Direct Interaction of Major Histocompatibility Complex Class II-derived Peptides with Class Iα Phosphoinositide 3-Kinase Results in Dose-dependent Stimulatory Effects*

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Peptides corresponding to residues 65–79 of human lymphocyte antigen class II sequence (DQA*03011) are cell-permeable and at high concentrations block activation of protein kinase B/Akt and p70-S6 kinase in T-cells, effects attributed to inhibition of phosphoinositide (PI) 3-kinase activity. To understand the molecular basis of this, we analyzed the effect this peptide had on activity of class I PI 3-kinases. Although there was no effect on the activity of class Ib PI 3-kinase or on the protein kinase activity of class I PI 3-kinases, there was a biphasic effect on lipid kinase activity of the class Ia enzymes. There was an inhibition of activity at higher peptide concentrations because of a formation of insoluble complexes between peptide and enzyme. Conversely, at lower peptide concentrations there was a profound activation of PI 3-kinase activity of class Ia PI 3-kinases. Studies of peptide variants revealed that all active peptides conform to heptad repeat motifs characteristic of coiled-coil helices. Surface plasmon resonance studies confirmed direct sequence-specific binding of active peptide to the p85α adapter subunit of class I PI 3-kinase. Active peptides also activated protein kinase B and extracellular signal-regulated kinase (ERK) in vitro in a wortmannin-sensitive manner while reducing recoverable cellular p85 levels. These results indicate that the human lymphocyte antigen class II-derived peptides regulate PI 3-kinase by direct interaction, probably via the coiled-coil domain. These peptides define a novel mechanism of regulating PI 3-kinase and will provide a useful tool for specifically dissecting the function of class Ia PI 3-kinase in cells and for probing structure-function relationships in the class Ia PI 3-kinase heterodimers.

Phosphoinositide (PI)1 3-kinases play a pivotal role in signaling pathways regulating a wide range of cellular processes including apoptosis, metabolism, cell growth, cell proliferation, and cytoskeletal rearrangements (1–3). Several classes of PI 3-kinase exist, but tyrosine kinase-linked receptors signal mainly through class Ia PI 3-kinases, which are bifunctional kinases, possessing both lipid and protein kinase activities (4). The class Ia PI 3-kinases can be regulated in a number of ways. These include interactions via the SH2, SH3, and Bcr homology domains of the adapter subunit and direct interaction of Ras through the Ras binding domain of the catalytic subunit (1–3).

A potentially novel mechanism for regulating PI 3-kinase activity has recently emerged from studies of the properties of biologically active peptides derived from class II MHC molecules (5). These studies identified a peptide fragment of DQA*03011 α chain allele of MHC class II that was capable of blocking IL2-induced T-cell proliferation and thus offered a possibly novel means of achieving immunosuppression. Further work found that treatment of T-cells with one of these peptides, corresponding to the residues 65–79 of DQA*03011, blocked IL2-induced activation of protein kinase B/Akt and p70-S6K activities (6) and also blocked the ability of IL2 to regulate levels of p21Cip1 and p27Kip1 (5). This was attributed to a direct inhibition of PI 3-kinase. How this occurs is unknown; nonetheless, the study noted a reasonable degree of sequence homology between the peptide and a conserved region in the catalytic subunit of PI 3-kinases, and the authors speculated this may be important.

In the present study, we sought to define the mechanism by which this peptide is able to regulate PI 3-kinase activity. At higher concentrations the peptide reduced class Ia PI 3-kinase activity in vitro. However, this is not due to direct inhibition of PI 3-kinase catalytic activity but as a result of the peptide causing the enzyme to become insoluble, probably as a result of complex formation between peptide multimers and the PI 3-kinase. A reduction in levels of class Ia PI 3-kinase adapter subunit was observed when high concentrations of peptide were added to cells indicating that in vitro it also induced a reduction in the amount of PI 3-kinase that could be solubilized. However, at lower concentrations, the peptide greatly stimulated class Ia PI 3-kinase activity. Low concentrations of peptide also activated protein kinase B and ERK in CHO-IR cells in a wortmannin-sensitive manner suggesting the peptides are activating PI 3-kinase directly in cells. Using variants of the wild type peptide it was found that only peptides conforming to a heptad repeat consensus were capable of interacting with PI 3-kinase suggesting the regulatory effects were mediated via the coiled-coil domains of PI 3-kinase. Overall these studies define a novel mechanism for the regulation of PI 3-kinase activity and indicate that these peptides will be useful reagents for studying PI 3-kinase function.

