Novel Mechanism of Interaction of p85 Subunit of Phosphatidylinositol 3-Kinase and ErbB3 Receptor-derived Phosphotyrosyl Peptides*§

Atsushi Suenaga‡‡, Naoki Takada‡‡, Mariko Hatakeyama‡‖, Mio Ichikawa‡, Xiaomei Yu‡, Kentaro Tomii‡, Noriaki Okimoto‡, Noriyuki Futatsugi‡, Tetsu Narumi‡, Mikako Shirouzu**‡‡, Shigeyuki Yokoyama***‡‡‡, Akihiko Konagaya‡, and Makoto Taiji‡

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Ligand-activated and tyrosine-phosphorylated ErbB3 receptor binds to the SH2 domain of the p85 subunit of phosphatidylinositol 3-kinase and initiates intracellular signaling. Here, we studied the interactions between the N-(N-SH2) and C-(C-SH2) terminal SH2 domains of the p85 subunit of the phosphatidylinositol 3-kinase and eight ErbB3 receptor-derived phosphotyrosyl peptides (P-peptides) by using molecular dynamics, free energy, and surface plasmon resonance (SPR) analyses. In SPR analysis, these P-peptides showed no binding to the C-SH2 domain, but P-peptides containing a phospho-YXXM or a non-phospho-YXXM motif did bind to the N-SH2 domain. The N-SH2 domain has two phosphotyrosine binding sites in its N- (N1) and C- (N2) terminal regions. Interestingly, we found that P-peptides of pY1180 and pY1241 favored to bind to the N2 site, although all other P-peptides showed favorable binding to the N1 site. Remarkably, two phosphotyrosines, pY1178 and pY1243, which are just 63 amino acids apart from the pY1241 and pY1180, respectively, showed favorable binding to the N1 site. These findings indicate a possibility that the pair of phosphotyrosines, pY1178-pY1241 or pY1243-pY1180, will fold into an appropriate configuration for binding to the N1 and N2 sites simultaneously. Our model structures of the cytoplasmic C-terminal domain of ErbB3 receptor also strongly supported the speculation. The calculated binding free energies between the N-SH2 domain and P-peptides showed excellent quantitative agreement with SPR data with a correlation coefficient of 0.91. The total electrostatic solvation energy between the N-SH2 domain and P-peptide was the dominant factor for its binding affinity.

Overexpression or mutation of ErbB receptors (ErbB1; epidermal growth factor receptor, ErbB2; Neu, ErbB3, and ErbB4) is implicated as a cause of various human cancers (1–4). ErbB3 is a kinase-impaired receptor; nevertheless ligand binding to the receptor can cause heterodimerization with another ErbB receptor and induces activation and transphosphorylation of the ErbB3 receptor in cells (5–7). Once activated and tyrosine-phosphorylated, the ErbB3 binds to the Src homology 2 (SH2) domain of p85 (a regulatory subunit of phosphatidylinositol 3-kinase (PI3K)) at its binding sites within the C-terminal regulatory region. Six binding sites have been identified for p85 binding; accordingly the ErbB3 receptor is considered as a scaffold protein for PI3K (2, 4, 8–9). Studies on insulin receptor substrate-1 (IRS-1) (10–12), platelet-derived growth factor receptor (13), and ErbB receptors (4, 8) revealed that the phospho-YXXM (pYXXM) motif is a specific binding site for SH2 domain of the p85, to which binding is necessary for activation of the p110 catalytic subunit of PI3K (12, 14). On the other hand, the p85 is reported to bind to a pYXXV motif in the hepatocyte growth factor receptor (15), and another study also suggested that there was no binding specificity in any binding motifs for SH2 domains of p85, PLCγ, RasGAP, and Shc (16). The p85 has a tandem SH2 region; two SH2 domains at N-terminal (N-SH2) and C-terminal (C-SH2) domains linked by the inter-SH2 (IS) region (Fig. 1). Generally, the existence of both N-SH2 and C-SH2 domains induces high affinity interaction with phosphotyrosine and activation of PI3K; however, binding of individual SH2 domains to the phosphotyrosyl peptides is quite specific. Phosphotyrosyl peptides derived from IRS-1 (17) and the platelet-derived growth factor receptor (13) preferred binding to the p85 C-SH2, and the ones derived from the ErbB3 receptor preferred the N-SH2 (4, 8). Furthermore, an NMR study of p85 N-SH2 and a doubly phosphorylated peptide derived from a polyomavirus middle T antigen (PvMT) complex revealed double phosphotyrosine binding in N-SH2 (18). Thus, the interaction mechanism of p85 SH2 and phosphotyrosyl peptides is quite complex. To study this complexity, we analyzed the interaction of ErbB3 receptor-derived phos-
phototyrosyl peptides and SH2 domains of p85 using SPR analysis and molecular dynamics (MD) simulation.

