Published in final edited form as:

Pflugers Arch. 2016 May ; 468(5): 817–824. doi:10.1007/s00424-016-1794-9.

The $I_{Cl,swell}$ inhibitor DCPIB blocks Kir channels that possess weak affinity for PIP_2

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Abstract

Inwardly rectifying K^+ (Kir) channels are important contributors to the resting membrane potential and regulate cellular excitability. The activity of Kir channels depends critically on the phospholipid PIP_2 . Several modulators of the activity of Kir channels alter the apparent affinity of the channel to PIP_2 . Channels with high apparent affinity to PIP_2 may not respond to a given modulator, but mutations that decrease such affinity can render the channel susceptible to modulation. Here, we identify a known inhibitor of the swelling-activated Cl^- current, DCPIB, as an effective inhibitor of a number of Kir channels both in native cardiac cells and in heterologous expression systems. We show that the apparent affinity to PIP_2 determines whether DCPIB will serve as an efficient blocker of Kir channels. These effects are consistent with a model in which DCPIB competes with PIP_2 for a common binding site.

Keywords

DCPIB; PIP_2 ; Kir channels; patch clamp; two-electrode voltage clamp

Introduction

Inwardly rectifying potassium channels (Kir) have been found in a wide variety of tissues and play important roles in numerous physiological and pathological processes (6). Seven subfamilies of Kir channels have been identified. Besides intracellular magnesium and polyamines that cause various degrees of inward rectification, phosphatidylinositol 4,5-bisphosphate (PIP_2) has been found to act as a common regulator of the activity of all Kir channels (9, 10, 30, 33).

Recent identification of the structures of several Kir channel has allowed a more detailed understanding of the structural basis of PIP_2 sensitivity. Based on available structures, it has been proposed that the positively charged residues critical for PIP_2 interaction form a pocket involving both the N- and C-termini of Kir channels (9, 10). PIP_2 is essential for the activities of all Kir channels and their regulators, including $G\beta\gamma$ (19, 29), intracellular Na^+ (7, 25, 28, 29), phosphorylation (13, 15, 23), and pH (1, 5, 21, 22), all of which modulate channel activity by altering channel- PIP_2 interactions (7, 9, 10, 19, 25, 29, 30, 33).

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Among Kir channels, the cardiac K_{ACh} and K_{ATP} channels, whose activity is highly modulated by a number of intracellular molecules, show some of the lowest apparent affinities and stereospecificities to phosphoinositides (24). In contrast, channels that exhibit high apparent affinities and stereospecificities for PIP_2 are ones that are least affected by intracellular modulators (24).

DCPIB is a sensitive and specific blocker of the swelling-activated chloride current ($I_{Cl,swell}$) (2). Previous studies showed that DCPIB has no effect on Ca^{2+} channels, Na^+ channels, the $CLC-Cl^-$ channel family, human cystic fibrosis transmembrane conductance regulator (hCFTR), and multiple K^+ channels including I_{Ks} , I_{Kr} , I_{K1} , I_{Kur} , and I_{to1} (2). Recently DCPIB was found to be an activator of TREK potassium channels in cultured astrocytes (17).

Here we show that DCPIB blocks members of the Kir channel family, and its inhibitory effect is dependent on the strength of channel- PIP_2 interaction. DCPIB (10 μM) blocked $I_{K_{ACh}}$ in native myocytes and reversed ACh-induced action potential duration (APD) shortening. DCPIB inhibited the currents elicited by Kir3.1/Kir3.4, Kir3.4, and Kir6.2, which are Kir channels with relatively weak affinity for PIP_2 . On the other hand, Kir2.1 and Kir2.3, which exhibit stronger channel- PIP_2 interaction, were insensitive to DCPIB. Strengthening channel- PIP_2 interaction in Kir3.4 by mutating I229 to Leu and D216 to Asn reduced the sensitivity of Kir3.4 to DCPIB, while mutating R312 in Kir2.1 to Gln weakened channel- PIP_2 interaction and rendered the mutant channel sensitive to DCPIB.

Methods

Molecular Biology and Channel Expression in *Xenopus* Oocytes

Point mutations were generated using *Pfu*-based the QuickChange site-directed mutagenesis kit (Stratagene). The cDNA was linearized and transcribed into cRNA using the MessageMachine kit (Ambion). Oocytes were isolated and microinjected with cRNA of different Kir channels. The amount of cRNA was adjusted based on the level of the expressed currents. All oocytes were maintained at 17 °C.

