The unique N-terminal domain of the cAMP phosphodiesterase PDE4D4 allows for interaction with specific SH3 domains

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Abstract Of the five PDE4D isoenzymes, only the PDE4D4 cAMP specific phosphodiesterase was able to bind to SH3 domains. Only PDE4D4 and PDE4A5, but not any other PDE4A, B, C and D isoforms expressed in rat brain, bound to src, lyn and fyn kinase SH3 domains. Purified PDE4D4 could bind to purified lyn SH3. PDE4D4 and PDE4A5 both exhibited selectivity for binding the SH3 domains of certain proteins. PDE4D4 did not bind to WW domains. We suggest that an important function of the unique N-terminal region of PDE4D4 may be to allow for association with certain SH3 domain-containing proteins.

Key words: cAMP phosphodiesterase; Src homology 3 domain; Src family tyrosyl kinase; Lyn; Protein-protein interaction; Compartmentalisation

1. Introduction

The second messenger cyclic AMP (cAMP) exerts control over a wide range of cellular processes in a cell type specific fashion [1]. There is an increasing body of evidence to support the notion that cAMP signalling within cells is functionally compartmentalised [1,2]. Over the past few years, the machinery which underpins this has been uncovered. This includes the targeted expression of specific adenylyl cyclase isoenzymes, of protein kinase A isoenzymes and of cAMP phosphodiesterase (PDE) isoenzymes [1,2].

A large multigene family of enzymes exhibits cAMP PDE activity, providing the sole route for cAMP degradation in cells [3–6]. These enzymes are thus poised to exert a profound influence on the intracellular levels of cAMP, as can be demonstrated by the action of inhibitors of these enzymes. Specific PDE enzyme classes appear to either control or influence distinct functional processes in cells as inhibitors, which are specific for certain PDE classes and can exert selective effects [3,7]. This selectivity has been exploited in the development of various therapeutic agents. Thus, for example, inhibitors which are selective for the PDE4 enzyme family provide potent anti-inflammatory agents, exerting selective cellular effects such as exemplified by the inhibition of TNF-α production and action [7].

The PDE4 family of enzymes is encoded by four distinct genes with additional heterogeneity occurring through alternative mRNA splicing [4,6]. This takes the form of 5′ domain swaps, producing proteins with distinct N-terminal regions. A key role of these N-terminal regions has been suggested [6] to be in the precise intracellular targeting of certain PDE4 isoenzymes. This was initially demonstrated [8] for PDE4A1, whose unique N-terminal region is responsible for targeting this protein to a specific intracellular membrane localisation [9–12]. More recently, other PDE4A isoenzymes [13–15] as well as various PDE4B [16] and PDE4D [17] isoenzymes have been shown to exhibit distinct patterns of intracellular distribution, suggesting that they are too selectively targeted within cells. In this regard, we have demonstrated [18] that the rat PDE4A5 isoenzyme as well as its human homologue PDE4A4 [19] can both interact with the src homology 3 (SH3) domains found in various proteins. SH3 domains are self-folding structures which allow for protein-protein interaction to occur through their ability to interact with proline- and arginine-rich regions in the coupled protein target [20,21]. These domains are utilised by a variety of signalling proteins, adapter proteins and cytoskeletal proteins.

In this study, we demonstrate that of the five known isoenzymes generated by the PDE4D gene [17], the brain specific PDE4D4 form can interact with certain SH3 domains expressed as GST fusion proteins. The screening of a library of SH3 fusion proteins shows a selectivity for interaction with those of certain src family tyrosyl kinases. We suggest that this may indicate a propensity of this isoenzyme to interact with specific SH3 domain-containing proteins.

2. Materials and methods

2.1. Materials

Glutathione-Sepharose was obtained from Pharmacia (St. Albans, Herts, UK). All tissue culture reagents were from Gibco Life Technologies (Paisley, UK). [8-^{3}H]Adenosine 3′:5′-cyclic monophosphate (24 Ci/mmol) and the enhanced chemiluminescence (ECL) detection system were from Amersham International (Amersham, Bucks, UK). All other reagents were from Sigma (Poole, Dorset, UK).

