Insect prothoracicotropic hormone: a new member of the vertebrate growth factor superfamily

Tosiyuki Noguti[a], Takashi Adachi-Yamada[b], Teruhiko Katagiri[a], Atsushi Kawakami[b,**], Masafumi Iwami[b,***], Jun Ishibashi[c], Hiroshi Kataoka[c], Akinori Suzuki[c], Mitiko Gō[a,*], Hironori Ishizaki[b,****]

[a]*Laboratory of Molecular Biophysics, Department of Biology, School of Science, Nagoya University, Nagoya 464-01, Japan*
[b]*Laboratory of Developmental Biology, Department of Biology, School of Science, Nagoya University, Nagoya 464-01, Japan*
[c]*Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Received 16 October 1995

Abstract Prothoracicotropic hormone (PTTH) is a brain neurosecretory protein that controls insect development. PTTH of the silkmoth *Bombyx mori* is a homodimeric protein, the subunit of which consists of 109 amino acids. Clear-cut sequence similarity to any other proteins has not been observed. By disulfide-bond pattern analysis and modeling of the PTTH structure based on the known three-dimensional (3D) structures of growth factor family with cystine-knot motif, we propose that the PTTH protomer adopts the fold unique to the structural superfamily of the growth factors, β-nerve growth factor (β-NGF), transforming growth factor-β2 (TGF-β2), and platelet-derived growth factor-BB (PDGF-BB). The insect neurohormone PTTH appears to be a member of the growth factor superfamily, sharing a common ancestral gene with the three vertebrate growth factors, β-NGF, TGF-β2 and PDGF-BB.

Key words: Cystine-knot; Growth factor; Insect neurohormone; Protein superfamily; Prothoracicotropic hormone

1. Introduction

Prothoracicotropic hormone (PTTH), a brain neurosecretory protein of insects, activates the prothoracic glands to synthesize and release ecdysone, the steroid essential to growth, moulting, and metamorphosis [1]. PTTH thus plays a central role in the endocrine network for insect development control. Amino acid sequence of the PTTH of the silkmoth *Bombyx mori* has been determined [2,3]: it is a 30 kDa homodimeric protein, the subunit of which consists of 109 amino acid residues containing seven cysteines. No strong sequence similarity to any other proteins has been detected by database homology search [3]. However, from determining locations of disulfide bonds of an *Escherichia coli* recombinant PTTH [4] (Fig. 1A), we were impressed with a striking similarity of the sequential arrangement of three intra-chain disulfide bonds in the *Bombyx* PTTH to that in β-nerve growth factor (β-NGF), transforming growth factor-β2 (TGF-β2) and platelet-derived growth factor-BB (PDGF-BB) (Fig. 1B–D). This fact has led us to investigate the evolutionary relatedness of the *Bombyx* PTTH to these growth factors, based on the comparison of the predicted 3D structure of PTTH with the known structures of the growth factors.

From the known 3D structures of β-NGF [5], TGF-β2 [6,7], and PDGF-BB [8], it has been argued that the protomers of these growth factors have the core structures very similar to one another [9–13]. The structure common to these growth factors has been characterized by two β-sheets, each containing a pair of antiparallel β-strands, and three intra-chain disulfide bonds that link the β-strands, forming a 'cystine-knot' motif. Despite the lack of significant sequence identity except for the conservation of cysteines, the 3D structural similarity in the three growth factors has led to the notion that they have been derived from a common ancestral protein [11–13]. Recent X-ray crystallographic studies have revealed that a heterodimeric glycoprotein hormone, human chorionic gonadotropin (hCG) belongs to the superfamily of these cystine-knot growth factors [14,15].

In this paper, we propose that the protomer of PTTH, the insect brain secretory peptide, has the core structure similar to those of the vertebrate growth factors (β-NGF, TGF-β2, and PDGF-BB), based on the analysis of its unique intra-chain disulfide bonding pattern and a modeled 3D structure. This result implies that the insect hormone molecule regulating development, PTTH, shares a common evolutionary origin with the three vertebrate growth factors.

2. Materials and methods

2.1. Data for the structures of β-NGF, TGF-β2, and PDGF-BB

The conformations of the protomers of β-NGF [5], TGF-β2 [6–7,16–17], and PDGF-BB [8] are shown schematically in Fig. 2A–C. They have been constructed from the data of the respective original papers, the X-ray coordinates of TGF-β2 (Brookhaven Protein Databank, entry P1TGI [6]), and the hydrogen-bonding pattern in the structure of PDGF-BB [13].

