Interfering PLD1-PED/PEA15 interaction using self-inhibitory peptides: An in silico study to discover novel therapeutic candidates against type 2 diabetes

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Diabetes type 2 (T2D) is a very complex disorder with a large number of cases reported worldwide. There are several reported molecular targets which are being used towards drug design. In spite of extensive research efforts, there is no sure shot treatment available. One of the major reasons for this failure or restricted success in T2D research is the identification of a major/breakthrough therapeutic target responsible for the progression of T2D. It has been well documented that one of the major causes mediating the insulin resistance is the interaction of PLD1 with PED/PEA15. Herein, we have performed in silico experiments to investigate the interaction between PLD1 with PED/PEA15. Furthermore, this study has explored pertinent molecular interactions involving the self-derived peptides. The peptides identified in this study are found to be capable of restricting the interaction of these two proteins. Accordingly, the study suggests that the “self-derived peptides” could be used as promising therapeutic candidate(s) against T2D.

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1. Introduction

Diabetes type 2 (T2D) is a complex disorder, which is usually diagnosed by the features of hyperglycaemia, commonly affects by defects in the secretion and action of insulin (Kaneto et al., 2016; American Diabetes, 2009; Olokooba et al., 2012). Recent findings give an impression that the environmental determinants on the human genome are very much responsible for current scenario of wide spread epidemics cases of T2D (Fiory et al., 2017; Prasad and Groop, 2015; Sears and Genuis, 2012; Tabish, 2007). However, the identification of most important therapeutic target genes mainly responsible for the progression of this disorder is still a quest (Stancakova and Laakso, 2016; Kwak and Park, 2016). Condorelli et al. conducted experimentation on skin fibroblast mRNAs from the T2D individuals and they adopted a differential display approach to identify the differentially expressed genes in T2D (Condorelli et al., 1998). They reported a 15 KDa protein which was found to be highly expressed in the skin fibroblasts of T2D individuals, along with the expression level of this protein was also high in the adipose tissues and the skeletal muscle of the T2D subjects (Condorelli et al., 1998). Later the cloning was performed and the identified 15 KDa protein was termed PED/PEA-15 (Araujo et al., 1993). Later several studies were conducted on this protein and reports suggest that this 15 KDa cytoplasmic protein is ubiquitously expressed in human tissues and is found to be highly conserved among species. It was found that PED/PEA-15 demonstrates no catalytic activity but it rather serves as a molecular...
adaptor playing crucial role in regulating several intracellular functions (Fiory et al., 2017). Despite being a small 15 KDa protein, PEA-15 also serves as a major modulator of apoptosis, proliferation, nutrient metabolism and survival in different cell types (Fiory et al., 2009; Condorelli et al., 1999; Renault et al., 2003; Renganathan et al., 2005; Vigliotta et al., 2004).

There are several reports highlighting the overexpression of PED/PEA15 in several tissues of type 2 diabetes affected individuals (Valentino et al., 2006; Viparelli et al., 2008). Finding also suggests that in intact cells and transgenic animal models, the overexpression of PEA15 impairs insulin regulation of glucose transport by a mechanism mediated by the interaction of PEA15 with the D4 region of Phospholipase D1 (PLD1) which consequently results in the increase of protein kinase C-α activity (Fiory et al., 2017; Viparelli et al., 2008).

PLDs are another ubiquitously expressed enzyme which are widely distributed in animals, bacteria, fungi, and plants and catalyzes the formation of phosphatidic acid (PA), which is an an intracellular messenger implicated in various cellular processes (Huang and Frohman, 2009; Doti et al., 2010). PA is further converted into several other mediators, one of them is diacylglycerol (DAG), which is a major activator of phosphokinase C isoforms (PKCs) (Cummings et al., 2002).