2.2. GST fusion proteins

The GST fusion proteins of the SH3 domains of human lck, lyn, src and fyn were generated by us previously [18] in pGEX-2T. Fusion proteins of the Csk and Crk SH3 domains were provided by Dr. Siegmund Fischer (Laboratory of Clinical and Molecular Oncology, INSERM, Paris, France) and that for c-Abl by Dr. David Baltimore (Department of Biology, MIT, Cambridge, MA, USA).

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Abbreviations: PDE, cyclic nucleotide phosphodiesterase; UCR, upstream conserved region; SH3, src homology 3 domain

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2.3. Purification of GST fusion proteins

The purification of GST fusion proteins was performed as described by us previously in some detail [18].

2.4. Pull down assay for protein-protein interaction

Pull down assays using purified GST fusion proteins, immobilised on glutathione-Sepharose resin, were performed on sufficient of the high speed supernatant fraction (100 000 × *g*) from transiently transfected COS-7 cells to contain 30–40 EU PDE activity as previously described by us [18].

2.5. Construction of maltose binding protein (MBP) fusion proteins

The full open reading frame of the cDNA encoding the human PDE4D4 isoform (pPDE39; GenBank accession number L20969; [17]) was inserted into the *Not*I site of pMALN [22] to produce pMALP39. This construct produces a fusion between the MBP and the amino-terminus of the PDE4D4 protein. The construct was prepared by the addition of *Not*I sites to the cDNAs by the use of PCR, as described previously [17]. The construct pMALP39A1 encodes a fusion between MBP and the amino-terminus of the first 169 amino acids of PDE4D4. To generate pMALP39A1, pMALP39 was digested with the restriction endonucleases *Bam*HI and *Xba*I. The 5′ overhangs produced by endonuclease digestion were made blunt by filling in with dNTPs and Klenow fragment and the resulting construct was allowed to recircularise and was religated with T4 DNA ligase. This procedure removed the entire insert from pMALP39 except the first 169 amino acids, of which the first 152 are unique to PDE4D4 and the remaining 15 are common to the PDE4D3, PDE4D4 and PDE4D5 isoforms [17]). It also moved the sequence TAG, originally part of the *Xba*I recognition sequence, into the correct reading frame at the end of codon 169, where it could serve as a stop codon. The structures of the constructs were verified by sequencing before use.

2.6. Purification of MBP fusion proteins

The induction and purification of MBP fusion proteins was by a method based on that already described for the purification of GST fusion proteins by us [18] but with the following differences. The *Escherichia coli* (JM109) cells were transformed with pMAL-c2 (for the production of MBP) or with pMAL-c2 containing either full length HSPDE4D4 or the amino-terminal region (residues 1–166) of HSPDE4D4 as in frame fusion proteins with MBP. The L-broth was supplemented with glucose (1 mg/ml) as well as with ampicillin (100 μg/ml). Expression of the fusion protein was induced by the addition of IPTG to a final concentration of 0.3 mM. The culture was then shifted to 30°C for the expression of the fusion protein. The sonicate was cleared by centrifugation at 9000 × *g* for 30 min and the supernatant was incubated with amylose resin (New England Biolabs) equilibrated in phosphate-buffered saline containing 1 mM DTT and protease inhibitor cocktail. The fusion protein was eluted from the washed amylose resin by three incubations in 1 ml elution buffer (KHEM supplemented with 10 mM maltose) for at least 10 min each, end-over-end at 4°C. The three eluted fractions were pooled and dialysed at 4°C against three 600 ml batches of dialysis buffer (50 mM NaCl, 20 mM Tris (pH7 .4)) before being snap frozen as aliquots. The aliquots were stored at −80°C until use.