EXPERIMENTAL PROCEDURES

Materials—Peptides were synthesized by the Wolfson Institute for Biomedical Research, University College London, London, UK. Recons
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Table I

**Residue substitutions with respect to the wild type sequence are in bold.**

| Peptide | Sequence | Remarks |
|---------|----------|---------|
| M1      | a b c d e f g a b c d e f g | wild type (DQ 65–79) |
| M2      | N I A V L K H S H L N I V I K R | K70S (more potent<sup>a</sup>) |
| M3      | N I A V L K H S H L N I V I K R | K74S (more potent) |
| M4      | N I A V L S H N L S I V I K R | K70S/K74S (double mutant) |
| M5      | N I A V L K H S N I M V K D | based on p110α<sup>a</sup> |
| M6      | N I A V L K S D N I M V K K | based on p110β |
| M7      | N I A V L K H S D N I M V K K | based on PI 3-kinase-C2α |
| Ser-608 | L G N E N D Q Y S L V E D D L | based on p85α Ser-608 |
| Ser-608A | L G N E N D Q Y A L V E D D L | |
| Ser-111 | R R P E D K R A G G E E S Q F E M D I | |
| Ser-111A | R R P E D K R A G G E E Q F E M D I | |

<sup>a</sup> "More potent" refers to peptide inhibitory effect on T-cell proliferation (5).

<sup>b</sup> Substitutions have been made to resemble closer to the designated PI 3-kinase.

**Table II**

**Homology of DQ 65–79 to the catalytic subunit of PI 3-kinase**

| Peptide | Sequence alignment | Identity | Homology |
|---------|-------------------|----------|----------|
| DQ 65–79 | K H N L N I V I K R | % | % |
| p110α | K N S N I M V K D | 50 | 80 |
| p110β | K N S D N I M V K K | 40 | 80 |
| p110γ | K N S D N I M I E | 50 | 70 |
| p110δ | K N S D N I M L R | 40 | 70 |
| PI-4K | E R N G N I M L R | 40 | 80 |
| mTOR | F H N N L I M L R | 30 | 70 |
| PI 3-kinase-C2α | F H N N L I M L R | 40 | 80 |

Immunoblotting using the phospho-specific antibodies was performed according to the manufacturer’s instructions. Visualization was performed with ECL. Images were analyzed with a Fuji LAS-1000 Luminescent Image analyzer and Fuji Image Gauge software.

**PI 3-Kinase Protein Kinase and Lipid Kinase Assays**—PI 3-kinase autophosphorylation assays were performed in a total volume of 30 µl in a buffer consisting of 50 mM Tris-HCl, pH 7.4, 50 mM NaCl. For treatment with various peptides, the enzyme was preincubated with the respective peptide or vehicle in a volume of 25 µl for 10 min on ice. Reactions were initiated by adding 5 µl of a mix of ATP (final concentration of 100 µM, plus 0.5 µC of [γ-32P]ATP/assay) and MnCl<sub>2</sub> (final concentration of 10 mM), incubated for 20 min at 25 °C, and terminated by the addition of 5× electrophoresis sample buffer and boiling. The reaction products were analyzed by SDS-PAGE and autoradiography.

**PI 3-kinase lipid kinase assays** were performed in a total volume of 50 µl in a buffer containing 50 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM dithiothreitol, 5 mM MgCl<sub>2</sub>, 100 µM ATP (plus 0.1 µC of [γ-32P]ATP/assay) using 200 µg/mg phosphatidylinositol as a substrate. For treatment with various peptides, the enzyme was preincubated with the respective peptide or vehicle in a volume of 10 µl on ice. Then, phosphatidylinositol was added, and reactions were initiated by adding a mix containing ATP and MgCl<sub>2</sub>. Reactions were incubated at 25 °C for 20 min and terminated by the addition of 100 µl of 0.1 M HCl and 200 µl of chloroform:methanol (1:1). The mixture was vortexed, and the phases were separated by centrifugation at 10,000 g for 2 min. The aqueous phase was discarded, and the lower organic phase was washed with 80 µl of methanol, 1 ml of HCl (1:1). After centrifugation the aqueous phase was again discarded, and the lower organic phase evaporated to dryness. Reaction products were resuspended in 30 µl of chloroform: methanol (4:1) and spotted onto thin layer silica gel-60 plates (Merck), which had been pretreated with 1% oxalic acid, 1 mM EDTA in water; methanol (8:4). TLC plates developed in chloroform, methanol, 4 µ ammonia (9:7:4). Images of radioiodinated protein and lipid products were analyzed using a Fuji FLA-2000 phosphorimager and Fuji Image Gauge software.