The activity of PLD1 is found to be synergistically regulated by small GTPases, and one of them are isoforms of protein kinase C mainly PKC-α (Sung et al., 1997; Powner and Wakeham, 2002; Exton, 2002). The increased activity of PLD1 is reported to be negatively regulating the glucose transport by a mechanism involving its interaction with PED/PEA15 (Condorelli et al., 1998; Valentino et al., 2006). As mentioned earlier, PEA15 is found to be overexpressed in in individuals affected by type 2 diabetes. A molecular mechanism has been proposed earlier, where the insulin resistance is mediated by the interaction of PLD1 with PED/PEA15 (Vigliotta et al., 2004; Zhang et al., 2000). Studies also reported that the blocking this PLD1-PED/PEA15 interaction with either the D4 region of PLD1 or with the peptide derive from the D4 region of PLD-1 or from PED/PEA15 results in the re-establishment of normal PKC signaling and the glucose uptake, which could be a useful way to encounter the progression of T2D (Fig. 1) (Fiory et al., 2017; Viparelli et al., 2008; Zhang et al., 2000; Viparelli et al., 2008).

Here in this study we have explore the interaction of PLD-1 with PED/PEA15 using in silico protein-protein interaction approach. Further we derived the self-inhibitory peptides from PLD-1 and PED/PEA15 proteins and these linear peptide segments were found to be maximum contributing in the PLD1-PED/PEA15 interaction. So we assume these peptides could be used as a inhibitory peptides capable of blocking the PLD1-PED/PEA15 interaction. These peptides are suggested to be used as an important segment for future studies aimed at developing novel compounds with great therapeutic potential in T2D.

2. Material and methods

2.1. Structure modelling

The crystal structure of PED/PEA15 as available with protein data bank (pdb id: 4iz5) (Mace et al., 2013), while the structure of PLD-1 was unavailable. Here we modelled the 3D protein structure of D4 region of PLD-1. The complete amino acid sequence of PLD-1 was retrieved from uniprot database (Apweiler et al., 2004) and the amino acids of the D4 region were selected for structural modelling purpose (residues 712–1024). I-tasser program (Zhang, 2008) was used to model the structure of PLD-1 (D4 Region). Five structures were generated by I-tasser. All the structures were refined using 3Drefine server (Bhattacharya et al., 2016). Further the structures were validated using Rampage and the structure passing the stereochemical properties check was used in this study (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

2.2. Protein-Protein interaction study:

The structure of PED/PEA15 and PLD1 were subjected to protein-protein interaction study using patch dock (Schneidman-Duhovny
et al., 2005). The results obtained from patchdock were further refined using firedock algorithm (Andrusier et al., 2007).

2.3. Deriving self-inhibitory peptides:

The peptiderive server was used to derive self-inhibitory peptides (Sedan et al., 2016). Here the PED/PEA15-PLD1 complex was uploaded in the peptiderive server and the self-inhibitory peptides were derived from the PLD1 and PED/PEA15 protein of the complex. Here the peptides derived are the linear peptide segments from each protein highly contributing to its interaction with another protein. Peptide of different length ranging from 5 to 25 amino acids was derived in this step. All the peptides were evaluated on the basis of the interface scores. The top most contributory peptides from each protein were selected.

3. Results and discussion

Due to the unavailability of the 3D structure of PLD-1, here we modelled the structure of PLD-1 using its amino acid sequence information. It is widely reported that the D4 region of PLD-1 is majorly involved in its interaction with PED/PEA15, suggesting that PED/PEA15 impairs insulin regulation of glucose transport, and this process is mediated by a mechanism involving its interaction with the D4 region of PLD-1 (Viparelli et al., 2008; Doti et al., 2010; Viparelli et al., 2008). Taking these information into account, here we modelled the structure of D4 region using the in silico approach. The program I-tasser was used for modelling the structure of PLD-1 (Yang et al., 2015). The structure generated by I-tasser was further refined using 3Drefine server (Bhattacharya et al., 2016). For efficient protein structure refinement, this server utilizes two-step process involving the iterative optimization of hydrogen bonding network combined with atomic-level energy minimization on the optimized model using a composite physics and knowledge-based force fields (Bhattacharya et al., 2016). Further the stereochemistry quality of the modeled structure was validated using RAMPAGE. The Ramachandran plot generated by RAMPAGE (Lovell et al., 2003) reveals that a large proportion of amino acid residues of the modeled structure we present in the in most favored region (93.3%), while just 1% residues were present in the outlier region. It is well evident that interfering the PED/PEA-15-PLD1 interaction restores insulin sensitivity (Viparelli et al., 2008).