2.7. Overlay assay

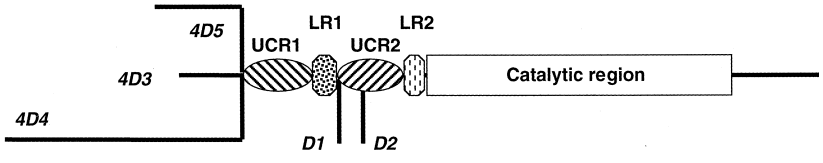
Purified GST fusion proteins were eluted from the glutathione-Sepharose beads by three incubations in 100 μl elution buffer (10 mM glutathione, 50 mM Tris-HCl (pH 8.0)) for at least 10 min each, end-over-end at 4°C. The three eluted fractions were pooled and assayed for their protein concentration, diluted 1:2 with 2 × Laemmli buffer [2] and then boiled for 5 min. The boiled samples were loaded onto a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel (10 μg/lane) which was run at 40 mA/gel until the dye front reached the bottom. The samples were then transferred from the gel onto a nitrocellulose membrane. The membrane was incubated for 1 h at room temperature with gentle shaking in 100 ml blocking buffer (137 mM NaCl, 20 mM Tris (pH 7.6), 5% skimmed milk powder). The blocked membrane was washed four times over a 20 min period with T-TBS (137 mM NaCl, 20 mM Tris (pH 7.6), 0.1% Tween-20) and then incubated overnight at 4°C with rapid shaking in the presence of 1 μg of either purified MBP or of MBP-HSPDE4D4 diluted in 10 ml dilution buffer (137 mM NaCl, 20 mM Tris (pH 7.6), 1% skimmed milk powder). The membrane was then washed as before and incubated for 1 h at room temperature, with rapid shaking, in the presence of an anti-MBP polyclonal antibody (New England Biolabs) diluted 1:10 000 in dilution buffer. The membrane was then washed as before and incubated for 1 h at room temperature, with rapid shaking, in the presence of peroxidase-conjugated anti-rabbit IgG (Sigma) diluted 1:20 000 in dilution buffer. Finally, the membrane was washed again as before and the labelled bands were detected according to the Amersham ECL western blotting visualisation protocol.

2.8. Other methods

All other methods used in this study have been described in some detail previously by us [17,18]. These include the cloning of the HSPDE4D cDNAs into mammalian expression vectors, the transient expression of these species in COS-7 cells, the harvesting and fractionation of transfected COS-7 cells, the Western blotting procedure, reverse transcriptase-PCR procedures and assays for cAMP PDE enzyme activity with 1 μM cAMP as substrate.

3. Results

SH3 domains are self-folding structures of around 60 amino acids whose globular nature and propensity for interaction with proline- and arginine-rich peptides has been determined both by NMR and XRD [23,24]. A number of investigators have screened for putative SH3 domain-interacting proteins by expressing the SH3 domains of specific proteins as fusion proteins with GST [20,21,25]. These have been used to capture interacting proteins in 'pull down' assays done upon the binding of GST to glutathionine agarose. We have utilised this



MEAEGSSAPARAGSGEGSDSAGGATLKAPKHLWRHEQHH
QYPLRQPQFRLLHPHHHLPPPPPPSPQPQPQCPLQPPPPPPLP
PPPPPPGAARGRYASSGATGRVRHRGYSDTERYLYCRAMD
RTSYAVETGHRPGLKKSRMSWPSSFQGLRR

Fig. 1. Schematic representation of the amino-terminal region of PDE4D4. The top panel shows a representation of the five known PDE4D isoenzymes [17]. These are PDE4D1–PDE4D5. The long isoenzymes, PDE4D4 through PDE4D5, contain both the UCR1 and UCR2 regions which provide unique signatures of the PDE4 enzyme family. The short isoforms, PDE4D1 and 4D2, lack the UCR1 region. The isoenzymes are characterised by unique N-terminal regions, with the sequence of that of PDE4D4 being shown in the lower panel. Underlined are the three proline-rich stretches of the sequence.