*Corresponding author. Fax: (81) (52) 789-2977.
E-mail: go@bio.nagoya-u.ac.jp

***Present address:* Department of Molecular Biology, School of Science, Nagoya University, Nagoya 464-01, Japan.

****Present address:* Department of Biology, Faculty of Science, Kanazawa University, Kanazawa 920-11, Japan.

*****Present address:* Aichi-Shukutoku University, Nagakute-cho, Aichigun, Aichi 480-11, Japan.

Abbreviations: PTTH, prothoracicotropic hormone; NGF, nerve growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; hCG, human chorionic gonadotropin; CD, circular dichroism.

SSDI 0014-5793(95)01296-6

2.2. Molecular modeling of the PTTH protomer

The conformation of the core structure of the PTTH protomer was modeled by replacing the side-chains of the TGF-β2 crystal structure (Brookhaven Protein Databank, entry P1TGI [6]) with the PTTH side-chains based on the sequence alignment in Fig. 3C. We examined whether the common fold of β-NGF, TGF-β2, and PDGF-BB could be realized in PTTH without any atomic collision. The modeling was done in the following procedures by using the program BIOGRAF (version 3.0, Molecular Simulations Inc., Waltham, MA, 1992) [18].

First, segments 1–14, 23–36, 49–76 and 93–94 were removed from the TGF-β2 crystal structure, and the atomic coordinates of the TGF-β2 common fold were left untouched. Second, Asp-82 and Lys-107 were deleted from the common fold, and the irregular geometry caused by the deletion was reformed by an energy minimization relaxing two residues on both the N- and C-terminal sides of the two deletion points each. Third, the side-chains were replaced by those of PTTH and the conformational energy was minimized to remove atomic overlaps. Fourth, based on the results of secondary structure prediction (data not shown), we assumed the main-chain structure of the segment connecting β1 to β2 as an extended antiparallel β-sheet by elongation of the first sheet of the common fold and built it manually with insertion of an octa-glycine. The main-chain structure of the segment between the β3 and β4 strands was modeled in a similar way. A tetra-glycine was inserted before and after Cys 48 manually. The N-terminal 12 residues and C-terminal 9 residues were not modeled but four and one residues before and after the ends of the common fold (residues 17–99 in PTTH), respectively, were manually modeled. After modeling the main-chain structures of these segments, the inserted glycines were replaced by the PTTH side-chains. Fifth, the whole structure was subjected to a molecular dynamics simulation for one picosecond at 400 K with a set of distance constraints on the hydrogen bonds of the β-sheets, followed by an energy minimization without the distance constraints. The minimization was terminated when the root-mean-square force was less than 0.1 kcal·mol^{-1}·Å^{-1} and no atomic collision was observed. The root-mean-square deviation of the Cα positions in the common fold of PTTH from those of the TGF-β2 crystal structure was 1.9 Å.

Atomic coordinates of the modeled core structure of PTTH protomer will be submitted to the Brookhaven Protein Databank.

3. Results and discussion

Six cysteines of the recombinant PTTH protomer form three intra-chain disulfide bonds between positions 17 and 54, 40 and 96, and 48 and 98, while the seventh one at position 15 participates in an inter-chain disulfide bond to form a dimeric molecule [4] (Fig. 1A). The sequential arrangement of the three intra-chain disulfide bonds in PTTH is obviously very similar to that in β-NGF [19], TGF-β2 [6,7] and PDGF-BB [8,20–21] (Fig. 1B–D). The basic pattern of disulfide bond arrangement common to the four proteins is schematically shown in Fig. 1E. Besides the same sequential order of C1 to C6 (half-cystine symbols; see Fig. 1E), the following three aspects shared by the four proteins are worth emphasizing. First, C5 and C6 are separated strictly by one residue. Second, C2 and C3 are localized relatively close to each other, being separated by 3–9 residues. Third, the distances between C1 and C2 and between C4 and C5 are large.

