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Prostaglandin endoperoxide synthase contains an EGF-like domain

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Prostaglandin endoperoxide synthase was subjected to the computer-assisted homology search in the protein primary structure database, in order to investigate the regulation mechanism of the expression of prostaglandin endoperoxide synthase As a result of that, it turned out that prostaglandin endoperoxide synthase shares sequence homology with epidermal growth factor (EGF) in the N-terminal region The implication of the existence of an EGF-like domain in prostaglandin endoperoxide synthase is discussed

Prostaglandin endoperoxide synthase, Epidermal growth factor, Sequence homology

1. INTRODUCTION

The first step of the pathway for the conversion of arachidonic acid and related unsaturated fatty acids to the prostanoids is initiated by prostaglandin endoperoxide synthase. The enzyme has two enzymatic activities, that is, a *bis*-oxygenase activity and a hydroperoxidase activity [1–5]. The former initiates the formation of the prostaglandin G and the latter is involved in the reduction of prostaglandin G to prostaglandin H. Recently, the primary structure of sheep prostaglandin endoperoxide synthase was determined and the homologies with heme proteins and the other peroxidases were reported [6–8].

Thus, prostaglandin endoperoxide synthase occupies an important position in the metabolism of prostanoid and the regulation of prostaglandin endoperoxide synthase controls the rate of prostanoid synthesis. Therefore, the regulation mechanism of the prostaglandin endoperoxide synthase has been studied extensively. One of the interesting observations is that EGF can affect the expression of the prostaglandin endoperoxide synthase [9].

In order to investigate the relationship of the regulation mechanism of the prostaglandin endoperoxide synthase with the known organic regulation system, homology search of the prostaglandin endoperoxide synthase in protein primary structure database was carried out. As a result of that, it turned out that the N-terminal region of the prostaglandin endoperoxide synthase shares significant sequence homology with EGF.

2. EXPERIMENTAL

Prostaglandin endoperoxide synthase was subjected to the computer-assisted homology search in the protein database, NBRF (release 19 0) [10] The homology search system used in this study was described in [11] The detected homologous sequences were aligned and subjected to the statistical test in order to check the significance of the similarity [12]

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3. RESULTS AND DISCUSSION

As a result of the computer-assisted homology search, it turned out that the N-terminal region of the prostaglandin endoperoxide synthase is homologous with the entire region of epidermal growth factor (EGF). The detected homologous regions were aligned (see fig.1) and subjected to the statistical test. The degree of similarity between the prostaglandin endoperoxide synthase and EGF is not so strong; however, the statistical test shows that those homologies between them are significant (see table 1). That is, the EGF-like domain of the prostaglandin endoperoxide synthase has an evolutionary relationship with EGF and the enzyme is considered to have appeared during the course of the evolution by exon-shuffling events [13].

There is a wide variety of proteins which contain EGF-like domains, for example, the products of neurogenic genes (Delta and Notch), coagulation factor precursors (IX, X, XII, and VII), Lin12 homeotic protein, protein C, and protein Z, etc., although the roles of those EGF-like domains have remained unknown. These EGF-like domains are classified into 3 groups, type 1, type 2 and type 3, discriminated from each other by the length and the sequence between the conserved cysteine residues [14]. The 6 conserved cysteine residues of the detected EGF-like domain have been numbered as Cys^1–Cys^6, following the order in which they appear in the alignment (see fig.1). Judging from the difference of the sequence and the length between Cys^5 and Cys^6, the EGF-like domain of prostaglandin endoperoxide synthase can be discriminated from the type 3 EGF-like sequence. The similarity of the sequence and the other characters (data not shown) suggest that the detected

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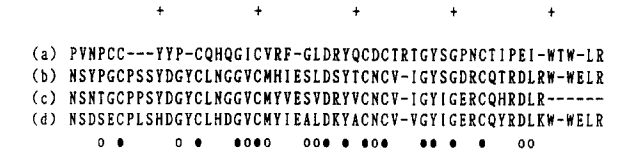

