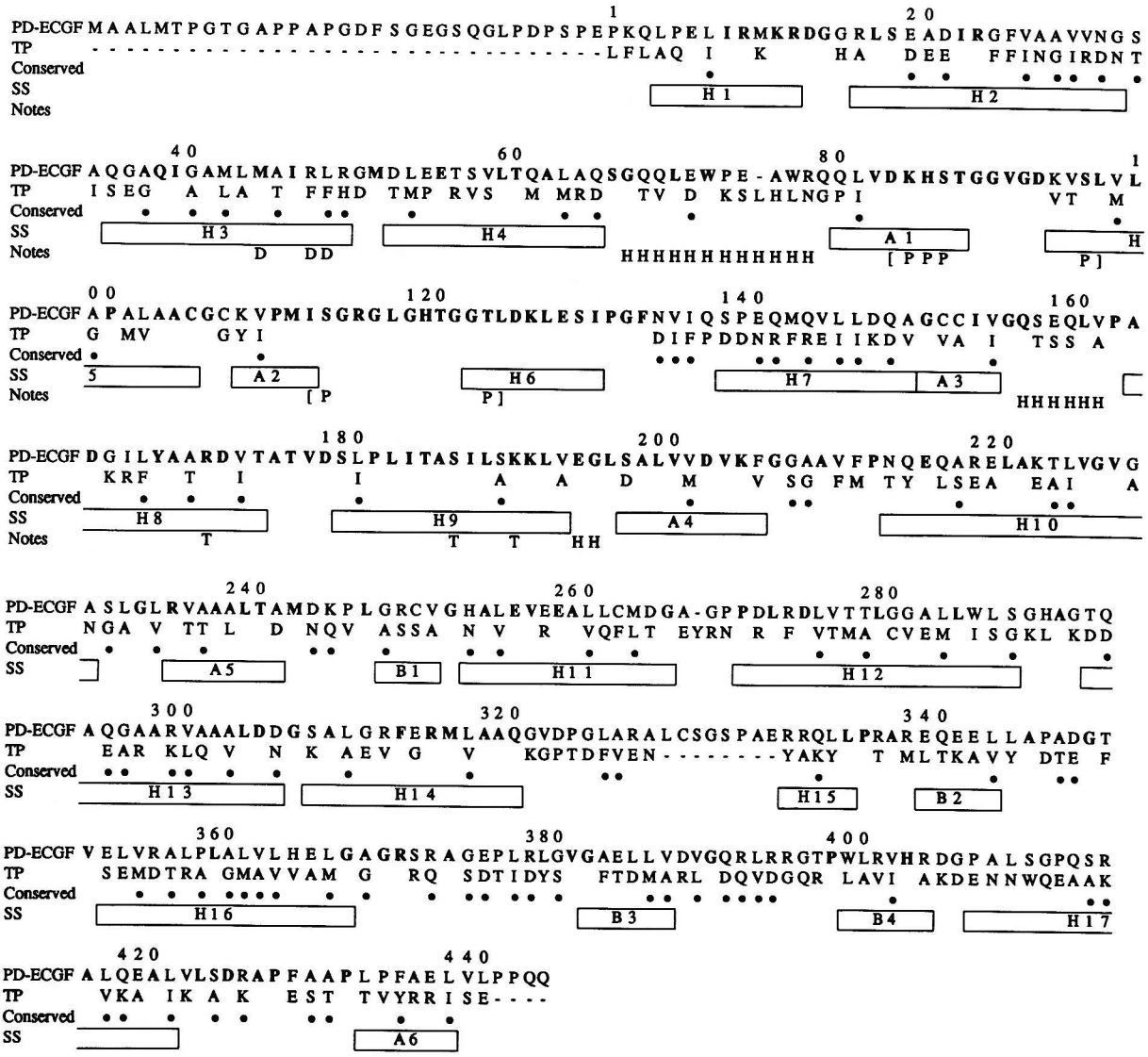

Fig. 1. Sequence alignment of human platelet-derived endothelial cell growth factor (PD-ECGF) and *E. coli* thymidine phosphorylase (TP). Sequence similarity was detected by scanning the NBRF-PIR database (V.28) with the PD-ECGF sequence using the Smith and Waterman (1981) local similarity algorithm with Dayhoff's MDM78 matrix and a length-dependent penalty of 8. The alignment shown was generated with the AMPS package (Barton, 1990) and shows 40% identity over 438 aligned positions. The similarity score is 30 standard deviation units from the mean of scores for randomized sequences of the same length and composition as PD-ECGF and TP. Aligned residues identical in PD-ECGF and TP are shown in bold in the PD-ECGF sequence and not repeated in the TP sequence. Conserved: • highlights residue pairs that have positive substitution values in Dayhoff's MDM78 matrix. SS: open boxes show the secondary structure of TP (Walter et al., 1990); H1–H17 are helices; A1–A6 and B1–B4 are β-strands in sheets A and B, respectively. Notes: Residues involved in the putative phosphate-binding site are identified by a P character; similarly, residues of the thymidine-binding site are shown by a T. Regions that are specifically involved in the dimer interface (H1, H3, and H8–H9 loop) are labeled with D. Putative hinge points at 66–79, 155–162, and 193–196 are shown by H characters.

PD-ECGF. There are two deletions of one residue in addition to the eight-residue insertion in the PD-ECGF sequence. These changes lie on the surface of the TP structure and are distant from the active site cleft. The deletion of Tyr at position 267 is spatially adjacent to the eight-residue insertion (position 329 of TP) in the structure, whereas the deletion of Leu 75 is in a region proposed as a hinge-point between the two domains (Walter et al., 1990). The TP dimer interface is centered on three hydrophobic residues in helix H3 (Met 44, Phe 47, and Phe 48). Of these, only the first (Met) is identical in PD-ECGF, and the second and third are replaced by Arg and Leu, respectively.