**RESULTS**

A previous report that synthetic peptides derived from the MHC class II sequences directly inhibit PI 3-kinase activity in p85 immunoprecipitates (6) prompted the present investigation of their effects on lipid and protein kinase activities of...
recombinant PI 3-kinases. Therefore, we synthesized the wild type peptide (M1) and three derivatives (M2–M4) of this peptide, two of which (M2 and M3) had been found previously to be active in blocking IL2 effects on T-cell proliferation (Table I) (5). Peptides M5–M7 were synthesized to correspond to the regions of PI 3-kinases that were homologous to the MHC class
II DQA*03011-derived peptides (Table II) as it was also speculated that this homology may contribute to the functionality of these peptides (6). Additionally, four other peptides were used as controls. Two of these were based on the region of the class Ia PI 3-kinase adapter subunit that contains the Ser-608 residue that is the target of the protein kinase activity of the catalytic subunit (4). The other two control peptides were based on the Ser-111 sequence in the eIF4E-binding protein-1, which is a wortmannin-sensitive phosphorylation site in vivo (7). These four were chosen as the Ser-608- and Ser-111-based peptides are substrates for the protein kinase activity of PI 3-kinase and could therefore act as competitors for the lipid kinase activity.

Initially the effects of peptides on the lipid kinase activity of recombinant p85α/p110β were tested. Surprisingly, in the presence of 100 μM peptide there was a strong activation observed with the MHC class II DQA 65–79 peptide and its three derivatives (M1–M4) (Fig. 1). The p110-based peptides M5, M6, and M7 and peptides based on the Ser-608 of p85α or the Ser-111 of the eIF4E-binding protein-1 had a minimal effect showing stimulation of less than 2-fold. As these results contrasted with those obtained previously (6) we next tested higher concentrations of peptide. At 1 mM peptide we find that PI 3-kinase activity of p85α/p110α is completely inhibited in our in vitro assay (Fig. 2). To test whether these effects were an isoform-specific phenomenon we next tested the effect of various peptides on the lipid kinase activity of recombinant p85α/p110β. This revealed a similar pattern as seen with p85α/p110α, with the wild type peptide (M1) causing a strong stimulation of the lipid kinase activity at 100 μM but with inhibition evident at 1 mM (Fig. 3). Peptides inactive against p85α/p110α were also inactive against p85α/p110β.

The effects of the MHC class II-derived peptide on protein and lipid kinase were then compared in all class I PI 3-kinases (p85α/p110α, p85α/p110β, p85α/p110δ, and p110γ). For each of the heterodimeric PI 3-kinases (p85α/p110α, p85α/p110β, or p85α/p110δ) a strong stimulation of lipid kinase activity was observed at 100 μM but with inhibition evident at 1 mM in all cases. Surprisingly, the lipid kinase activity of the monomeric class Ib p110γ was not increased by 100 μM peptide and was only slightly decreased by 1 mM peptide (Fig. 4, A, top, and B). Interestingly, under the same experimental conditions the protein kinase activity of the heterodimeric class Ia PI 3-kinases (assessed as phosphorylation of p85α by p110α or autophosphorylation of p110β and p110δ) was not significantly affected by the peptide at 100 μM (Fig. 4, A, middle, and C), whereas at 1 mM peptide the same inhibition as for the lipid kinase activity was observed. When the gels from the above experiment were