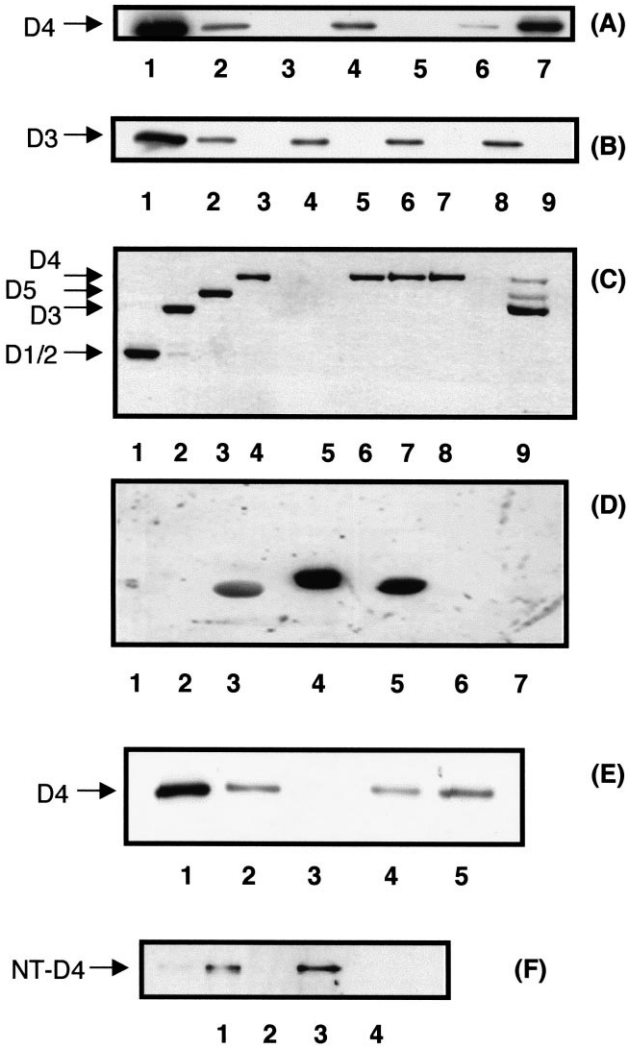


Fig. 2. Interaction of PDE4D4 with SH3 domains. (**A**) shows a PDE4D immunoblot of a 'pull down' assay for PDE4D4 with bound (tracks 3, 5 and 7) and unbound (tracks 2, 4 and 6) fractions. A PDE4D4 standard is shown (track 1) and pull downs with agarose beads only (tracks 2 and 3), with GST agarose (tracks 4 and 5) and a GST fusion protein of lyn SH3 bound to agarose (tracks 6 and 7). (**B**) shows a PDE4D immunoblot of a 'pull down' assay for PDE4D3 with bound (tracks 3, 5, 7 and 9) and unbound (tracks 2, 4, 6 and 8) fractions. A PDE4D3 standard is shown (track 1) and pull downs with agarose beads only (tracks 2 and 3), with GST agarose (tracks 4 and 5), with a GST fusion protein of src SH3 bound to agarose (tracks 6 and 7) and with a GST fusion protein of lyn SH3 bound to agarose (tracks 6 and 7). (**C**) shows a PDE4D immunoblot for standards of PDE4D1+2 (track 1), PDE4D3 (track 2), PDE4D5 (track 3) and PDE4D4 (track 4), a cytosol extract from rat brain (track 9) and the bound fractions from pull down assays done on rat brain cytosol with GST fusion proteins of lyn SH3 (track 8), fyn SH3 (track 7) and src SH3 (track 6) as well as GST alone (track 5). (**D**) shows an immunoblot of an overlay assay done using PDE4D4 to probe lanes containing either GST itself (track 1) or various GST fusion proteins of the SH3 domains of lck (track 2), src (track 3), lyn (track 4), fyn (track 5), crk (track 6) and csk (track 7). (**E**) shows a PDE4D immunoblot of a 'pull down' assay using purified MBP fusion protein of PDE4D4 with bound (tracks 3 and 5) and unbound (tracks 2 and 4) fractions. The MBP-PDE4D4 standard is shown (track 1) as are pull downs with purified fusion proteins of GST alone (tracks 2 and 3) and lyn SH3 (tracks 4 and 5). The MBP fusion protein of PDE4D4 had a size of 152 ± 3 kDa. (**F**) shows a pMal immunoblot of a 'pull down' assay using purified MBP fusion protein of the N-terminal region of PDE4D4 with bound (tracks 2 and 3) and unbound (tracks 1 and 4) fractions. The pull downs were done with purified fusion proteins of GST alone (tracks 1 and 2) and GST-lyn SH3 (tracks 3 and 4). The MBP fusion protein of the N-terminal region of PDE4D4 had a size of 84 ± 2 kDa. These data are typical of experiments done at least three times on separate occasions.
←

procedure [18] to show that the isoenzyme, PDE4A5 (rpde6), can interact with the SH3 domains of a variety of proteins, exhibiting a preference for those of src family tyrosyl kinases lyn and fyn as well as src itself. This interaction was determined by the unique N-terminal region of PDE4A5 which contains proline- and arginine-rich motifs.