MD simulation has been acknowledged as a powerful method to investigate protein-protein, protein-ligand, and protein-DNA interactions at the atomic level. The molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method has been recently proposed for calculating free energies of macromolecules in solution (19, 20). This method combines MD simulations in explicit solvent with implicit solvation model, Poisson-Boltzmann (PB) analysis (21–24), and solvent accessible surface area (25) for estimating binding free energies. Successful estimations of binding free energy using the MM-PBSA method have been reported in previous studies (26–34).

In this study, we first investigated the interactions between the tandem SH2 (N-SH2, IS, and C-SH2 domains), N-SH2 and C-SH2 domains of the p85 subunit of PI3K, and eight phototyrosyl peptides derived from the ErbB3 receptor (P-peptides: pY1035, pY1178, pY1180, pY1241, pY1243, pY1257, pY1270, and pY1309) using SPR and cellular interaction analyses. Positive interactions observed for the N-SH2 domain and P-peptides were further analyzed by MM-PBSA and MD on a dedicated MD computer, the Molecular Dynamics Machine (MDM) (34–39).

**MATERIALS AND METHODS**

**SPR Analysis**

**P-peptide Synthesis**—Human ErbB3 receptor-derived P-peptides in which the N terminus was biotinylated were synthesized as previously described (34). The amino acid sequences of P-peptides are listed in Table I. Expression and Purification of Proteins—cDNA encoding N-SH2 (amino acids 333–428), C-SH2 (amino acids 624–724), and tandem SH2 (amino acids 333–724), N1 and N2 indicate the two phototyrosine binding sites in the N-SH2.

**System Setup for MD Simulation**

The production MD trajectory was collected for a 300 ps period (from 1 to 4 °C) at constant pressure (100 μ) for 1 h at 4 °C and washed three times with PBS. Each immobilized peptide bead was incubated with the MCF-7 cell lysate (1 g/mL) at 4 °C for 1 h. The beads were washed three times with PBS and applied to Western blot. Dissolved protein bands were detected with an anti-p85 antibody (Santa Cruz Biotechnology).

**Equilibration**

All MD simulations were carried out using modified Amber 6.0 (43) for MDM on a PC (Intel Pentium4, 2.53 GHz) equipped with an MDRape-2 board, which is a main component of the MDM (4 chips; 64 Giga bytes of memory). The parm96 force field (44) was adopted, and the time step was set at 1 fs. All non-bonded interactions, van der Waals, and Coulomb forces and energies were calculated using the MDM accurately. The bond lengths involving hydrogen atoms were constrained to equilibrium lengths using the SHAKE method (45). The temperature of each system was gradually heated to 300 K during the first 100 ps period, and additional 900 ps MD simulations were performed for data collection. The temperature was kept constant at 300 K using the method of Berendsen et al. (46) coupled to a temperature bath with coupling constants of 0.2 ps.

**Free Energy Calculations**

The production MD trajectory was collected for a 300 ps period (from 700 to 1,000 ps) with each snapshot saved every 10 ps. The set of structures obtained by an MD trajectory was sampled for use in estimating binding free energies. In the analysis of binding free energies,
the water molecules were replaced with implicit solvation models.

The procedures for estimating the binding free energy have been described in detail in the previous report (34). Briefly, the Delphi program (47, 48) was used to solve the PB equation. The PARSE (49) and FORTE search, we used the AL2TS program that converts sequence structure alignment (AL) format to tertiary structure (TS) format. To build a backbone model from the alignment derived from the FORTE search, we used the AL2TS program that converts sequence structure alignment (AL) format to tertiary structure (TS) format.

