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Electrostatic Interactions of Peptides Flanking the Tyrosine Kinase Domain in the Epidermal Growth Factor Receptor Provides a Model for Intracellular Dimerization and Autophosphorylation

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ABSTRACT **The mechanism by which ligand-activated EGFR induces autophosphorylation via dimerization is not fully understood. Structural studies have revealed an extracellular loop mediated receptor dimerization. We have previously presented experimental data showing the involvement of a positive 13 amino acid peptide (R645–R657; $P13^+$) from the intracellular juxtamembrane domain (JM) of EGFR important for intracellular dimerization and autophosphorylation. A model was presented that suggest that $P13^+$ interacts with a negative peptide (D979–E991; $P13^-$) positioned distal to the tyrosine kinase domain in the opposite EGFR monomer. The present work shows additional data strengthening this model. In fact, by analyzing protein sequences of 21 annotated ErbB proteins from 9 vertebrate genomes, we reveal the high conservation of peptides $P13^+$ and $P13^-$ with regard to their sequence as well as their position relative to the tyrosine kinase (TK) domain. Moreover *in silico* structure modeling of these ErbB intracellular domains supports a general electrostatic $P13^+/P13^-$ interaction, implying that the C-terminal of one receptor monomer is facing the TK domain of the other monomer in the receptor dimer and vice versa. This model provides new insights into the molecular mechanism of ErbB receptor activation and suggests a new strategy to pharmacologically interfering with ErbB receptor activity. Proteins 2006;62:1036–1043.** © 2005 Wiley-Liss, Inc.

Key words: ErbB; charge cluster; conserved peptides; dimer; monomer

INTRODUCTION

The receptor tyrosine kinases (RTKs) play a crucial role in recruiting and activating signaling proteins. As a consequence they are involved in many cell processes such as proliferation, differentiation, cell cycle, and cell metabolism and survival.[1] The epidermal growth factor (EGF) receptor was the first RTK to be characterized and isolated as a cell surface protein involved in oncogenesis.[2] Extensive biochemical and molecular studies allowed the identification of the EGF receptor (EGFR) family composed of four members: EGFR/HER1/ErbB1, HER2/neu/ErbB2, HER3/ErbB3, and HER4/ErbB4.[3] Several ligands, mainly growth factors, were shown to bind and activate the EGF family receptors except HER2, which has no known ligand. The presence of numerous ligands having distinct biochemical properties, in combination with the existence of four different ErbB receptors, gives rise to signaling diversity. Moreover, the four receptors can potentially form many distinct homo- and heterodimers in a ligand-dependant fashion, leading to an expansion in the number of possible signaling pathways stimulated by a single ligand.[4]

The finding that EGFR family members are overexpressed in many cancers has accelerated research to target these RTKs in therapies.[5] In fact, antibodies blocking the ligand binding were developed in parallel with the identification of tyrosine kinase (TK) inhibitors.

Like most RTKs, the EGFR contains an extracellular ligand binding domain, a single α-helical transmembrane domain, and a well conserved intracellular TK domain.[6] The ligand binding activates the receptor, which dimerizes and induces autophosphorylation of key tyrosine residues. It was shown that dimerization can be obtained without ligand binding and receptor activation.[7] Any member of EGFR family can have a homodimer or a heterodimer preferentially with HER2. The phosphorylated tyrosines provide docking sites for intracellular signalling proteins that trigger the MAP kinase pathway, linking the receptor activation within the plasma membrane to gene expression modulation in the nucleus that provides the appropriate biological response.[6]

Understanding the proper dimerization mechanism became a necessity to increase the efficiency of targeting the EGFR in cancer therapy. Structural studies of the EGFR extracellular domain bound to EGF[8] or TNF-α[9] have

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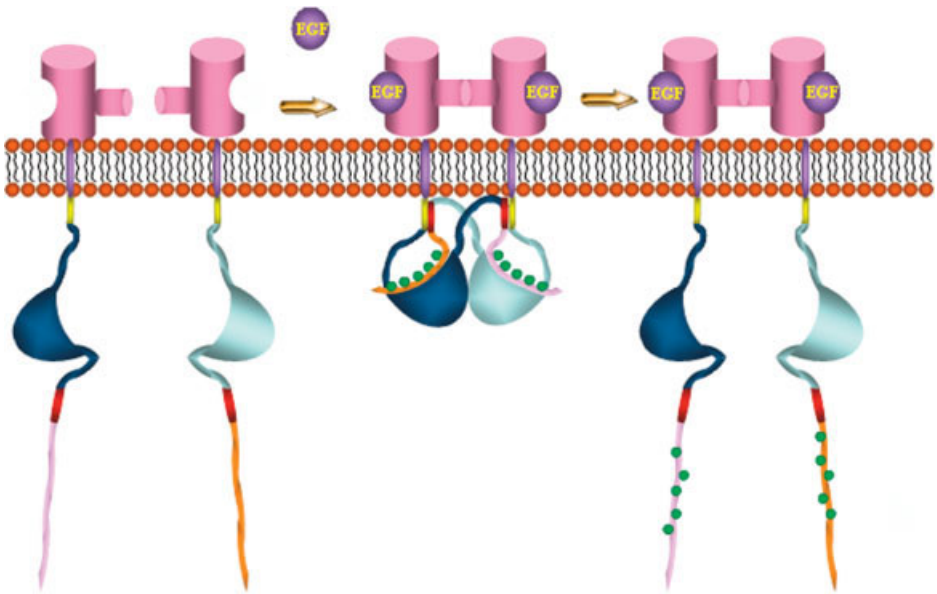