The PDE4D gene encodes so called 'long' and 'short' isoenzymes (Fig. 1), due to alternative mRNA splicing occurring at two distinct sites [6,17]. These isoenzymes have unique N-terminal regions which are encoded by single 5′ exons. However, the long isoenzymes exhibit two domains which are unique to the PDE4 enzyme family. These are called upstream conserved region (UCR) 1 and UCR2. In contrast to this, the short form, PDE4D1, exhibits only the UCR2 region and PDE4D2 exhibits only the carboxy-terminal portion of UCR2.

Recently, we have cloned two novel long human PDE4D isoenzymes [17]. One of these, called PDE4D4, has a unique 136 residue N-terminal region of which 30 residues are prolines and 16 residues are arginines (Fig. 1). The clusters of proline residues found in this region are indicative of a propensity for interaction with SH3 domains. Here, we demonstrate, in a 'pull down' assay with the SH3 domain of lyn tyrosyl kinase, expressed as a GST fusion protein, that human PDE4A4 can indeed interact with this SH3 domain (Fig. 2A). In these assays, we mixed recombinant PDE4D4, expressed in the cytosol of transiently transfected COS-7 cells, with a purified lyn SH3-GST fusion protein that was immobilised on glutathione agarose. This was harvested by centrifugation and washed before being subjected to SDS-polyacrylamide gel electrophoresis (PAGE) with subsequent detection of bound PDE4D4 determined by immunoblotting. This was done with a monoclonal antibody (mAb) specific for a common C-terminal region found uniquely in PDE4D isoenzymes [17]. Such analyses identified the 119 kDa immunoreactive PDE4D species that was bound to lyn SH3-GST but did not bind to GST alone (Fig. 2A). In similar studies, we were able to demonstrate that neither recombinant PDE4D3 (Fig. 2B) nor PDE4D5 (data not shown) could bind to the SH3 domains of src and lyn kinase. These two long PDE4D isoenzymes only differ from PDE4D4 by virtue of their unique N-terminal regions (Fig. 1). This suggests that PDE4D4 associates with the SH3 lyn domain through its unique alternatively spliced N-terminal region. This is consistent with the hypothesis that the proline-rich sequences (Fig. 1), found in the N-terminal region of PDE4D4 but not in the N-terminal regions of either PDE4D3 or PDE4D5, allow PDE4D4 to interact with SH3 domains.

In order to ascertain if endogenously expressed PDE4D4 could bind to SH3 domains, we analysed a soluble extract from rat brain. We have previously shown [18] that endogenous PDE4A5 found in such an extract can bind to src SH3. Brain is a good source of a very wide range of PDE4 isoenzymes [6] and we have previously shown [17] that species

Table 1
Interaction of PDE4D4 with SH3 domains

Fusion protein	PDE4D4 bound	PDE4A5 bound
Src SH3	1 (4)	1
Fyn SH3	0.990 ± 0.042 (3)	3.100 ± 0.600
Abl SH3	0.890 ± 0.028 (3)	0.480 ± 0.037
Fodrin SH3	0.882 ± 0.085 (3)	nd
Lyn SH3	0.863 ± 0.034 (22)	9.100 ± 0.850
PI3K SH3	0.343 ± 0.114 (3)	nd
Grb C SH3	0.289 ± 0.075 (3)	nd
Cortactin SH3	0.142 ± 0.076 (3)	nd
Csk SH3	0.115 ± 0.022 (5)	0.060 ± 0.020
Lck SH3	0.087 ± 0.015 (3)	0.070 ± 0.016
Grb N SH3	0.068 ± 0.023 (3)	nd
Crk SH3	0.058 ± 0.016 (4)	0.050 ± 0.016
P53BP2 SH3	0.033 ± 0.009 (3)	0.516 ± 0.117
Nedd4 WW2	0.055 ± 0.075 (3)	nd
GST	0.012 ± 0.007 (26)	0.040 ± 0.050