Protein-protein interaction study was carried out using patchdock to get an insight into the interaction of PED/PEA15 with PLD1. The complex generated by patchdock were further refined using firedock for 1000 steps and the complex with the best global

![Fig. 2. Interaction of PED/PEA15 with PLD1 and the residues involved in their binding.](image)

| Peptide length | Interface score | Relative interface score (%) | Sequence |
|----------------|----------------|-----------------------------|----------|
| 5              | -4.192         | 30.74                       | AEYGT    |
| 6              | -5.046         | 37.01                       | YGTLLQ   |
| 7              | -5.435         | 39.86                       | EYGTLLQ  |
| 8              | -6.224         | 45.65                       | AEYGTLLQ |
| 9              | -6.928         | 50.81                       | MAEYGTLLQ|
| 10             | -6.823         | 50.04                       | MAEYGTLLQD|
| 11             | -6.881         | 50.47                       | MAEYGTLLQDL|
| 12             | -6.889         | 50.53                       | MAEYGTLLQDLT|
| 13             | -7.701         | 56.48                       | GTLLQDLTNITL|
| 14             | -9.148         | 67.09                       | YGTLLQDLTNITL|
| 15             | -9.537         | 69.95                       | EYGTLLQDLTNITL|
| 16             | -10.327        | 75.74                       | AEYGTLLQDLTNITL|
| 17             | -11.03         | 80.9                        | MAEYGTLLQDLTNITL|
| 18             | -11.024        | 80.85                       | MAEYGTLLQDLTNITL|
| 19             | -11.61         | 85.15                       | MAEYGTLLQDLTNITL|
| 20             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|
| 21             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|
| 22             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|
| 23             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|
| 24             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|
| 25             | -11.601        | 85.09                       | MAEYGTLLQDLTNITL|

Table 1 The linear peptides derived from PLD-1 and their Interface score and other details.
energy score was selected for further investigation. Here we found that PED/PEA15 interacts with PLD-1 with global free energy score of −71.69 (Fig. 2). Later, the PED/PEA15-PLD1 complex was investigated using peptiderive server which predicts the interfacing linear peptides from both PLD1 and PED/PEA15 which contribute most to the interaction of these two proteins. Here our approach was to find out the fragments constituting a large portion of the binding energy, these selected fragments were further selected and can be considered to be candidates used for competing with the existing PED/PEA15-PLD1 interaction. The peptide ranging from 5 to 25 residues in each protein along with their contribution in complex formation was analyzed. The peptide found to be showing significant binding energy is reported. The interface score corresponds to the binding energy of the protein-peptide/protein-protein complex. In our study, the total interface score for PLD1- PED/PEA15 complex was found to be -13.634. While the peptides spanning residues [782–805] and [782–806] were showing maximum contribution in the binding of PED/PEA15 with PLD1, where these peptide binds with the interface score of −7.389 and −7.382 respectively. This suggests that these two peptides were contributing 54.20% and 54.15% of the total interface score of PLD1- PED/PEA15 complex (Table 1). These selected hot segments derived from the peptiderive tool could compete with their originally derived proteins (PLD1 or PED/PEA15) from for binding to the partner. Suggesting these short 23 mers and 24 mers PLD1 mimicking peptides can be used as effective antagonists capable of disrupting the PLD1-PED/PEA15 interaction. In the previous studies it has been well reported that the synthetic peptides derived from the spanning residues 762–801 of PLD-1 act as a potent binding competitors (Doti et al., 2010). Our finding are also very much in harmony with the previous results reported by different research groups. Likewise another group of linear peptides were derived from PED/PEA15 protein. It was earlier reported that the linear segment of PED/PEA15 comprising of residues 1–24 abrogates the formation of PED/PEA15-PLD1 complex and subsequently reducing the activity of protein kinase C-α (Viparelli et al., 2008). An important finding in this study was that the administration of this peptide successfully restores the insulin-stimulated glucose uptake (upto 70%). In our study we found that PED-(1-19) was the most actively participating segment and this peptide was found to show interface score of -11.610, followed by PED/PEA15 -(1-20), (1-21), (1-23), (1-24) and (1-25) (Table 2). The selected peptides were