Atrial Myocyte Isolation

Atrial myocytes were freshly isolated from adult New Zealand White rabbits (2.8–3.1 kg) of either gender. Hearts were excised, immediately mounted on a Langendorff column, and perfused for 5 min with Tyrode solutions containing (in mM): 130 NaCl, 5 KCl, 1.8 $CaCl_2$, 4 KH_2PO_4 , 3 $MgCl_2$, 5 HEPES, 15 taurine, 5 creatine, 10 glucose, pH 7.25, then for 5 min with a Ca^{2+} -free solution containing (in mM): 130 NaCl, 5 KCl, 0.5 K_2EGTA , 4 KH_2PO_4 , 3 $MgCl_2$, 5 HEPES, 15 taurine, 5 creatine, 10 glucose, pH 7.25. The heart was then perfused with enzyme solution for ~20 min. Atria were excised, minced, and placed in fresh enzyme solution containing collagenase (0.45 mg/ml; Cls 4, Worthington) and pronase (0.015 mg/ml, Type XIV, Sigma-Aldrich) in Ca^{2+} -free Tyrode solution without EGTA. The tissue chunks were bubbled with an O_2/CO_2 (95%:5%) gas mixture and gently shaken for two 15-min cycles in a 37 °C shaker bath. At the end of each cycle the supernatant was collected. Isolated myocytes were washed twice and stored in a modified Kraft–Brühe (KB) solution

until use. The modified KB solution contained (in mM): 120 K-glutamate, 10 KCl, 10 KH_2PO_4 , 0.5 K_2EGTA , 10 taurine, 1.8 MgSO_4 , 10 HEPES, 20 glucose, 10 mannitol, pH 7.2 (adjust with KOH). Rod-shaped quiescent cells with clear striations were studied within 10 h of isolation.

Electrophysiology

Two-electrode Voltage-clamp Recording and Analysis (*Xenopus* Oocytes)—

Two-electrode voltage clamp (TEVC) recordings were performed 2–3 days after injection. TEVC with a GeneClamp 500 amplifier (Axon Instruments) was used to measure whole-cell currents expressed in oocytes. Electrodes were filled with 1.5% agarose in 3 M KCl. The electrodes had resistances of 0.5–1 M Ω . Oocytes were perfused with a high-potassium (HK) solution containing (in mM): 96 KCl, 1 NaCl, 1 MgCl_2 , and 5 Hepes, pH 7.4. Once the currents reached steady state, HK supplemented with 10–200 μM DCPIB was added. At the end of the experiment, barium (2 mM) was used to block potassium currents and obtain measurements of the remaining leak current.

Macropatch Recording (*Xenopus* Oocytes)—

Macropatch recordings were performed 2–6 days after injection. Inside-out patches were excised and currents were recorded using an Axopatch 200A patch-clamp amplifier and pClamp8 data acquisition software (Molecular Devices). Electrodes were made using Sutter P-97 microelectrode puller (Sutter Instrument) and the tip diameters were 10–25 μm . The bath and pipette solutions of ND96K+EGTA contained (in mM): 96 KCl, 1 MgCl_2 , 5 EGTA, and 10 HEPES, pH 7.4. Dose-response curve for current reactivation were constructed by adding different concentrations of diC8-PIP₂ (Avanti Polar Lipids) to the bath solution. Currents were recorded at a holding membrane potential of –80 mV.

Whole-Cell Patch Clamp and Electrophysiological Recordings (Atrial

Myocytes)—Pipettes were pulled using a P-97 micropipette puller (Sutter Instrument) and then fire polished. The final pipette tip diameter was 2–3 μm , and resistance was 2–4 M Ω . Junction potentials were corrected, and a 3-M KCl-agar bridge served as the ground electrode. Freshly isolated atrial myocytes were dispersed over a glass-bottomed cell chamber (~0.3 ml) and extracellular solution was superfused at a rate of 2–3 ml/min. Typical seal resistances were 5–10 G Ω . Myocytes were dialyzed for at least 5 min before data were collected. After obtaining the whole-cell configuration, successive 250 ms long steps were applied from –50 mV or –80 mV to test potentials between –100 and +40 mV in +10 mV increments, and current-voltage (I–V) relationships were plotted from quasi steady-state currents. Currents were recorded with an Axoclamp 200B and Digidata 1322A under pClamp 9 (MDS Analytical Technology) and digitized (5 kHz) after low-pass filtering (Bessel, 2 kHz). The high-K/low-Cl extracellular solution contained (in mM): 90 NaMeSO_3 , 30 KMeSO_3 , 20 KCl, 0.5 CaCl_2 , 1.0 MgCl_2 , 10 HEPES, pH 7.4. The pipette solution contained (in mM): K-aspartate 110, KCl 20, NaCl 10, MgCl_2 1.0, $\text{Na}_2\text{-ATP}$ 2.0, EGTA 2.0, $\text{Na}_2\text{-GTP}$ 0.01, HEPES 10, pH 7.4. To obtain I_{KACH} , ACh (10 μM) was added into the bath solution and 100 nm tertiapin-Q (Tocris) was used to block I_{KACH} currents. To examine the effect of GTP γS , GTP γS (100 μM) were added to the pipette solution, and the myocytes were dialyzed for at least 10 min before data were collected. Currents were normalized for