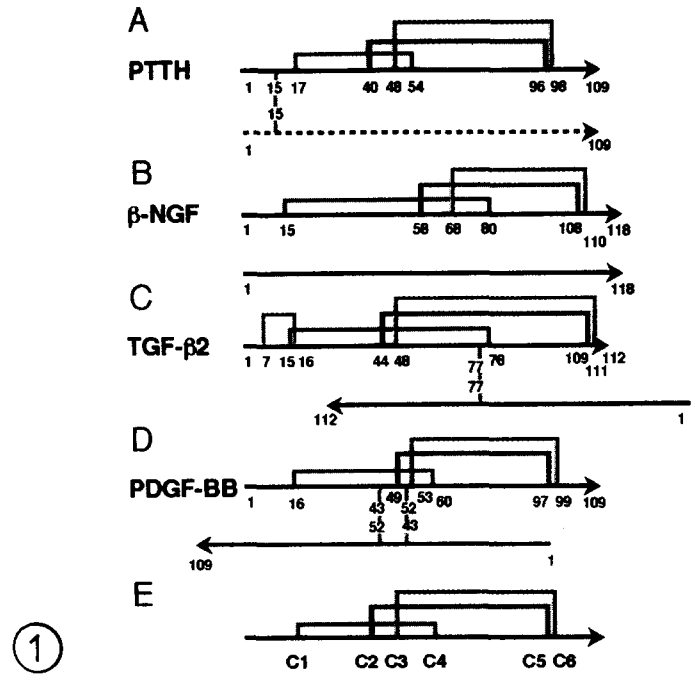
Fig. 2A–C depict schematically the structures of the β-NGF, TGF-β2 and PDGF-BB protomers, which were constructed by referring to the 3D structural studies of these growth factors [5–8,13,16–17]. We define a common fold of these growth factor protomers as diagrammed in Fig. 2D, which highlights the β-strands, disulfide bonds, and hydrogen bonds that contribute to the formation of this fold. All half-cystines except for C3 are located at the exactly equivalent positions in the three proteins, providing them with the structural basis for the formation of the common fold. The β4 strand has a large twist. At the three places indicated by arrowheads, we observe irregularities in the β-sheet due to the insertion of one or two amino acid residues, but they do not disturb the hydrogen bonds outside these irregular sites [13,17].

The protomers of these growth factors lack a typical hydrophobic core because of their elongated nonglobular folds [6,9,16–17]. However, hydrophobic residues make at least two clusterings common to the growth factor protomers (Fig. 2A–C). The first clustering is formed by the six half-cystines (red) and the hydrophobic residues around them (pink). Both of the β-sheets in the common fold are twisted and the hydrophobic side-chains located at face-to-face positions between the two β-sheets form a core of the second common hydrophobic clustering (yellow). In TGF-β2, for example, the core consists of the side-chains of Phe-24, Tyr-39, Leu-86, Ile-88, Leu-101, Met-104 and Ile-105. The hydrophobic side-chains at the equivalent positions in β-NGF and PDGF-BB form similar cores. The second cores in β-NGF and TGF-β2 have also been noted by McDonald et al. [5] and Daopin et al. [16], respectively. The core of the second common hydrophobic clustering is not buried so completely as in typical hydrophobic cores. This core seems to play an important role in stabilization of the common fold of the protomers, as well as the three disulfide bonds in the first cluster whose contribution to the stabilization is self-evident. The third hydrophobic cluster (green) is observed in the