**Fig. 3.** Effect of various MHC-derived peptides on recombinant p85α/p110β lipid kinase activity. Recombinant p85α/p110β was incubated with either 0.1 or 1 mM each peptide for 10 min on ice followed by an in vitro lipid kinase assay with [γ-32P]ATP and phosphatidylinositol as the substrates. The reaction products were analyzed by thin layer chromatography and autoradiography. The autoradiogram is shown in A. Relative PI 3-kinase activities were plotted in the graph shown in B. Peptides M1, M5, M6, and M7 were dissolved in Me2SO (DMSO). Ser-608 peptides were dissolved in water. The lipid kinase activity obtained after addition of water only was assumed to be 1. The experiments were repeated at least three times with similar results. Data from one representative experiment are shown here. PI3K, PI 3-kinase. PI3P, phosphatidylinositol 3-phosphate.
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It was observed previously that the DQ 65–79 class II MHC-derived peptide is cell-permeable (6), which lead us to test whether the stimulatory effects observed in vitro with low concentrations of peptide could manifest as activation of PI 3-kinase-dependent signaling pathways in cells. Here we find three of the MHC class II-derived peptides (M2–M4) were able to stimulate phosphorylation of protein kinase B/Akt and of ERK1/2 in a wortmannin-sensitive manner (Figs. 6 and 7A). This suggested an activation of PI 3-kinase in the cell by the peptides. As the peptides were not activating tyrosine kinases in the cell (Fig. 7B) it was unlikely that they were acting via cell surface receptors, and the most likely scenario was a direct interaction between the peptides and PI 3-kinase as was observed in vitro. This is further supported by the finding that high concentrations of peptide caused a reduction in recoverable p85 adapter subunit levels with no apparent effects on actin or Akt levels (Fig. 7A) suggesting that the peptides were permeating the cell membrane and directly interacting with PI 3-kinase to specifically induce it to become insoluble.

**DISCUSSION**

The current findings extend previous studies by demonstrating that the inhibition of PI 3-kinase activity induced by MHC class II DQA 65–79 peptide is specific to the class Ia isoforms.
FIG. 6. Treatment of cells with certain MHC class II-derived peptides activates protein kinase B and mitogen-activated protein kinase. CHO-IR cells were either treated with 100 or 500 μM each of the peptides designated for 30 min at 37 °C or stimulated with 100 nM insulin (ins) for 10 min in the presence or absence of 100 nM wortmannin (the Me 2SO concentration in the culture medium was adjusted to be equal in all experiments). Subsequently, the cells were lysed, and the lysates were analyzed by SDS-PAGE and immunoblotting with a phosphorylation state-specific antibody against Ser-473 of Akt. The same filter was consecutively stripped and reprobed with antibodies against total Akt, the PI 3-kinase specific antibody against Ser-473 of Akt. The same filter was consecutively stripped and reprobed with antibodies against Thr-308 of Akt and Thr-202/Tyr-204 of ERK1/2 and antibodies against total Akt and total ERK1/2. Six independent experiments were performed with identical results. Blots from one representative experiment are shown here. IB, immunoblot; MAPK, mitogen-activated protein kinase.

FIG. 7. Similar mechanisms underlie both in vitro and in vivo PI 3-kinase activation. A, CHO-IR cells were either treated with 100 or 500 μM peptide M2 for 30 min at 37 °C or stimulated with 100 nM insulin (ins) for 10 min (the Me 2SO concentration in the culture medium was adjusted to be equal in all experiments). Subsequently, the cells were lysed, and the lysates were analyzed by SDS-PAGE on an 8% acrylamide gel and immunoblotting with a phosphorylation state-specific antibody against Ser-473 of Akt. The same filter was consecutively stripped and reprobed with antibodies against total Akt, the PI 3-kinase regulatory subunit p85, and β-actin. IB, immunoblot; B, activation of the PI 3-kinase pathway by MHC-derived peptides does not involve apparent activation of a receptor tyrosine kinase. An identical series of samples was analyzed by SDS-PAGE and immunoblotting using the antiphosphotyrosine antibody PY99. Two independent experiments were performed with identical results. Blots from one representative experiment are shown here. MW, molecular weight.

The results further suggest the inhibition is not due to direct inhibition of catalytic activity but is in fact due to formation of insoluble peptide multimer-enzyme complexes. This implies that the peptide can directly interact with class Ia PI 3-kinase, and as this was not observed in class Ib isoforms it suggests that the effect requires the presence of the p85 adapter subunit of class Ia PI 3-kinase. This is supported by SPR analysis which revealed sequence-specific binding of the MHC peptides to recombinant p85α. However, at lower peptide concentrations the class Ia PI 3-kinases remain in solution, and their activity is actually greatly increased. This stimulatory effect is not an artifact of peptide affecting the environment of the lipid assay as some closely related peptides do not cause the stimulation, and none of the peptides activate the class Ib PI 3-kinase. Therefore this presumably results from a direct interaction between the peptide and PI 3-kinase outside the catalytic site.

A number of mechanisms have been defined by which class Ia PI 3-kinase can be activated by interactions outside the catalytic site. The most well studied of these involves the binding of tyrosine-phosphorylated peptides to the SH2 domains of p85 adapter subunits (8–12). Other mechanisms have also been characterized that involve the binding of Ras to a specific Ras binding domain in p110 (13, 14) or the binding of Rac or Cdc42 to the Bcr homology domain of p85 (15, 16). However, the peptides described in the current study are unlikely to activate PI 3-kinase by any of these mechanisms and thus represent a novel mechanism.