cell size by dividing current amplitude (pA) by membrane capacitance (pF) to obtain current density.

For action potential recordings, myocytes were stimulated at 1 Hz with 1-ms pulses, and the membrane potential was digitized (10 KHz, Bessel filtered at 2 KHz). At least 30 action potentials were recorded to verify that the APD was stable, and the last 10 action potentials were averaged. APD at 50% and 90% repolarization (APD₅₀, APD₉₀) were calculated from averaged records.

Data Analysis

All electrophysiology data were analyzed using the Clampfit 8 and Sigmaplot software. Data are reported as mean $\pm$ SEM. Statistical significance, taken as $P < 0.05$, was evaluated by Student's *t* test or One-way Repeated Measures ANOVA and the Holm-Sidak method (SigmaStat 3.11).

Molecular Docking

Docking was performed using AutoDock Vina (31). DCPIB molecular structure [IUPHAR database: (27)] was docked to both conformations of the Kir3.1 chimera structure (PDB code: 2QKS) (18). Missing sidechains and residues in the channel structures were built using the loop-modeling routine of Modeller (26). All 9 rotatable bonds on DCPIB were allowed to rotate, and global docking was performed within a 120 $\times$ 120 $\times$ 135 angstrom box encompassing the entire channel structure at an exhaustiveness level of 2000. Three independent docking runs using different random seeds were performed for each conformation of the channel, and the top 5 binding modes predicted by each run were analyzed. Docking to the “open” conformation of the Kir3.1 chimera structure consistently predicted binding modes within the same channel pocket as the known PIP2 binding site and achieved predicted binding affinities as great as -9.4 kcal/mol. Docking results for the “closed” conformation of the channel structure produced variable results with predicted binding modes in the transmembrane, pore, and intracellular regions of the channel and binding affinities as great as -8.9 kcal/mol. The final selected model thus reflects the highest predicted affinity structure from docking to the “open” conformation of the channel. Contact residues of the channel were defined as those having at least one atom within 4 Å of the ligand structure.

Results

DCPIB inhibits I_{KACH} in adult atrial myocytes

Firstly, we examined the effect of DCPIB on I_{KACH} in freshly dissociated atrial myocytes. As shown in Fig. 1a–c, ACh elicited an inwardly rectifying current with an amplitude of -7.71 ± 1.36 pA/pF at -80 mV ($n = 5$, $P < 0.001$). Addition of DCPIB (10 μ M; 10 min) in the presence of ACh inhibited $112 \pm 13\%$ of the ACh-induced current ($n = 5$, $P < 0.001$). The current after the addition of DCPIB was not significantly different than the basal current ($n = 5$, $P = 0.84$). Fig. 1d, e shows that ACh shortened the APD₅₀ and APD₉₀ by 25 ± 5 and 37 ± 4 ms, respectively ($n = 6$, $P < 0.05$ for both), and the APD shortening effect of ACh was completely reversed by DCPIB (10 μ M, 10 min).

Intracellular PIP₂ abrogates the inhibitory effect of DCPIB on I_{KACH} in adult atrial myocytes

Since the activity of I_{KACH} is dependent on PIP₂, next we studied the effect of intracellularly applied DiC8 PIP₂ (200 μM) on the inhibitory effect of DCPIB. As Fig. 2a and b show, with 200 μM DiC8 PIP₂ in the pipette, DCPIB (10 μM) failed to inhibit I_{KACH} currents (n = 4, P = 0.22), while tertiapin-Q (100 nm) completely blocked the ACh-induced currents of the same cell (n = 4, P < 0.001).