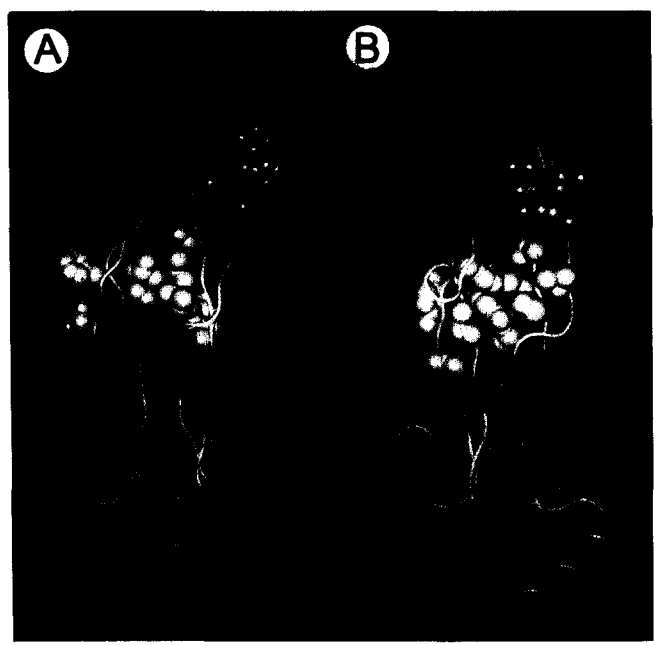
→

Fig. 1. Sequential arrangement of disulfide bonds. (A–D) Schematic representation of the primary structures of PTTH [4], β-NGF [19], TGF-β2 [6,7] and PDGF-BB [8,20–21] highlighting disulfide bonds. Note a sequential pattern of three intra-chain disulfide bonds (red, purple, and green) which is common to the four proteins. TGF-β2 has an additional intra-chain disulfide bond (sky). The inter-chain disulfide bonds (brown) are shown. The position numbers of the half-cystines are indicated. The subunit lengths of the four proteins are similar, ranging from 109 to 118, as shown by the position number of the C-terminus. The associated subunits in the dimeric forms are shown with their orientation indicated by arrow. The broken line for PTTH represents the associated subunit with hypothetical orientation. (E) Schematic representation of the basic pattern in the sequential arrangement of the half-cystines forming the three intra-chain disulfide bonds, common to the four proteins. C1 to C6 represent half-cystine symbols, being numbered in the order from the N- to C-terminus.

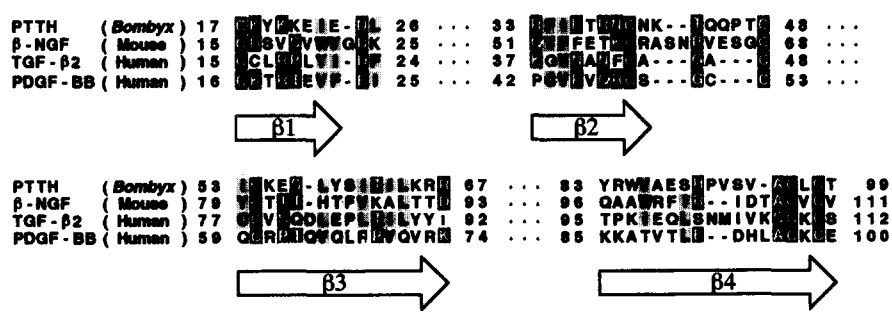
Fig. 3. 3D structures of the modeled PTTH and crystallized TGF-β2, and the sequence alignment of the PTTH and the three growth factors. (A) The main-chain structure in tube model and the hydrophobic clusters of the modeled PTTH protomer. (B) Same as (A), for the TGF-β2 protomer, based on the X-ray coordinates [6]. The side-chain atoms involved in the hydrophobic clusters in the common fold are shown in a space filling model in different colors according to the clusters they belong to. Coloring is same as in Fig. 2E and B. (C) The amino acid sequences of PTTH, β-NGF, TGF-β2, and PDGF-BB that form the common folds are aligned according to the topological equivalence represented in Fig. 2. The six conserved cysteines are highlighted in red. When three or all of the aligned amino acid residues of the four proteins belong to the same group with respect to the hydrophobic (Val, Ile, Leu, Phe, Tyr, Cys, Met and Trp), hydrophilic (His, Asp, Glu, Asn, Gln, Arg and Lys) and small ambivalent (Gly, Ala, Pro, Ser and Thr) groups [22], they are highlighted in yellow (hydrophobic), blue (hydrophilic) or green (small ambivalent). Arrows indicate the β-strands of the common fold. Murray-Rust et al. [13] reported a sequence alignment of β-NGF, TGF-β2, and PDGF-BB, based on a structural alignment. Their result and the present one agree completely in the region of the common fold, except for the position of a gap in the β3-strand.


A
PTTH
1 15 17 40 48 54 96 98 109
15
1
109
B
β-NGF
1 15 58 68 80 108 110 118
C
TGF-β2
1 7 15 16 44 48 77 76 109 112 111
77
112 1
D
PDGF-BB
1 16 43 49 52 53 60 97 99 109
52 43
109 1
E
C1 C2 C3 C4 C5 C6