RESULTS AND DISCUSSION

**SPR and Cellular Interaction Analyses**—Binding of tandem SH2, N-SH2, and C-SH2 domains of p85 and the ErbB3 receptor-derived eight P-peptides were analyzed (Table II). Results showed specific binding of tandem SH2 to the P-peptides that include the pYXXM motif (pY1035, pY1178, pY1241, pY1257, and pY1270). None of the C-SH2, or SH3 and BCR domains of p85 showed detectable binding to P-peptides (data not shown). These results on tandem SH2 binding were consistent with the earlier study describing that PXXMX is a specific binding motif for p85 SH2 (4, 8, 10–13). However, in our study, p85 N-SH2 bound to most of the P-peptides including pY1180 and pY1243, except for pY1309. The pY1309 has been known as a Src binding site and pY1180 and pY1243 as growth factor receptor binding protein 7 (Grb7) binding sites (9, 53). The interaction analysis of the p85 with P-peptides and non-phosphopeptides in HRG-stimulated MCF-7 cells clearly supported the SPR data on tandem SH2 (Fig. 2). Our data suggested a difference in binding patterns between the p85 N-SH2 and the tandem SH2 or p85 subunit (SH3, BCR and tandem SH2). To understand the binding mechanism of p85 N-SH2 to the P-peptides, MD simulation and structure modeling were performed.

**MD Simulations**—We performed 25 series of 1-ns MD simulations for estimating binding free energies between the p85 N-SH2 and P-peptides. From the backbone heavy atom root mean square deviations (r.m.s.d.) of the p85 N-SH2 and P-peptides from the initial structures (Supplemental Fig. 2), the mean square deviations (r.m.s.d.) of the p85 N-SH2 and P-peptides were smaller than 1.0%, and those of the free P-peptides were checked. Each standard deviation of the binding free energy, although the r.m.s.d. of the p85 N-SH2/P-peptide complexes and the free p85 N-SH2 and P-peptides fluctuated (7.6 ± 0.82 Å) (Supplemental Table I).

**Binding Free Energies between p85 N-SH2 and P-peptides**—To examine whether the calculated energies can evaluate binding free energies precisely, fluctuations of the calculated energies were examined. The average values and the standard deviations of calculated enthalpy H (sum of the internal energy of protein (E_{int}), the electrostatic solvation energy (G_{ASA}), and non-polar solvation energy (G_{solv})) and entropic contribution TS were checked. Each standard deviation of the H of the p85 N-SH2/P-peptides complexes and the free p85 N-SH2 were smaller than 1.0%, and those of the free P-peptides were also smaller than 3.0% (Supplemental Table II). These results indicate that the calculated energies are stable during the simulations; thus, these values can be utilized for evaluating the binding free energy, although the r.m.s.d. of the p85 N-SH2/P-peptide complexes and the free P-peptides fluctuated to some extent (Supplemental Fig. 2). As a result of analysis, the calculated binding free energies on P-peptides except for pY1309 and p85 N-SH2 obtained from MM-PBSA method showed excellent qualitative agreement with those obtained from SPR analysis (correlation coefficient was 0.91) (Table III and Fig. 3). Unexpectedly, the binding free energy of the p85 N-SH2/pY1309 complex obtained from N1 and N2 simulations showed no good agreement with that obtained from experimental analysis. The r.m.s.d. of the pY1309 extracted from the p85 N-SH2/pY1309 complex was unstable, increased monotonously, and greatly fluctuated from 700 to 1,000 ps (Supplemental Fig. 2 and Supplemental Table I). The root mean square fluctuations (r.m.s.f.) of phosphotyrosines in all P-peptides highly correlated with the binding free energies obtained from experimental analysis (correlation coefficient was 0.92). The r.m.s.f. of the pY1309 showed the largest value among all P-peptides tested (2.46 Å). In addition, residues around the