DCPIB inhibits Kir3.1/Kir3.4 heteromeric channels expressed in oocytes

In order to pursue the molecular determinants of the inhibitory action of DCPIB on K_{ACh} currents, we expressed Kir3.1/Kir3.4 in *Xenopus* oocytes. Fig. 3a, c-f demonstrate that DCPIB suppressed the basal whole-cell Kir3.1/Kir 3.4 currents in a dose-dependent manner with an EC₅₀ = 22.1 ± 3.3 μM. The inhibition was not voltage dependent. At 200 μM, DCPIB inhibited 89 ± 3% of the Kir3.1/Kir 3.4 current at -100 mV (n = 18, P < 0.001). Application of ACh induced additional current, when M2 receptors were co-expressed with Kir3.1/Kir3.4, and DCPIB also dose-dependently inhibited the Kir3.1/Kir3.4 current in the continuous presence of ACh (n=11, P < 0.001, Fig. 3b and g). Because the TEVC recordings of *Xenopus* oocytes may not be ideal for a blocker that might work inside the cells, we performed inside-out macropatch recordings. As shown in Fig. 3h, DCPIB in the bath solution inhibited Kir3.1/Kir3.4 heteromeric currents in a dose-dependent manner with an EC₅₀ = 3.3 ± 0.6 μM. The lower EC₅₀ obtained when DCPIB was applied at the cytosolic surface of the channel further supports the interpretation that its site of action is intracellular.

The inhibitory effect of DCPIB is determined by channel-PIP₂ interactions

Since not only Kir3 but all Kir family members are regulated by PIP₂, we investigated the effect of DCPIB on other cardiac Kir channels. Fig. 4a shows that DCPIB (200 μM) inhibited whole-cell currents from oocytes expressing Kir3.4 (n = 7, P < 0.001), Kir 6.2/SUR (n = 7, P < 0.001), and Kir6.2Delta36 (n = 5, P < 0.001). However, DCPIB had minimal effect on Kir2.1 (n = 8, P = 0.97) and Kir2.3 currents (n = 7, P = 0.85). Fig. 4b shows the dose-response curve for reactivation of Kir channels by DiC8 PIP₂. The EC₅₀ values indicate the apparent affinity of the Kir channels tested with PIP₂. Compared to other Kir channels, the Kir2.1 and Kir2.3 channels that have the highest apparent affinities for PIP₂, as indicated by their lower EC₅₀ values (3.1 ± 0.4 μM, n = 5; 23.0 ± 4.7 μM, n = 4; respectively), were insensitive to DCPIB. On the other hand, Kir3.4 and Kir6.2Delta36, which showed weaker apparent affinities for PIP₂ with higher EC₅₀ values (46.4 ± 4.3 μM, n = 4; 75.9 ± 11.2 μM, n = 3, respectively), were largely inhibited by DCPIB. Similar results were obtained with the non-cardiac Kir7.1 channel that exhibits a low EC₅₀ for PIP₂ (45.5 ± 2.3 μM) and a high fractional block by 200 mM DCPIB (>80%).

In order to further investigate the relationship between the channel apparent affinity for PIP₂ and DCPIB blockade, we modulated channel-PIP₂ interactions by mutating critical residues. As shown in Fig. 5a and b, mutation of R312 of Kir2.1 to Gln not only weakened channel-PIP₂ interactions, as indicated by the higher EC₅₀ for DiC8 PIP₂ activation (3.1 ± 0.4 μM vs. 52.2 ± 2.3 μM) but also rendered the channel sensitive to DCPIB (n = 6, P < 0.001). Compared to Kir2.1(R312Q), the Kir2.1(L222I) mutant that had a higher apparent affinity for PIP₂ (EC₅₀ = 18.8 ± 4.5 μM) remained insensitive to DCPIB (n = 7, P = 0.52). Similarly,

increasing the Kir3.4 channel's apparent affinity for PIP₂ by mutating I229 to Leu (35) or D216 to Asn (9–25) rendered them less sensitive to DCPIB ($EC_{50} = 30.7 \pm 9.8 \mu\text{M}$, $29.6 \pm 3.0\%$ blockade, $n = 6$, $P < 0.05$ and $EC_{50} = 24.6 \pm 4.5 \mu\text{M}$, $27.1 \pm 5.3\%$ blockade, $n = 5$, $P < 0.05$, respectively). Furthermore, DCPIB did not significantly affect the currents given by the double mutant Kir(I229L, D216N) ($EC_{50} = 13.8 \pm 2.7 \mu\text{M}$, $n = 7$, $P = 0.88$) (Fig. 5e and f).