① ② ③

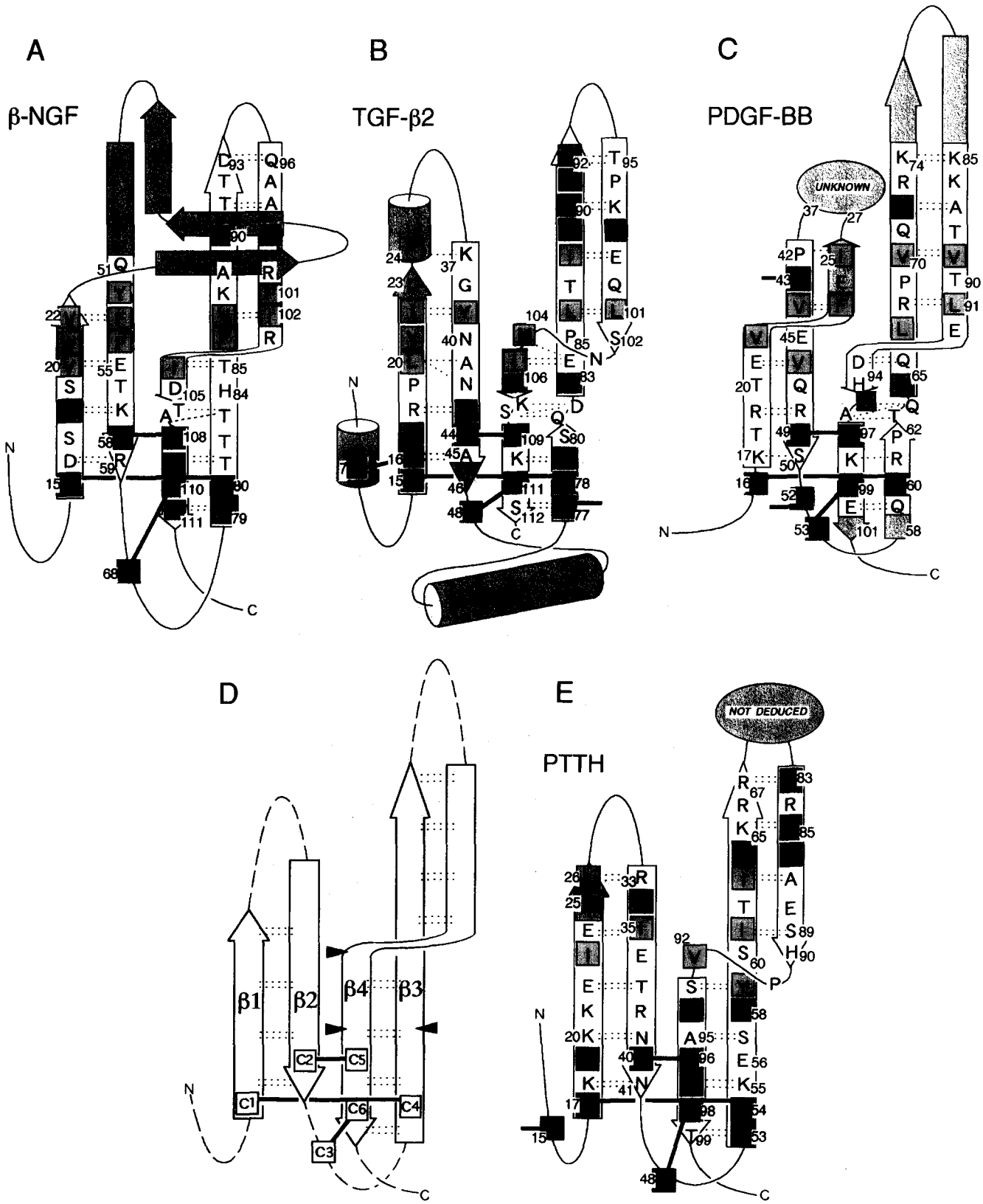

A
B

C


PTTH (Bombyx) 17 26 33 48
β-NGF (Mouse) 15 25 51 68
TGF-β2 (Human) 15 24 37 48
PDGF-BB (Human) 16 25 42 53
β1
β2
PTTH (Bombyx) 53 67 83 99
β-NGF (Mouse) 79 93 96 111
TGF-β2 (Human) 77 92 95 112
PDGF-BB (Human) 59 74 85 100
β3
β4

common fold of TGF-β2, but the corresponding cluster is ambiguous in β-NGF and PDGF-BB.

Since the strictly conserved topology of the three disulfide bonds in the three growth factors is obviously a prerequisite to the formation of their common fold, and since the sequential distribution of the three intra-chain disulfide bonds of PTTH resembles definitely that of the three growth factors, we tried to model the PTTH protomer structure assuming that the PTTH protomer adopts the same fold as the growth factor common fold. We considered that a putative PTTH fold may



have irregularities in the three sites of the β3 and β4 strands where irregularities are observed for the three growth factors (Fig. 2D, arrowheads). Each irregularity may be caused by insertion of one or two residues, as observed in the three growth factors. Thus, eight variants, with respect to the irregularity pattern, of the putative PTTH fold are possible, depending on being either regular or irregular at each of the three sites. In four variants out of the eight, hydrophobic residues were accommodated to face-to-face positions between the two β-sheets so as to form a core similar to that of the second hydrophobic clustering in the growth factor common fold. One of the four variants is shown in Fig. 2E. Clearly, the putative PTTH fold is very similar to the growth factor common fold.

