Volume 269, number 2, 454–456 FEBS 08823 September 1990

EF-hand motifs in inositol phospholipid-specific phospholipase C

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Received 4 July 1990

Computer sequence analysis with a linear weight matrix revealed the presence of one canonical EF-hand motif in the δ isoform of inositol phospholipid-specific phospholipase C and one ancestral EF-hand in the γ form.

Inositol phospholipid-specific phospholipase C; EF-hand

1. INTRODUCTION

Protein sequence data banks are gold mines of information, whose extraction continuously needs new tools. It is exemplified here by the identification of a typical EF-hand motif [1] and of an ancestral EF-hand motif in the δ and γ form of inositol phospholipid-specific phospholipase C (PI-PLC). PI-PLCs are involved in the hormonal and growth factor induced production of the second messengers inositol 1,4,5-trisphosphate and diacylglycerol [2]. There are different types of PI-PLC, subdivided by Rhee et al. [3] into 5 groups: α (57 kDa), β (138 kDa), γ (148 kDa), δ (88 kDa) and ϵ (85 kDa). The sequences of the first 4 are available and reveal some homology between β, γ and δ in two rather short (ca 140 residues) domains, X and Y.

Our tool consisted of a linear weight matrix capable of detecting EF-hand type motifs with very high efficiency. A weight matrix is a two-dimensional array of values that represent the scores for finding each of the possible sequence characters at each position in the signal or pattern that the matrix is supposed to detect (for a recent review, see [4]).

So as to obtain a matrix that would pick up efficiently true EF-hand domains, we followed a two step procedure. In the first step we created a matrix of 12 positions (corresponding to the length of the Ca^{2+}-binding loop in the EF-hand domain) from the alignment of 57 well characterized EF-hand regions. From the result of this preliminary scan we created a new matrix of 16 positions (corresponding to the Ca^{2+}-binding loop plus two residues on both sides) from 101 EF-hand regions: the 57 original domains as well as 44 additional ones from various known Ca^{2+}-binding proteins [5]. This 'second generations' matrix is shown in Fig. 1.

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When the SWISS-PROT databank (release 14 of April 1990) was scanned with a cut-off score set to 9 (out of a maximum number of 27), the matrix picked up 99.5% of all real and 38% of all ancestral EF-hand motifs. In addition 14 proteins, not known to contain EF-hand motifs, also obtained a score of 9 or above. Among these an exceptionally high score of 20 was generated by a segment in bovine and rat δ PI-PLC. By means of a low cut-off score we then checked whether other PI-PLCs contained the vestiges of an EF-hand motif. Such segments were indeed found in rat and bovine γ PI-PLC (score of 3.6) and in the recently sequenced [6] γ' isoform (score of 3.9). Fig. 2 shows the sequences of the EF-hand motifs in δ, γ and γ' PI-PLC compared to the EF-hand consensus sequence of Kretsinger [1], more specifically to the consensus sequence of the '1 and 3 type' domains (for details, see [1]). It confirms that according to this well established criterion δ PI-PLC contains one canonical EF-hand motif and γ PI-PLC an EF-hand segment that likely no longer binds Ca^{2+}. It should be noted that in all δ and γ PI-PLCs this 29 residue-long segment is located ca 150 residues N-terminalwards of the conserved X region [3], in a region that is otherwise very poorly conserved in the different isoforms. Our data suggest that δ PI-PLC only can be selectively detected by Stains-all [7] or by the ^{45}Ca-overlay [8], which would be very useful in the classification of the PI-PLC family.

What functional meaning could have the EF-hand motif in one particular isoform? Different cytosolic and membrane forms of PI-PLC show different Ca^{2+} dependencies which differ moreover with the substrates [9–12]. Extraction and purification as well as the use of detergents have pronounced effects on the Ca^{2+} dependency of most forms. There is strong evidence that in the brain all PI-PLCs are down-regulated [3] and that up-regulation by external signals occurs through different mechanisms for the different isoforms [13]. It is