A molecular model of DCPIB interactions with a Kir channel reveals sites shared with PIP₂

A docked model of DCPIB on a Kir3.1 chimera structure (18) (see Methods) was compared to a model of PIP₂ interacting with the same channel (16). As can be appreciated by the direct comparison of the two models, DCPIB and PIP₂ both occupy the same binding pocket, which includes the basic residues K79, K183, and K188 that are thought to bind directly to PIP₂ (16, 32) (Fig. 6a, b).

Discussion

The list of ion channels and transporters that depend on interactions with phosphoinositides to maintain their activity continues to grow (11, 12). Studies on Kir channels in the past two decades have provided the greatest structural insights of how channel PIP₂ interactions might control channel gating. There are only a couple examples of small molecules that enhance or compete with the effects of PIP₂ (20, 36). Here, we identify a previously well-characterized inhibitor of swelling-activated Cl⁻ currents (2), DCPIB, as a blocker of Kir channels that show weak apparent affinity to PIP₂. Our data support a model in which DCPIB is able to compete with PIP₂ to occupy the same binding pocket, provided that the affinity of the channel for PIP₂ is low.

Since channels that show the lowest apparent affinity for PIP₂ are the ones most susceptible to modulation by intracellular agents, it may be useful to have a general blocker that acts in pathophysiological situations in which activation of such channels leads to unwanted effects. In heart for example, constitutive activation of K_{ACh} by unknown mechanisms has been implicated in chronic atrial fibrillation (4). Whether DCPIB could prove useful in this setting remains to be examined.

The action of DCPIB on Kir channels raises the question whether its effect on I_{Cl,swell} proceeds via a similar mechanism. Addition of 10 μM DiC8 PIP₂ or DiC8 PIP₃ to the patch pipette solution strongly suppresses α_{1A} -adrenoreceptor and Gq signaling-induced attenuation of I_{Cl,swell} (8, 34). These data suggest I_{Cl,swell} activity critically depends on PIP₂ and PIP₃ binding. Yet, it remains uncertain whether DCPIB acts directly on the I_{Cl,swell} channel protein or on its upstream signaling cascade (3). The possibility remains that other channels and transporters are susceptible to similar block by DCPIB when their affinity for PIP₂ is low enough because DCPIB seems able to compete effectively with PIP₂ for its protein target.

Recently DCPIB was found to activate TREK potassium channels in cultured astrocytes (17). Since TREK channels are activated by PIP₂ (14) it is possible that the DCPIB effect is

mediated via an allosteric enhancement of the PIP₂ effect rather than a direct competition with it.

Acknowledgments

The authors are grateful to Heikki Vaananen and Sophia Gruszecki for oocyte isolation. Thanks go to all members of the Logothetis laboratory for critical feedback on this work at every stage of its development. The work was supported by National Institutes of Health grants R01HL59949-18 and R01HL090882-4 to D.E.L. WD was supported by NIH training grant T32HL094290: Lipid Signaling and Functional Lipidomics in Cardiovascular and Respiratory Diseases (LCRD). RM was supported by predoctoral NRSA fellowship F30HL097582 from NHLBI.

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Fig. 1. DCPIB inhibits $I_{K_{ACh}}$ in adult atrial myocytes

a Families of currents (-120 to +40 mV, 250 ms) in control (Ctrl), after ACh (10 μ M, steady state), and after addition of DCPIB (10 μ M, 5 min) in the presence of ACh (+DCPIB). **b** I-V relationships from **a**. **c** Current densities at -80 mV ($n = 5$; Ctrl vs. ACh, $**P < 0.001$; ACh vs. +DCPIB, $**P < 0.001$; Ctrl vs. +DCPIB, $P = 0.84$, NS). **c, d** ACh (10 μ M) significantly decreased APD₅₀ and APD₉₀, and the shortening of the APD was completely reversed by DCPIB ($n = 6$, ACh vs. Ctrl, $*P < 0.05$; ACh vs. +DCPIB, $*P < 0.05$; Ctrl vs. +DCPIB, $P = 0.25$, NS).

Fig. 2. Intracellular DiC8 PIP₂ (200 μM) attenuates the inhibitory effect of DCPIB on I_KACh
a I-V relationships of the currents (-120 to +40 mV, 250 ms) in control (Ctrl), after ACh (10 μM, steady state), after addition of DCPIB (10 μM, 5 min) in the presence of ACh (+DCPIB), and after adding tertiapin-Q (100 nM) (n=4). **b** Current densities at -80 mV (n = 4; Ctrl vs. ACh, **P < 0.001; DCPIB vs. ACh, P = 0.22, NS; ACh vs. +tertiapin Q, **P < 0.001; ctrl vs. + tertiapin Q, P = 0.90, NS). DiC8 PIP₂ (200 μM) was added into the pipette solution.