Fig 1 Alignment of the N-terminal region of the prostaglandin endoperoxide synthase and the epidermal growth factors from different sources • indicates the site occupied by an identical amino acid and ○ indicates the site occupied by a similar amino acid group [12] Gaps (—) were introduced to align the regions which have been subjected to the insertion/deletion events (a) The amino acid sequence from the prostaglandin endoperoxide synthase (residue positions 8-55, see [6]). (b) The amino acid sequence of mouse EGF (residue positions 977-1029, see [20]) (c) The amino acid sequence of rat EGF (residue positions 1-48, see [21]) (d) The amino acid sequence of the human EGF (residue positions 1-53, see [22])

EGF-like domain is related to type 1 and type 2 EGF-like sequences. However, the length between Cys^3 and Cys^4 of the detected EGF-like domain is a little different from those of type 1 and type 2 EGF-like sequences. Therefore, the purpose of the detected EGF-like domain has not been identified.

It was reported that prostaglandin endoperoxide synthase is homologous with the other peroxidases and the homology was also detected by our homology search. A region of prostaglandin endoperoxide synthase, residue positions 198-330 [6], is homologous with a region of thyroperoxidase, residue positions 318-459 [15], and a region of myeloperoxidase, residue positions 331-468 [16]. However, the structure of these peroxidases are different from each other. Prostaglandin endoperoxide synthase has the following conformation: N-terminal-EGF-like domain-(transmembrane region which has not been identified yet)-the region shared by the 3 peroxidases. Contrary to that, myeloperoxidase has the following conformation: N-terminal-the region shared by 3 peroxidases-cytochrome *c* oxidase-like domain-C-terminal. Thyroperoxidase has a conformation similar to that of myeloperoxidase, although it contains a C-terminal extension: N-terminal-the region shared by 3 peroxidases-cytochrome *c* oxidase-like domain-C46-like domain - EGF-like domain-transmembrane-C-terminal. It is an interesting observation that thyroperoxidase also contains an EGF-like domain, although the position of the EGF-like domain in thyroperoxidase is different from that in prostaglandin endoperoxide synthase and the EGF-like domain of thyroperoxidase belongs to the type 3 EGF-like sequence. Therefore, it is considered that the fusions of EGF-like domain and peroxidase found in prostaglandin endoperoxide synthase and thyroperoxidase are considered to have occurred independently during the course of their evolution.

Table 1

The results of the statistical test - identities of each aligned pair

	PES (sheep)	EGF (mouse)	EGF (rat)	EGF (human)
PES (sheep)	-	6 55 S D	4 17 S D	5 60 S D
EGF (mouse)	37 7%	-	12 24 S D	13 06 S D
EGF (rat)	32 6%	77 1%	-	12 25 S D
EGF (human)	34 0%	69 8%	68 8%	-

The normalized deviations of the alignment scores from the mean value of the scores for the randomized sequences (the upper half of the table) and the identity (%) of the aligned sequence (the lower half of the table). PES = prostaglandin endoperoxide synthase and EGF = epidermal growth factor The names in the parentheses indicate the source animals of the proteins The alignment used in this calculation is shown in fig 1 The score matrix MDM_{78} was used for the statistical test [12] The continuous gaps were treated as single substitutions regardless of their length and a score of −60 was assigned to the gaps [12]

The cysteine residues found in EGF bind with each other by disulfide bonds. As shown in fig.1, all the cysteine residues corresponding with those of EGF are conserved in the EGF-like domain of prostaglandin endoperoxide synthase. Therefore, it is strongly suggested that these cysteine residues in the EGF-like domain may also bind with each other by disulfide bonds. The N-terminal region of the prostaglandin endoperoxide synthase is considered to be one of the luminal domains from the hydropathy profile and the pattern of the distribution of putative glycosylation sites [5-7]. The existence of the cysteine residues which may participate in forming disulfide bonds also supports this hypothesis. One of the putative glycosylation sites is found in the detected EGF-like domain (Asn residue of alignment position 42; see fig.1).