| P-peptide | Binding protein | \( K_f \times 10^4 \text{ M}^{-1} \text{s}^{-1} \) | \( K_a \times 10^{-3} \text{s}^{-1} \) | \( K_D \text{ nm}^{-1} \) |
|-----------|-----------------|----------------|----------------|----------------|
| pY1035    | Tandem SH2*     | 38.4 ± 1.82    | 2.06 ± 0.04    | 5.38 ± 0.36    |
| N-SH2     | 568 ± 83        | 16.7 ± 2.2     | 2.94 ± 0.82    |
| pY1178    | Tandem SH2      | 70.6 ± 1.3     | 2.42 ± 0.07    | 3.42 ± 0.16    |
| N-SH2     | 16.9 ± 0.7      | 4.28 ± 0.48    | 25.4 ± 3.9     |
| pY1180    | Tandem SH2      | 11.0 ± 1.1     | 7.43 ± 0.24    | 67.5 ± 6.7     |
| N-SH2     | 11.0 ± 0.7      | 1.35 ± 0.06    | 12.3 ± 1.3     |
| pY1241    | Tandem SH2      | 108 ± 5.2      | 6.81 ± 0.73    | 6.33 ± 0.99    |
| N-SH2     | 824 ± 131       | 12.9 ± 1.7     | 1.56 ± 0.46    |
| pY1243    | Tandem SH2      | 4.81 ± 0.97    | 10.5 ± 2.36    | 218 ± 93      |
| N-SH2     | 2.75 ± 0.54     | 2.03 ± 0.22    | 7.52 ± 2.33    |
| pY1257    | Tandem SH2      | 50.0 ± 6.36    | 5.31 ± 1.06    | 10.6 ± 3.4     |
| N-SH2     | 243 ± 23.8      | 5.17 ± 0.51    | 2.13 ± 0.42    |
| pY1270    | Tandem SH2      | 24.7 ± 3.47    | 1.29 ± 0.14    | 5.20 ± 1.29    |
| N-SH2     | 4.17 ± 0.44     | 0.31 ± 0.01    | 7.53 ± 1.13    |
| pY1309    | Tandem SH2      | 2.94 ± 0.51    | 7.58 ± 1.92    | 258 ± 110     |
| N-SH2     | 2.42 ± 0.44     | 2.54 ± 0.16    | 105 ± 25.8     |

* Tandem SH2 (amino acids 333–724) of the p85 subunit.

* N-terminal SH2 domain (amino acids 333–429) of the p85 subunit.

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**Table II**

**SPR analysis of P85 SH2 domain/P-peptide binding affinity**

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2 AL2TS service by the Protein Structure Prediction Center (Lawrence Livermore National Laboratory) provides a method to translate structure alignment (AL) format to tertiary structure (TS) format.
The corresponding non-phosphopeptides B MCF-7 cell lysate (A). Peptides (pY) or the corresponding non-phosphopeptides (Y) were also incubated with HRG-stimulated or non-stimulated MCF-7 cell lysate (B) and quantified by densitometer. Specific binding of p85 with P-peptides was obtained by subtracting non-phosphopeptide binding (C). Note that pY1180 and pY1243 share the same non-phosphopeptide sequences of Tyr-1178 and Tyr-1241, respectively. Peptide-protein complexes were then analyzed by Western blot. PI3K p85 was detected with a specific antibody against p85.

TABLE III
The calculated binding free energies between p85 N-SH2 and P-peptides

| P-peptide | $\Delta G_{\text{calc}}(N1)^a$ | $\Delta G_{\text{calc}}(N2)^b$ | $\Delta G_{\text{exp}}$ |
|-----------|-----------------|----------------|----------------|
| pY1035    | -131.64         | -76.61         | -11.63         |
| pY1178    | -56.65          | -54.35         | -10.35         |
| pY1180    | 199.19          | -29.71         | -10.78         |
| pY1241    | -92.11          | -164.33        | -12.00         |
| pY1243    | -85.17          | -8.16          | -11.07         |
| pY1257    | -177.44         | -142.49        | -11.82         |
| pY1270    | -114.58         | -29.22         | -11.07         |
| pY1309    | -210.53         | -91.48         | -9.51          |

$^a$ The values were obtained from N1 simulation.
$^b$ The values were obtained from N2 simulation.
$^c$ All energies are in kcal/mol.

Interestingly, the result of calculated binding free energies showed that pY1180 and pY1241 favored the N2 site and the p85 N-SH2, though all other P-peptides favored the N1 site. There are two tyrosines, Tyr-1178 and Tyr-1243, which are just 63 amino acids apart from pY1241 and pY1180, respectively, and these two tyrosines showed favorable binding to the N1 site when they are phosphorylated. These findings indicate the possibility that the pair of phosphotyrosines, pY1178-pY1241 or pY1243-pY1180, will fold into appropriate configuration for binding to N1 and N2 sites at once. Our model structures of the cytoplasmic C-terminal domain of the ErbB3 receptor strongly supported this speculation (Fig. 4).