Analyses were done as described in Section 2 using the 'pull down' assay procedure. This utilised GST fusion proteins of the indicated SH3 and WW domains which were probed against human recombinant PDE4D4 expressed in COS-7 cells. The conditions described in Section 2 allowed for the association of around 70% of the PDE4D4 in the assay with the SH3 domain of src kinase. For ready comparison, data were expressed as binding relative to that to the SH3 domain of src kinase, set at unity, with means and errors as S.D. and the indicated number of experiments given in parentheses. We also give, for comparison, previously published data for PDE4A5 done under similar conditions [18]. nd = not determined.

which co-migrate with recombinant human PDE4D3, D4 and D5 are expressed there and confirm this here (Fig. 2C). Using the GST fusion proteins of the SH3 domains of src, lyn and fyn, we show here that these all could bind only rat PDE4D4 (Fig. 2, tracks 6–8). The major PDE4D species in this extract was PDE4D3, which along with PDE4D5 did not bind to these SH3 domains. We were also able to confirm (data not shown) previous studies [6,18] that rat brain contains PDE4B1, PDE4B2, PDE4C2, PDE4A8, PDE4A1 isoenzymes. However, none of these became associated with the SH3 domains of either src, fyn or lyn (data not shown). This implies that at least in brain only two PDE4 isoenzymes can interact with the SH3 domains of these src family tyrosyl kinases, namely PDE4A5 and PDE4D4.

As an independent method of monitoring potential SH3 domain-PDE4D4 interaction, we also developed an 'overlay' assay which also allowed us to demonstrate that the SH3 domains of the src family tyrosyl kinases src, lyn and fyn could all bind to PDE4D4 (Fig. 2D). The overlay assay was based upon that used to probe for protein kinase A anchor proteins [1]. In these experiments, GST and GST fusion proteins of src, lyn and fyn were subjected to SDS-PAGE. Proteins were then transferred to nitrocellulose, the membrane blocked and then 'probed' with a purified MBP-PDE4D4 fusion protein. The membranes were then washed to remove unbound MBP-PDE4D4 before being subjected to Western blotting using either a PDE4 mAb or an anti-MBP Ab so as to identify bound PDE4D4. The position of binding on the gel was compared to that where GST and the various GST fusion proteins were located. This showed that PDE4D4 did not interact with GST but did bind to the fusion proteins of src, lyn and fyn SH3 domains (Fig. 2D). These experiments also showed that PDE4D4 did not bind to the SH3 domains of lck, crk and csk (Fig. 2D). As a control, we also performed an overlay probing the blot with MBP alone (data not shown). This failed to show any interaction with the various SH3 fusion proteins, ensuring that the detected interactions could not be attributed to the MBP moiety of the fusion protein.

The overlay assays indicated (Fig. 2D) that PDE4D might show a preference for interaction with the SH3 domains of certain proteins. Here (Table 1), we have examined the ability of PDE4D4 to interact with a panel of different GST fusion proteins of SH3 domains. We also investigated an example of a WW domain [26], namely that found in the Nedd4 protein. This, like SH3 domains, also interacts with proline-rich peptides but, in contrast to SH3 domains, the target motif usually includes a tyrosyl residue. Interactions were analysed by pull down experiments with quantitative assessment given in Table 1 as a ratio of binding relative to that seen for src SH3. This showed that PDE4D4 bound similarly to the SH3 domains of the src family tyrosyl kinases lyn, fyn and src as well as to the SH3 domains of abl tyrosyl kinase and the cytoskeletal protein fodrin. PDE4D4 also appeared to interact, albeit less strongly, with the SH3 domains of PI3-kinase and the C-terminal SH3 domain of the adapter protein Grb2. Interestingly, PDE4D4 did not interact with the N-terminal SH3 domain of Grb2, which may indicate a different specificity of these two SH3 domains in Grb2. Neither did PDE4D4 bind to a range of other SH3 domains, namely those of crk, lck, csk, p53BP2 and cortactin, nor did it bind to the WW domain of Nedd4. These data imply a distinct specificity for the potential interaction of PDE4D4 with the SH3 domains of various proteins.