### Table 2

The linear peptides derived from PED/PEA15 and their Interface score and other details.

| Peptide length | Interface score | Relative interface score (%) | Sequence |
|----------------|-----------------|-------------------------------|----------|
| 5              | -2.946          | 21.61 FFISC                  |          |
| 6              | -5.162          | 37.86 QKYRRV                 |          |
| 7              | -5.162          | 37.86 NQKRYRVY               |          |
| 8              | -5.175          | 37.96 ENQKRYRV               |          |
| 9              | -5.175          | 37.96 RENQKRYRV              |          |
| 10             | -5.428          | 39.81 HRENQKRYRV             |          |
| 11             | -5.545          | 40.67 AHERNQKRYRV            |          |
| 12             | -5.873          | 43.08 FFSCADDKVF             |          |
| 13             | -6.592          | 48.35 QFFSCADDKVF            |          |
| 14             | -6.592          | 48.35 QFFSCADDKVF            |          |
| 15             | -6.396          | 46.71 RILKAHRENQKRYRV        |          |
| 16             | -6.396          | 46.71 RILKAHRENQKRYRV        |          |
| 17             | -6.725          | 49.32 QFFSCADDKVFNKCIG       |          |
| 18             | -6.725          | 49.32 QFFSCADDKVFNKCIG       |          |
| 19             | -6.725          | 49.32 QFFSCADDKVFNKCIG       |          |
| 20             | -6.725          | 49.32 QFFSCADDKVFNKCIG       |          |
| 21             | -6.734          | 49.39 QFFSCADDKVFNKCIGDA     |          |
| 22             | -6.923          | 50.78 IGDAIQRLKAHERNQKRYRV   |          |
| 23             | -6.733          | 49.38 QFFSCADDKVFNKCIGDAQRI  |          |
| 24             | -7.389          | 54.2 QFFSCADDKVFNKCIGDAAQRI  |          |
| 25             | -7.382          | 54.15 QFFSCADDKVFNKCIGDAAQRI |          |

**Fig. 3.** Interaction of PED/PEA15 with PLD1 in complex with their self-derive inhibitors. (a) Interaction of PED/PEA15 with PLD1 (b) Interaction of PED/PEA15 with PLD1 in presence of PLD-1 derived peptide (c) Interaction of PED/PEA15 with PLD1 in presence of PED/PEA15 derived peptide.
further tested to confirm their accuracy. The selected peptides in complex with their respective receptor protein were subjected to dock against another partner protein and the binding affinity was further tested to confirm their accuracy. The selected peptides in sores of PED/PEA15–PLD-1 complex in the presence and absence of PED/PEA15 to PLD-1.

4. Conclusion

It has been well established that interrupting the PLD1-PED/PEA15 interaction is one of the important target for type 2 diabetes. Here in this study we have screened out some of the linear peptide segments from PLD1 and PED/PEA15 which are majorly contributing to their interaction. The peptide may act as a competitive inhibitor interrupting the PLD1-PED/PEA15 interaction resulting in the restoration of insulin-stimulated glucose uptake.

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