Our studies with variants of the wild type peptide show there is a high degree of specificity in the sequences capable of activating PI 3-kinase. Previously it was noted that there was a high homology between part of the sequence of this peptide (70–79) and a region (916–924) of the p110 catalytic subunit (Table II), and it was suggested this was functionally important. However, there is no evidence that this homology is related to the effect of the peptides, and indeed we find that peptides that fully mimic the catalytic subunit sequences are in fact inactive. Our results reveal a more likely mechanistic basis of the action of the peptides as all the active peptides, but none of the inactive ones, fall within the abcd heptad repeat consensus motif (see Table I), where a and d are hydrophobic residues, whereas b, c, e, f, and g are hydrophilic residues (17). The possibility that this is important in the functional effects of the peptides is supported by the results of a complete serine mutagenesis scan of the DQA65–89 peptide where mutations that caused loss of function, as assessed by blocking T-cell proliferation, were also mutations that disrupted the heptad consensus sequence (5).

Any explanation for the mechanism by which the peptides act must also take into account the finding that higher peptide...
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concentrations cause insolubility and loss of function of the PI 3-kinase. The answer to this can also be explained by the heptad arrangement as this is indicative of coiled-coil amphipathic helices where residues $a$ and $d$ form the helix interface and residues $b, c, e, f$, and $g$ form the solvent-exposed part of the coiled coil. These properties cause oligomerization, which is a common property of coiled-coil proteins (17). The most likely binding partner for this heptad repeat lies in the p110 binding domain in the iSH2 domain of the p85 subunit, which was predicted to form a coiled coil (18), a view supported by recent electron paramagnetic resonance studies (19). In addition, secondary structure prediction of the rather hydrophobic N terminus of p110, where the p85 binding domain resides, predicts 60% of it forms $\alpha$-helices and 40% forms $\beta$-sheet conformations, and thus it has been proposed that hydrophobic helices in this region interact with the coiled coil of the iSH2 domain (20). The interaction of a peptide capable of multimer formation with this domain would also explain the insolubility seen at high concentration of the peptide, assuming that high molecular weight heteroligomeric complexes are formed under these conditions. The interaction of peptide with this region could also explain the activation observed as it is established that the binding of the regulatory subunit p85 to the catalytic subunit p110 inhibits the enzymatic activity of the latter (21). Thus, binding of peptide to the coiled-coil region of the p85 iSH2 domain or the N terminus of p110 (or both) could disrupt the interaction and relieve the inhibition. It has been proposed that activation of the lipid kinase activity of PI 3-kinase by SH2 domain-mediated interaction with tyrosine-phosphorylated proteins is mediated by conformational changes that partially relieve inhibition in this way (21). In this regard it is interesting that the protein kinase activity of class Ia PI 3-kinase is not increased by tyrosine-phosphorylated proteins interacting with the SH2 domains indicating different mechanisms controlling lipid and protein kinase activities (10). Consistent with this we do not see any activation of protein kinase activity by the MHC-derived peptides.

These studies suggest a number of potential applications for peptides based on those described here. One advantage of the peptides in the current study compared with other means of activating modulating PI 3-kinase is that these peptides are cell-permeable, as opposed to other PI 3-kinase activators such as Ras, Rac, and tyrosine-phosphorylated proteins. Therefore this allows a means to specifically regulate PI 3-kinase inside cells. If higher concentrations are used it would inhibit PI 3-kinase activity, and this could be used as a basis of immunosuppressive therapy (5, 22) or potentially as a basis for anti-tumor therapy (23). There is evidence that soluble MHC molecules circulate in the serum (22, 24, 25), so it is possible that such a mechanism may even have localized functional significance for PI 3-kinase regulation in vivo. As a research tool the peptides may be also useful in dissecting the specific contribution of class Ia PI 3-kinase in several signaling pathways as using these allows specific activation of PI 3-kinase-dependent signaling pathways and allows investigation of these in isolation from the other pathways that would inevitably be activated by, for example, growth factor stimulation of cells.

In summary, these results suggest a novel mechanism of regulating PI 3-kinase activity in cells. These peptides will also provide a useful tool for specifically dissecting the function of class Ia PI 3-kinase in cells and for probing structure-function relationships in the p85/p110 heterodimers.

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