Fig. 3. DCPIB inhibits Kir3.1/Kir3.4 heteromeric currents expressed in oocytes

a Representative time course of whole-cell currents inhibited by DCPIB at -100 mV in oocytes expressing Kir3.1/Kir3.4. **b** Representative time course of whole-cell currents at -100 mV in oocytes co-expressing Kir3.1/Kir3.4 and the muscarinic M2 receptor. **c** Example of a family of currents (-120 to +50 mV, 250 ms) in uninjected oocytes (top), or oocytes injected with Kir3.1/Kir3.4 in the absence (middle) or presence (bottom) of DCPIB. **d** I-V plots at different concentrations of DCPIB. **e** Fitted dose-response curve of basal TEVC currents. **f** Normalized TEVC basal currents of oocytes expressing Kir3.1/Kir3.4 at different concentrations of DCPIB, $n = 18$, $**P < 0.001$. **g** Normalized TEVC basal plus ACh-induced currents of oocytes co-expressing Kir3.1/Kir3.4 and M₂ receptor at different concentrations of DCPIB (in continuous presence of ACh), $n = 11$, Ctrl vs. ACh $**P < 0.001$; ACh vs. different concentrations, $**P < 0.001$. **h** Fitted DCPIB dose-response curve of inside-out macropatch currents.

Fig. 4. DCPIB inhibits Kir channels with weak channel-PIP₂ interactions

a Fractional blockade of whole-cell currents by maximal concentration of DCPIB (200 μM) at -100mV in oocytes expressing Kir2.3, Kir2.1, Kir3.4, Kir6.2/SUR, and Kir6.2Delta36. Kir2.1 (n = 8) vs. Kir3.4 (n = 7), Kir6.2/SUR (n = 7), and Kir6.2Delta36 (n = 5), **P < 0.001; Kir2.1 vs Kir2.3 (n = 7), P = 0.62, NS. **b** Dose-response curve of DiC8 PIP₂ activation of Kir2.3, Kir2.1, Kir3.4, and Kir6.2Delta36.

Fig. 5. Kir2 and Kir3 channel block by DCPIB depends on apparent affinity for PIP₂
a, c Fractional blockade of whole-cell currents by maximal concentration of DCPIB (200 μ M) at -100 mV in oocytes expressing Kir2.1, Kir2.1(R312Q), Kir2.1(L222I), Kir3.4, Kir3.4(I229L), Kir3.4(D216N), and Kir3.4(I229L,D216N). Kir2.1(R312Q) ($n=6$) vs. Kir2.1 ($n=7$), $**P < 0.001$; Kir2.1(R312Q) vs. Kir2.1(L222I) ($n=7$), $**P < 0.001$; Kir2.1 vs. Kir2.1(L222I), $P=0.55$, NS. Kir3.4 ($n=5$) vs. Kir3.4(I229L) ($n=6$), Kir3.4(D216N) ($n=5$), and Kir3.4(I229L,D216N) ($n=7$), $###P < 0.001$; Kir3.4(I229L) vs. Kir3.4(D216N), $P = 0.66$, NS; Kir3.4(I229L) vs. Kir3.4(I229L,D216N), $**P < 0.001$; Kir3.4(D216N) vs. Kir3.4(I229L,D216N), $**P < 0.001$. **b, d** Dose-response curve of DiC8 PIP₂ activation of Kir2.1, Kir2.1(R312Q), Kir2.1(L222I), Kir3.4, Kir3.4(I229L), Kir3.4(D216N), and Kir3.4(I229L,D216N).

Fig. 6. DCPIB occupies the same binding pocket within interaction distance from similar residues as PIP₂

a PIP₂ docked to the Kir3.1 chimera was adapted from the study by Meng and colleagues (25). **b** DCPIB was docked to the Kir3.1 chimera (see Methods) and was seen to occupy the same pocket as PIP₂ in A. Predicted contact residues, which were common to PIP₂ and DCPIB, are indicated in stick depiction. Basic residues are highlighted blue, acidic red, polar green, and non-polar white. The PIP₂ and DCPIB docked ligands are shown in ball-and-stick representation: carbon atoms are cyan, oxygen red, chlorine orange, and phosphorus tan.