We have carried out molecular modeling of the common fold in PTTH using the crystal structure of TGF-β2 [6] as a template. The residues of PTTH have been accommodated in the common fold without any serious atomic collision. Three hydrophobic clusters are formed in the model-built structure (Fig. 3A). In spite of the fact that the total number of the hydrophobic residues of PTTH is smaller than that of TGF-β2, a similar distribution of the hydrophobic residues is clearly observed in the 3D structure model (Fig. 3A and B). The core of the second cluster is realized between the two β-sheets in the model-built structure of PTTH. This self-consistent result of the molecular modeling supports further the existence of the common fold in PTTH.

The amino acid sequences of PTTH, β-NGF, TGF-β2 and PDGF-BB are aligned by matching the topologically equivalent residues in their common folds (Fig. 3C). Twenty kinds of amino acids have been classified into hydrophobic, hydrophilic and small ambivalent groups [22]. Assuming that the occurrence of each group at a site of a protein is random, the probability of the appearance of the same group at the same site of at least three out of the four proteins would be one-third. At most sites of β1, β2 and β3 strands, the amino acid residues which belong to the same group are observed, in at least three out of the four proteins. The amino acid sequences of the common fold are thus evidently conserved in the four proteins.

The occurrence of the common fold in PTTH is supported further by the following facts. First, we have predicted by secondary structure prediction [23,24] that PTTH consists mainly of β-strands (data not shown). Second, Ishibashi et al. measured the CD spectrum of PTTH and found that the molecule was β-rich (about 80%) (unpublished data).

We searched the Brookhaven protein data bank [25], in which information for 3D structure is available, for proteins having three disulfide bonds with the aforementioned sequential arrangement common to β-NGF, TGF-β2, PDGF-BB, and PTTH. Complement C5A, trypsin inhibitor II, wheat germ agglutinin, scorpion neurotoxin, and ribonuclease A were found to have three intra-chain disulfide bonds with a similar half-cystine arrangement in the order of C1 to C6 as diagrammed in Fig. 1E. However, none of these proteins shows the features characteristic of the three growth factors and PTTH, i.e. one-residue separation between C5 and C6, a relatively short distance between C2 and C3, and wide separations between C1 and C2 and between C4 and C5. Their 3D structures are also distinct from the core structure common to the three growth factors and PTTH, suggesting that the characteristic disulfide arrangement and the core structure specific to the growth factor superfamily are most probably linked. We further searched the amino acid sequence data bank, SWISS-PROT, for proteins having all the features of the specific disulfide arrangement pattern. No proteins other than the members which belong to the NGF, TGF, and PDGF families have been found to possess these characteristics. These results strongly support that the growth factor structural superfamily is a distinct, well-defined superfamily. The relative spacing pattern of seven cysteine residues on amino acid sequences of glial cell line-derived neurotrophic factor [26], Norrie disease protein [27] and C-terminal domains of mucin and von Willeband factor [28] is similar to that of the seven half-cystines (including those of cystine-knot and the inter-chain disulfide bond) of TGF-β. These protein and domains were proposed to be members of the cystine-knot superfamily [26–28], although the locations of their disulfide bonds were not known.

PTTH assumes a dimeric form like β-NGF, TGF-β2, and PDGF-BB. The mode of the dimer formation is quite different among the three growth factors, in contrast with the resemblance of having the common fold in their monomeric structures [10,12–13]. The X-ray crystallographic studies of the hCG heterodimer, the fourth member of the cystine-knot growth factor superfamily, have shown that its subunits are associated in a still different mode [14,15]. PTTH has one inter-chain disulfide bond between the 15th Cys residues, which are located near an end of the elongated, modeled PTTH protomer (Fig. 3A). If we hypothesize the anti-parallel association of two PTTH protomers as in TGF-β2 and PDGF-BB (Fig. 1C and D), the interface area between the PTTH protomers would be too small to assure the conformational stability. Rather, a parallel association of PTTH protomers seems likely (Fig. 1A).