Human TP and PD-ECGF appear to have a common tissue distribution (platelets, placenta, and some tumors) (Heldin et al., 1977; Shaw et al., 1988; Miyazono & Hel-

din, 1989; Yoshimura et al., 1990), to share a cytosolic cellular location (Shaw et al., 1988; Ishikawa et al., 1989; Usuki et al., 1989), and to have similar monomeric molecular weights (Yoshimura et al., 1990). Given the strong overall sequence similarity between human PD-ECGF and *E. coli* TP especially around the active site, it is tempting to suggest that PD-ECGF is human TP. The principal objections to this hypothesis are that PD-ECGF has an additional 22 residues at the N-terminus relative to *E. coli* TP, and that there is only limited residue conservation in the core of the dimer interface. These observations may explain why active PD-ECGF is apparently found in both monomeric and dimeric forms (King & Buchwald, 1984). We currently cannot rule out the possibility that the differences in length and in the dimer interface are simply due to interspecies variation rather than functional differences.

The expression and purification of recombinant PD-ECGF from the yeast *Saccharomyces cerevisiae* has enabled us to establish that recombinant PD-ECGF is mitogenic toward endothelial cells (Finnis et al., 1992). Preliminary results also suggest that recombinant PD-ECGF possesses TP activity, and that TP purified from *E. coli* is mitogenic toward endothelial cells (unpubl.). Moreover, the recently reported covalent modification of PD-ECGF by nucleotides in vitro and in vivo (Usuki et al., 1991) probably occurs as a result of the nucleoside- and phosphate-binding properties of PD-ECGF.

We conclude that PD-ECGF is a human TP homologue, the mitogenic and angiogenic activities of which are a result of its TP activity. The endothelial cell specificity of PD-ECGF (Ishikawa et al., 1989) might be achieved via a specific endothelial cell receptor; however, it seems more likely that endothelial cells respond specifically to a modulation in intracellular DNA precursor pools brought about by the TP activity of PD-ECGF.

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