Component Analysis of Binding Free Energies—Next, the dominant factor for binding affinity (with the exception of pY1309) was identified by component analysis of the binding free energies. The regression coefficients between the calculated binding free energies and its components indicate contribution of the energetic component to the binding affinity (Table IV). Since the absolute values of the regression coefficients of the electrostatic energy $\Delta E_{\text{ele}}$ (regression efficient of $-5.26$) and the electrostatic solvation energy $\Delta G_{\text{esol}}$ (regression efficient of $6.25$) were larger than that of the other components, these components seem to greatly contribute to binding affinity. The $\Delta G_{\text{esol}}$ and experimental binding free energy $\Delta G_{\text{exp}}$ showed a strong positive correlation coefficient of $0.80$. In contrast, the $\Delta E_{\text{ele}}$, the $\Delta G_{\text{exp}}$ showed negative correlations of $-0.79$. Interestingly, total electrostatic solvation energy $\Delta G_{\text{esol}}$, which is the sum of the $\Delta E_{\text{ele}}$ and the $\Delta G_{\text{esol}}$, and the $\Delta G_{\text{exp}}$ showed the highest correlation among all energy components (correlation coefficient of $0.84$). This result indicates that the $\Delta G_{\text{esol}}$ and the $\Delta E_{\text{ele}}$ compensate for each other, and the $\Delta G_{\text{exp}}$ between the p85 N-SH2 and the P-peptides is the dominant factor for binding affinity. To clarify the reason for the negative correlation between the $\Delta E_{\text{ele}}$ and the $\Delta G_{\text{exp}}$, the hydrogen bonds between p85 N-SH2 and the P-peptides were investigated. The number of hydrogen bonds and the $\Delta G_{\text{exp}}$ showed high correlation (correlation coefficient of $0.82$), and these results indicate that a lesser number of hydrogen bonds induces higher binding affinity (Supplemental Fig. 3). This fact also explains the strong negative correlation between $\Delta E_{\text{ele}}$ and the
Novel Interaction of PI3K p85 and ErbB3 Receptor

CONCLUSION

Binding of p85 to the tyrosine-phosphorylated proteins is an essential step for the activation of the p110 catalytic subunit of PI3K. Our SPR analysis showed that p85 N-SH2 mainly contributes to the interaction with the ErbB3 receptor. From the computational analysis, we found that pY1180 and pY1241 favored the N2 site in the p85 N-SH2 for binding, though all other P-peptides favored the N1 site. There are two tyrosines, Tyr-1178 and Tyr-1243, just 63 amino acids apart from the pY1241 and pY1180, respectively. These tyrosines are also targets for phosphorylation and these P-peptides (pY1178 and pY1243) favored the N1 site for binding. The model structure of the cytoplasmic C-terminal region of the ErbB3 receptor indicates that the pair of phosphotyrosines, pY1178-pY1241 or pY1243-pY1180, will act for simultaneous binding of “double phosphotyrosines.” Actually, pY1180 and pY1243 have been recognized as Grb7 binding sites (53). However, those phosphopeptides corresponding to Grb7 binding sites showed a slight rise in PI3K activity (14) or an affinity for p85 (9) in earlier studies, indicating that the non-pYXXM motif is also capable of binding to p85 SH2. Our SPR and computational analyses suggest that the amino acid sequence is not the sole determinant for specificity of the phosphotyrosyl peptide-SH2 interaction.

In general, binding to double phosphotyrosines enhances interaction and enzymatic activity of PI3K than binding to a single phosphotyrosine, and bivalent bindings of p85 at two tyrosine residues have been reported at 13 amino acids apart (pY1257 and pY1270) for the ErbB3 receptor, 7 amino acids apart for PvMT (18), and 20 amino acids apart for IRS-1 (12). Optimal amino acid distance for the p85 bivalent binding is not known. However, since different growth factor ligands induce discriminative phosphorylation patterns at multiple tyrosine sites within the ErbB receptor (55), those binding sites and patterns may differ in response to the various kinds of stimuli, and double phosphotyrosines at a greater distance may facilitate stabilizing the association with p85 at N1 and N2 sites.

Our experimental and computational analyses showed that the p85 N-SH2 domain itself is capable of binding to the non-pYXXM motif; however, the tandem SH2 and cellular p85 subunit did not bind to the same peptides. We estimated that the steric constraint of the p85 structure might alter the binding property. We made a homology model of p85 tandem SH2 based on the structure of ZAP-70 tandem SH2 (56); however, such constraint was not observed for P-peptide binding (data not shown). For another reason, the presence of the IS domain in p85 raises the binding affinity of the SH2 domain to that of ErbB3-derived phosphopeptides (8), and conformation of the IS domain is changed after N-SH2 binding to phosphopeptide (52). Accordingly the IS domain might induce a conformational change and contribute to the specificity of p85 binding to the pYXXM motif.