So as to determine if SH3 domain interaction with PDE4D4 was direct, we generated PDE4D4 as a fusion protein with MBP (PDE4D4-pMAL), expressed it in *E. coli* and then purified it to apparent homogeneity on an amylose resin. Similarly, we purified, to apparent homogeneity, the lyn SH3 domain as a GST fusion protein on glutathionine agarose. These two proteins were then mixed together and pull down assays were done using either glutathione agarose (Fig. 2E) or amylose resin (data not shown), so as to immobilise the one or other of the partners. These experiments demonstrated that both species could be pulled down (Fig. 2E). We also generated a fusion protein of the unique N-terminal 136 residues of PDE4D4 (NT-PDE4D4-pMAL) with MBP. This fusion protein was purified on amylose resin and could be demonstrated to bind to lyn SH3-GST in a pull down assay (Fig. 2F). These various experiments are consistent with a direct interaction between PDE4D4 and a protein SH3 domain.

Using PDE4D4 expressed in the cytosol of transiently transfected COS-7 cells, we failed, however, to identify any change in PDE activity ($< 5\%$ difference, 1 μM cAMP substrate) when it became bound to lyn SH3. Neither did we see any change in the dose-dependent ability of Mg^{2+} to activate PDE4D4 upon binding lyn SH3 ($< 5\%$ difference) nor any difference in the ability of the PDE4 selective inhibitor rolipram (4-(3-(cyclopentoxyl)-4-methoxyphenyl)-2-pyrrolidone) to inhibit PDE4D4 upon binding lyn SH3 (IC_{50} = 0.10 ± 0.02 = unbound, 0.08 ± 0.02 = bound; means ± S.D.).

4. Discussion

The PDE4D gene encodes five distinct isoenzymes [17]. Of these, PDE4D5 has recently been shown [22] to interact with RACK1 via a small motif within its unique N-terminal region. Here, we demonstrate that only PDE4D4 can interact with

protein SH3 domains, indicating that such an interaction occurs by virtue of the unique proline-rich N-terminal region which characterises the PDE4D4 isoenzyme. It appears that this interaction is direct, as purified fusion proteins of both PDE4D4 and its N-terminal region are both capable of binding to the purified recombinant lyn SH3 domain.

Whilst the SH3 domains seen in various proteins have a common three-dimensional globular structure and a propensity for interaction with proline- and arginine-rich peptides in target proteins, they do show a pronounced specificity towards the proteins they select to interact with [23,24]. This underlies the selective recruitment of proteins to signalling complexes, such as is seen for the interaction of Grb2 with SOS. There is, seemingly, a preference for interaction of PDE4D4 with the SH3 domains of certain proteins (Table 1). From the panel of SH3 domain fusion proteins investigated in this study, we have uncovered a preference for those of src family tyrosyl kinases lyn, fyn and src itself as well as the cytoskeletal protein fodrin and abl tyrosyl kinase (Table 1). The PDE4A5 isoenzyme has also been shown to exhibit a preference for the SH3 domains of src family tyrosyl kinases [18]. However, PDE4A5 shows ([18] and see Table 1) a distinct preference within the src family tyrosyl kinase family, namely for the SH3 domains of lyn $>>$ fyn $>$ src. It seems plausible that the selectivities of these two enzymes might reflect differences in the form of the proline-rich segments of their N-terminal regions. Thus that of PDE4A5 is characterised by three PxxPxxR motifs [18], whereas polyproline stretches predominate in PDE4D4 (Fig. 1). This may underlie the observation we make here that PDE4D4 and PDE4A5 show a different pattern of interactions for SH3 domain-containing proteins. This, we suggest, is likely to be reflected by differences in the range of partners these PDE4 enzymes select in various cell types and which may thus generate functional differences in the roles of these two PDE4 species.