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00145793/90/$3.50

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ala	7	10	0	7	0	5	0	0	4	0	1	7	8	0	0	8
Arg	10	1	0	8	0	5	0	2	8	0	0	7	1	0	0	12
Asn	4	0	0	3	33	11	20	4	2	0	9	1	7	2	0	2
Asp	9	0	99	2	61	3	50	0	2	0	31	1	28	7	0	1
Cys	1	3	0	1	0	0	0	0	0	1	2	1	0	0	0	2
Gln	2	0	0	3	0	2	1	1	7	0	4	0	11	0	0	6
Glu	15	1	0	5	4	5	0	0	10	0	9	7	24	91	0	6
Gly	0	5	0	0	0	46	1	88	0	0	8	6	1	0	0	6
His	4	0	0	0	0	5	1	0	2	0	0	1	1	0	0	0
Ile	6	11	0	5	0	0	1	0	0	52	0	5	0	0	2	7
Leu	9	19	0	5	0	1	0	0	3	21	0	13	1	1	35	12
Lys	10	1	0	27	0	14	0	4	14	0	0	1	8	0	0	16
Met	8	2	0	3	0	1	0	1	0	5	1	2	1	0	8	4
Phe	2	25	1	4	0	0	0	0	8	0	0	26	0	0	46	3
Pro	0	0	0	0	0	0	0	0	0	0	0	5	3	0	0	1
Ser	3	2	0	7	2	2	26	1	9	0	26	3	4	0	0	4
Thr	6	0	0	17	1	1	1	0	17	0	10	4	1	0	0	2
Trp	0	2	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Tyr	0	8	1	0	0	0	0	0	11	0	0	4	1	0	7	0
Val	5	11	0	4	0	0	0	0	4	22	0	7	1	0	0	9

Fig. 1. Matrix to detect EF-hand regions. Protein weight matrix of 16 columns derived from 101 EF-hand sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Coordinates										X		Y		Z		-Y		-X				-Z								
Consensus sequence /alternatives	E	n	x	x	n	n	x	x	n	D	x	D N	x	D N S	G	x	I L V	G S T D N E	x	x	E	L	x	x	n	n	x	x	n	
delta (rat)	**W**	**I**	**H**	**S**	**C**	**L**	**R**	**K**	**A**	**D**	**K**	**N**	**K**	**D**	**N**	**K**	**M**	**N**	**F**	**K**	**E**	**L**	**K**	**D**	**F**	**L**	**K**	**E**	**L**	144-172
delta (bov)	**W**	**I**	**H**	**S**	**C**	**L**	**R**	**K**	**A**	**D**	**K**	**N**	**K**	**D**	**N**	**K**	**M**	**S**	**F**	**K**	**E**	**L**	**Q**	**N**	**F**	**L**	**K**	**E**	**L**	83-111
gamma (bov & rat)	**W**	**L**	**R**	**K**	**Q**	**F**	**Y**	**S**	**V**	**D**	**R**	**N**	**R**	**E**	**D**	**R**	**I**	**S**	**A**	**K**	**D**	**L**	**K**	**N**	**M**	**L**	**S**	**Q**	**V**	156-184
gamma' (rat)	**W**	**L**	**R**	**K**	**Q**	**I**	**Y**	**S**	**V**	**D**	**Q**	**T**	**R**	**R**	**N**	**S**	**I**	**S**	**L**	**R**	**E**	**L**	**K**	**T**	**I**	**L**	**P**	**L**	**V**	145-173

Fig. 2. EF-hand type Ca^{2+}-binding motifs in PI-PLC. Upper line: numbering from 1 to 29. X, Y, Z, -Y, -X and -Z are the residues positioned at the vertices of the Ca^{2+}-coordinating octahedron. The consensus sequence of the '1 and 3 type' EF-hand domain was taken from [1]; n stands for any residue with a hydrophobic side chain, x for any residue. Bold letters correspond to residues that strictly obey to the consensus sequence. The numbering at the right refers to the position of the EF-hand motif in the sequence.

then attractive to speculate that δ PI-PLC, with its particularly well expressed EF-hand motif, is the main (perhaps unique) operative one in in vivo PI hydrolysis provoked by Ca^{2+} influx. Or Ca^{2+} binding to the EF-hand domain may change the substrate selectivity of δ PI-PLC. Or the EF-hand Ca^{2+}-binding site may be completely dissociated from the catalytic activity of the enzyme and regulate other aspects of the enzyme, such as its location or interaction with other proteins.

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