On the other hand, there are several lines of evidence that EGF can affect the biosynthesis of prostaglandin [17-19]. Particularly, one interesting observation is the recovery of the activity of the prostaglandin endoperoxide synthase by EGF and TGF-β after inhibition by aspirin [9]. The experiments showed that the stimulation by EGF and TGF-β produces the increase of mRNA of the prostaglandin endoperoxide synthase, although the details of the mechanism for the control of the expression of prostaglandin endoperoxide synthase by these growth factors has remained unknown. From the existence of the EGF-like domain, together with the experimental observations described above, we speculate that the prostaglandin endoperoxide synthase may regulate the expression of itself by positive or negative feedback mechanism, using the EGF-like domain.

The structural and functional implications of the EGF-like domain detected by computational analysis should be investigated by experimental work.

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REFERENCES

[1] Miyamoto, T , Ogino, N , Yamamoto, S and Hayaishi, O (1976) J Biol Chem. 251, 2629-2636

[2] Ohki, S , Ogino, N , Yamamoto, S and Hayaishi, O. (1979) J Biol Chem 254, 829–836.
[3] Samuelsson, B (1973) Fed Proc. 31, 1442–1450.
[4] Hamberg, M. and Samuelsson, B (1973) Proc. Natl. Acad Sci. USA 70, 899–903.
[5] Merlie, J P , Fagan, D , Mudd, J and Needleman, P. (1988) J. Biol Chem 263, 3550–3553
[6] Yokoyama, C , Takai, T and Tanabe, T (1988) FEBS Lett 231, 347–351
[7] DeWitt, D L and Smith, W L. (1988) Proc. Natl. Acad. Sci. USA 85, 1412–1416.
[8] Nugteren, D.H and Hazelhof, E. (1973) Biochim. Biophys Acta 326, 448–461
[9] Bailey, J M., Makheja, A N , Pash, J and Verma, M. (1988) Biochem Biophys. Res. Commun. 157, 1159–1163
[10] Barker, W C , Hunt, L T., George, D.G , Yeh, L S., Chen, H R., Blomquist, M C , Seibel-Ross, E I., Hong, M.K., Bair, J K., Chen, S.L. and Ledley, R.S (1986) Protein Identification Resource, National Biochemical Research Foundation, Washington, DC.
[11] Nishikawa, K , Nakashima, H , Kanehisa, M and Ooi, T (1987) Protein Sequences and Data Analysis 1, 107–116
[12] Toh, H., Hayashida, H. and Miyata, T (1983) Nature 305, 827–829
[13] Doolittle, R.F., Fend, D F., Johnson, M.S. and McClure, M.A. (1986) Cold Spring Harbor Symp. Quant. Biol. 51, 447–455.
[14] Appella, E., Weber, I.T. and Blasi, F. (1988) FEBS Lett. 231, 1–4.
[15] Libert, F., Ruel, J , Ludgate, M., Swillens, S., Alexander, N , Vassart, G. and Dinart, C. (1987) EMBO J 6, 4193–4196
[16] Morishita, K., Kubota, N , Asano, S., Kaziro, Y and Nagata, S. (1987) J Biol. Chem. 262, 3844–3851
[17] Zakar, T and Olson, D.M (1989) Can. J Physiol Pharmacol. 66, 769–775
[18] Atwell, M.M and Hanson, P.J (1989) Biochim Biophys. Acta 971, 283–288.
[19] Hatt, J.F. and Hanson, P.J (1989) Biochem. J 255, 789–794.
[20] Scott, J., Urdea, M , Quiroga, M., Sanchez-Pescador, R., Fang, N., Selby, M , Rutter, W.J. and Bell, G I. (1983) Science 221, 236–240.
[21] Simpson, R J , Smith, J A , Moritz, R.L., O'Hare, M J., Rudland, P S., Morrison, J.R., Lloyd, C.J., Grego, B , Burgess, A.W. and Nice, E.C. (1983) Eur. J Biochem. 153, 629–637
[22] Gregory, H. and Preston, B.M. (1977) Int J Peptides Protein Res 9, 107–118.