The studies described here indicate the propensity of the PDE4D4 isoenzyme to interact with SH3 domain-containing proteins. This property is conferred on the enzyme by its unique N-terminal region. We also show that there is a distinct specificity in the type of SH3 domain that can interact with PDE4D4. Such analyses provide an initial step in trying to identify the protein or range of proteins that can interact with PDE4D4 in the various cell types where it is expressed. Based on pull down assays done on rat brain, we suggest that PDE4A5 and PDE4D4 may be either the sole or major PDE4 species able to interact with SH3 domain-containing proteins. Thus, the potential of these two enzymes to interact with SH3 domain-containing proteins may be related to a major functional role. As with RACK1 binding to PDE4D5 [22], we were, however, unable to identify any changes occurring in the catalytic function of PDE4D4 upon its association with the SH3 domain of lyn. We suggest that the functional importance of any binding of these enzymes to SH3 domain-containing proteins may be in targeting the PDE4 species to a specific location within the cell and thus controlling local cAMP levels.

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References

[1] Murphy, B.J. and Scott, J.D. (1998) Trends Cardiovasc. Med. 8, 89–95.
[2] Houslay, M.D. and Milligan, G. (1997) Trends Biochem. Sci. 22, 217–224.
[3] Beavo, J.A. (1995) Physiol. Rev. 75, 725–748.
[4] Conti, M., Nemoz, G., Sette, C. and Vicini, E. (1995) Endocr. Rev. 16, 370–389.
[5] Manganiello, V.C., Taira, M., Degerman, E. and Belfrage, P. (1995) Cell. Signal. 7, 445–455.
[6] Houslay, M.D., Sullivan, M. and Bolger, G.B. (1997) Adv. Pharmacol. 44, 225.
[7] Torphy, T.J. (1998) Am. J. Respir. Crit. Care Med. 157, 351–370.
[8] Shakur, Y., Pryde, J.G. and Houslay, M.D. (1993) Biochem. J. 292, 677–686.
[9] Shakur, Y. et al. (1995) Biochem. J. 306, 801–809.
[10] Smith, K.J., Scotland, G., Beattie, J., Trayer, I.P. and Houslay, M.D. (1996) J. Biol. Chem. 271, 16703–16711.
[11] Pooley, L., Shakur, Y., Rena, G. and Houslay, M.D. (1997) Biochem. J. 271, 177–185.
[12] Scotland, G. and Houslay, M.D. (1995) Biochem. J. 308, 673–681.
[13] Huston, E., Pooley, L., Julien, J., Scotland, G., McPhee, I., Sullivan, M., Bolger, G. and Houslay, M.D. (1996) J. Biol. Chem. 271, 31334–31344.
[14] Bolger, G.B., McPhee, I. and Houslay, M.D. (1996) J. Biol. Chem. 271, 1065–1071.
[15] McPhee, I., Pooley, L., Lobban, M., Bolger, G. and Houslay, M.D. (1995) Biochem. J. 310, 965–974.
[16] Huston, E. et al. (1997) Biochem. J. 328, 549–558.
[17] Bolger, G.B. et al. (1997) Biochem. J. 328, 539–548.
[18] O'Connell, J.C. et al. (1996) Biochem. J. 318, 255–262.
[19] McPhee, I., Yarwood, S.J., Huston, E., Scotland, G., Beard, M.B., Ross, A.H., Houslay, E.S. and Houslay, M.D. (1999) J. Biol. Chem. 274, 11796–11810.
[20] Mayer, B.J. and Baltimore, D. (1993) Trends Cell Biol. 3, 8–13.
[21] Pawson, T. and Gish, G.D. (1992) Cell 71, 359–362.
[22] Yarwood, S.J., Steele, M.R., Scotland, G., Houslay, M.D. and Bolger, G.B. (1999) J. Biol. Chem. 274, 14909–14917.
[23] Dalgarno, D.C., Botfield, M.C. and Rickles, R.J. (1997) Biopolymers 43, 383–400.
[24] Musacchio, A., Wilmanns, M. and Saraste, M. (1994) Prog. Biophys. Mol. Biol. 61, 283–297.
[25] Quilliam, L.A. et al. (1996) J. Biol. Chem. 271, 28772–28776.
[26] Bork, P. and Sudol, M. (1994) Trends Biochem. Sci. 19, 531–533.