The receptor binding sites of β-NGF [29,30] and PDGF-BB [31] have been located in the peripheral, conformationally variable regions outside the common fold that we defined, though

←

Fig. 2. Schematic representation of the structures of the three growth factors and a putative fold of PTTH. (A–C) The structures of protomers of β-NGF, TGF-β and PDGF-BB. Arrows and cylinders represent β-strands and α-helices, respectively. Thick and dotted lines represent disulfide bonds and hydrogen bonds, respectively. The amino acid residues contributing to the common fold of the three growth factors are indicated by one-letter codes. The hydrophobic residues that form three distinct clusters are colored green, yellow, or pink, depending on the clusters they form. The pink residues form a hydrophobic cluster together with the half-cystines (red). Position numbers of some residues are indicated. The peripheral regions that adopt variable conformations are shown in grey. (D) Schematic drawing of the fold common to the protomers of the three growth factors. The common fold consists of two β-sheets, each containing a pair of antiparallel β-strands (β1/β2 and β3/β4). The β3/β4 sheet is sharply twisted in the same place of all proteins. The strands are linked by three interlocking disulfide bonds. The disulfide bond C1–C4 passes through a ring formed by C2–C5, C3–C6 and the intervening residues. Arrowheads point to the three places where irregularities occur in the β-sheet, due to the insertion of one or two amino acid residues. Broken lines represent the peripheral regions that form variable conformations. The half-cystine symbols, C1–C6, are as in Fig. 1E. (E) Putative core structure of the PTTH protomer that we propose, based on the assumption that PTTH adopts the same fold as in the three growth factors. The β1 strand has been assumed to extend up to Leu-26, to favor the stabilization of the core of the yellow hydrophobic cluster by forming the Leu-26–Arg-33 hydrogen bonds.

in some cases the binding site protruded into the core region. Based on our conclusion that PTTH adopts the same fold as the three growth factors, it seems highly probable that the receptor binding sites of PTTH reside also in the region peripheral to the common fold. Thus, our putative molecular modeling of PTTH may provide a structural basis for future study on the biological function of this hormone.

The conclusion that PTTH and the three growth factors adopt the same fold to form a structural superfamily implies that these proteins share a common ancestral molecule. Bombyxin, another brain secretory peptide of *Bombyx* that is capable of activating the prothoracic glands of the heterologous moth *Samia cynthia ricini*, has previously been shown to belong to the insulin family, and accordingly to be homologous with insulin-like growth factors [32,33]. This conclusion was based on the data of the amino acid sequence [34,35], disulfide bonding pattern [36], 3D structure modeling [37], and gene structure [32,38]. The present result showing that PTTH shares an ancestral molecule with the vertebrate growth factors and hCG adds to the evidence for the common origin of some hormones and growth factors, irrespective of divergence of vertebrates and invertebrates.

Acknowledgements: We thank Dr. C. Oefner for providing the information about the hydrogen-bonding pattern in the PDGF-BB protomer before publication of the work [13]. This work was partly supported by Grant-in-Aids for Scientific Research on Priority Areas to T.N. and M.G. from the Ministry of Education, Science and Culture, Japan and by a grant to H.I. from Takeda Science Foundation.