As for MD simulation, the calculated binding free energies showed excellent qualitative agreement with experimental data with the correlation coefficient of 0.91, although they showed no quantitative agreement with experimental data. The binding free energies obtained from computational analysis were smaller than those obtained from experiment. This fact may be caused by the short time scale of our MD simulations for equilibration of the small peptide (34). However, our present study shows that the current method for estimating binding free energy is effective for in silico screening.

The electrostatic solvation contribution and electrostatic interaction compensate each other, and the sum of these compo-

\[ \Delta G_{\text{exp}} \text{ (Table IV). This means that loss of hydrogen-bonded energy between p85 N-SH2 and P-peptides are compensated by the solvation of the p85 N-SH2 and P-peptides.} \]

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**FIG. 4.** Stereoviews of predicted interactions between the model structure of the cytoplasmic C-terminal domain of ErbB3 receptor (green) and p85 N-SH2 (red). The ErbB3 receptor and the p85 N-SH2 are represented by the ribbon model. Four tyrosines (Tyr-1178, orange; Tyr-1180, cyan; Tyr-1241, brown; and Tyr-1243, magenta) in the ErbB3 receptor and two phosphotyrosine binding residues at N1 (yellow) and N2 (blue) sites in the p85 N-SH2 are highlighted by a space-filled model. The model structure of ErbB3 receptor and p85 N-SH2 are docked manually to reproduce the interactions of N1-pY1178 and N2-pY1241 (A) and N1-pY1243 and N2-pY1180 (B).

**TABLE IV**

Component analysis of calculated binding free energies

| P-peptide | \( \Delta F_{\text{ele}}^a \) | \( \Delta E_{\text{vdW}} \) | \( \Delta G_{\text{vdW}} \) | \( \Delta G_{\text{ele}} \) | \( \Delta G_{\text{int}} \) | \( -T \Delta S \) | \( \Delta G_{\text{calc}}^b \) | \( \Delta G_{\text{calib}} \) | \( \Delta G_{\text{exp}} \) |
|-----------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| pY1035    | -77.56              | -340.48             | 172.37              | -5.55               | 50.30               | 69.28               | -168.11             | -131.64             | -11.63              |
| pY1178    | -60.79              | -892.18             | 798.18              | -5.73               | 34.04               | 69.83               | -94.00              | -56.65              | -10.35              |
| pY1180    | -89.75              | -1232.11            | 1176.84             | -6.73               | 31.33               | 90.71               | -55.27              | -29.71              | -10.78              |
| pY1241    | -60.02              | -508.27             | 300.45              | -3.25               | 53.83               | 52.93               | -207.82             | -164.33             | -12.00              |
| pY1243    | -21.89              | -618.20             | 438.44              | -3.19               | 56.15               | 63.52               | -179.76             | -85.17              | -11.07              |
| pY1257    | -66.80              | -333.38             | 119.29              | -4.43               | 37.67               | 70.21               | -214.09             | -177.44             | -11.82              |
| pY1270    | -88.48              | -739.06             | 583.14              | -7.05               | 56.27               | 80.60               | -155.92             | -114.58             | -11.07              |
| pY1309    | -45.64              | -630.81             | 363.46              | -4.3                | 35.97               | 70.79               | -267.35             | -210.53             | -9.51               |

\(^a\) All energies are in kcal/mol.

\(^b\) \( \Delta G_{\text{calc}} = \Delta F_{\text{ele}} + \Delta G_{\text{vdW}} \).

\(^c\) The values of pY1180 and pY1241 were obtained from N2 simulations, and others were from N1 simulations.

\(^d\) Correlation coefficient between energetic component and experimental binding free energies (except for pY1309).

\(^e\) Regression coefficient between energetic component and calculated binding free energies (except for pY1309).
ments, total electrostatic solvation energies $\Delta G_{\text{ele}}$ is the dominant factor in binding affinity. In the case of the interaction between the SH2 domain of Grb2 and P-peptides derived from ErbB1 and ErbB4 receptors, the binding of the dominant factor for its binding affinity was the van der Waals interaction (34). The difference between two dominant factors for binding affinity indicates that the Grb2 recognizes the "molecular shape" of the ErbB1 and ErbB4 receptor, and the binding of the p85 subunit of PI3K and the ErbB3 receptor is controlled by an "electrostatic field," and may be very important for receptor-adaptor recognition.

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