References

[1] Bollenbacher, W.E. and Granger, N.A. (1985) in: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Kerkut, G.A. and Gilbert, L.I. eds.) vol. 7, pp. 109–151, Pergamon, Oxford.
[2] Kataoka, H., Nagasawa, H., Isogai, A., Ishizaki, H. and Suzuki, A. (1991) Agric. Biol. Chem. 55, 73–86.
[3] Kawakami, A., Kataoka, H., Oka, T., Mizoguchi, A., Kimura-Kawakami, M., Adachi, T., Iwami, M., Nagasawa, H., Suzuki, A. and Ishizaki, H. (1990) Science 247, 1333–1335.
[4] Ishibashi, J., Kataoka, H., Isogai, A., Kawakami, A., Saegusa, H., Yagi, Y., Mizoguchi, A., Ishizaki, H. and Suzuki, A. (1994) Biochemistry 33, 5912–5919.
[5] McDonald, N.Q., Lapatto, R., Murray-Rust, J., Gunning, J., Wlodawer, A. and Blundell, T.L. (1991) Nature 354, 411–414.
[6] Daopin, S., Piez, K.A., Ogawa, Y. and Davies, D.R. (1992) Science 257, 369–373.
[7] Schlunegger, M.P. and Grütter, M.G. (1992) Nature 358, 430–434.
[8] Oefner, C., D'Arcy, A., Winkler, F.K., Eggimann, B. and Hosang, M. (1992) EMBO J. 11, 3921–3926.
[9] Swindells, M.B. (1992) Science 258, 1160–1161.
[10] Daopin, S., Cohen, G.H. and Davies, D. (1992) Science 258, 1161–1162.
[11] Murzin, A.G. and Chothia, C. (1992) Curr. Opin. Struct. Biol. 2, 895–903.
[12] McDonald, N.Q. and Hendrickson, W.A. (1993) Cell 73, 421–424.
[13] Murray-Rust, J., McDonald, N.Q., Blundell, T.L, Hosang, M., Oefner, C., Winkler, F. and Bradshaw, R.A. (1993) Structure 1, 153–159.
[14] Lapthorn, A.J., Harris, D.C., Littlejohn, A., Lustbader, J.W., Canfield, R.E., Machin, K.J., Morgan, F.J. and Isaacs, N.M. (1994) Nature 369, 455–461.
[15] Wu, H., Lustbader, J.W., Liu, Y., Canfield, R.E. and Hendrickson, W.A. (1994) Structure 2, 545–558.
[16] Daopin, S., Li, M. and Davies, D.R. (1993) Proteins 17, 176–192.
[17] Schlunegger, M.P. and Grütter, M.G. (1993) J. Mol. Biol. 231, 445–458.
[18] Mayo, S.L., Olafson, B.D. and Goddard III, W.A. (1990) J. Phys. Chem. 94, 8897–8909.
[19] Angeletti, R.H. and Bradshaw, R.A. (1971) Proc. Natl. Acad. Sci. USA 68, 2417–2420.
[20] Haniu, M., Rohde, M.F. and Kenney, W.C. (1993) Biochemistry 32, 2431–2437.
[21] Östman, A., Andersson, M., Bäckström, G. and Heldin, C.-H. (1993) J. Biol. Chem. 268, 13373–13377.
[22] Gō, M. and Miyazawa, S. (1980) Int. J. Peptide Protein Res. 15, 211–224.
[23] Chou, P.Y. and Fasman, G.D. (1974) Biochemistry 13, 222–245.
[24] Garnier, J., Osguthorpe, D.J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.
[25] Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[26] Lin, L.-F.H., Doherty, D.H. Lile, J.D. Bektesh, S. and Collins, F. (1993) Science 260, 1130–1132.
[27] Meitinger, T., Meindl, A., Bork, P., Rost, B., Sander, C., Haasemann, M. and Murken, J. (1993) Nature Genet. 5, 376–380.
[28] Bork, P. (1993) FEBS Lett. 327, 125–130.
[29] Ibáñez, C.F., Ebendal, T., Barbany, G., Murray-Rust, J., Blundell, T.L. and Persson, H. (1992) Cell 69, 329–341.
[30] Ibáñez, C.F., Ilag, L.L., Murray-Rust, J. and Persson, H. (1993) EMBO J. 12, 2281–2293.
[31] LaRochelle, W.J., Pierce, J.H., May-Siroff, M., Giese, N. and Aaronson, S.A. (1992) J. Biol. Chem. 267, 17074–17077.
[32] Ishizaki, H. and Suzuki, A. (1992) Prog. Brain Res. 92, 1–14.
[33] Ishizaki, H. and Suzuki, A. (1994) Int. J. Dev. Biol. 38, 301–310.
[34] Nagasawa, H., Kataoka, H., Isogai, A., Tamura, S., Suzuki, A., Ishizaki, H., Mizoguchi, A., Fujiwara, Y. and Suzuki, A. (1984) Science 226, 1344–1345.
[35] Nagasawa, H., Kataoka, H., Isogai, A., Tamura, S., Suzuki, A., Mizoguchi, A., Fujiwara, Y., Suzuki, A., Takahashi, S.Y. and Ishizaki, H. (1986) Proc. Natl. Acad. Sci. USA 83, 5840–5843.
[36] Nagasawa, H., Maruyama, K., Sato, B., Hietter, H., Kataoka, H., Isogai, A., Tamura, S., Ishizaki, H., Semba, T. and Suzuki, A. (1988) in: Peptide Chemistry 1987 (Shiba, T. and Sakakibara, S. eds.) pp. 123–126, Protein Research Foundation, Osaka.
[37] Jhoti, H., McLeod, A.N., Blundell, T.L., Ishizaki, H., Nagasawa, H. and Suzuki, A. (1987) FEBS Lett. 219, 419–425.
[38] Iwami, M., Kawakami, A., Ishizaki, H., Takahashi, S.Y., Adachi, T., Suzuki, Y., Nagasawa, H. and Suzuki, A. (1989) Dev. Growth Differ. 31, 31–37.