Fig. 1. Schematic representation of EGFR activation model. Two EGFR monomers are colored the same: pink for the extracellular domain, purple for the transmembrane domain, yellow for the 13 positive amino acid peptide ($P13^+$) and red for the 13 negative amino acid peptide ($P13^-$). The kinase and C-terminal domains are colored differently. The extracellular domain and the kinase domain are connected via a transmembrane helix and a short juxtamembrane segment containing $P13^+$ (yellow). The kinase and the C-terminal domains (containing the tyrosine residues known as the autophosphorylation sites) are connected via $P13^-$ (red segment). In the inactive conformation (left), with no ligand binding, the two monomers are separate. Activation (middle) occurs when ligands (purple circles, here EGF) bind to the extracellular domains, leading to the formation of a stable extracellular contact, enabling an intracellular contact and dimerization via $P13^+/P13^-$ interaction. The two monomers are positioned back to back in the model and the positively charged $P13^+$ and the negatively charged $P13^-$ interact due to their electrostatic complementarity. The kinase domain of one monomer can now transautophosphorylate the tyrosine residues of the C-terminal domain of the other monomer (Phosphates are represented by green dots). The intracellular domains of the active monomers are thereafter separated (right) to allow the recruitment of tyrosine phosphate binding proteins responsible for the intracellular signalling of EGFR.

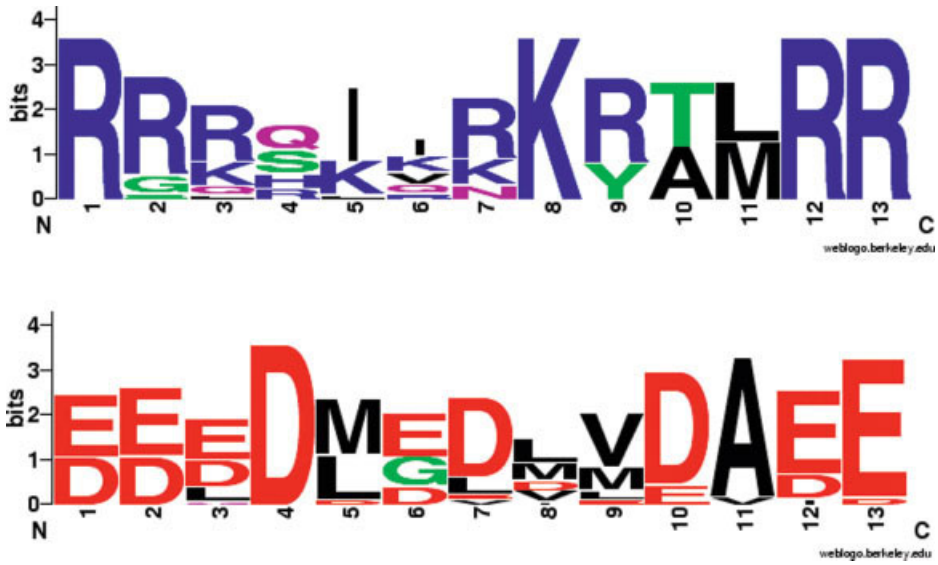

Fig. 2. Logo presentation of the consensus sequences in vertebrate species of (**a**) $P13^+$ peptide and (**b**) $P13^-$ peptides.

revealed that receptor dimerization is mediated by a loop protruding from neighboring receptors. Active EGFR dimers consist of a 2:2 EGF:EGFR complex formed from stable intermediates of 1:1 EGF:EGFR complexes and the dimerization loop-mediated mechanism is mediated entirely by receptor-receptor interactions. The ligand bind-

ing induces or stabilizes the conformation change responsible for the formation of dimers.

Few studies have been conducted to understand the conformational changes, initiated by ligand binding to the extracellular domain and leading to the activation of the TK domains allowing the autophosphorylation.[10,11]

The intracellular domain of EGFR contains the TK domain flanked by a juxtamembrane domain (JM) and the C-terminal tail.[12]

The JM domain has a 13 amino acid-positive peptide ($P13^+$; R645–R657), which was shown to play a role in the conformational change of the receptor[13] in the interaction with calmodulin *in vitro*[14,15] and *in vivo*[16] and in the regulation of Gs proteins.[17,18] It was also suggested that $P13^+$ functions as a nuclear localization signal (NLS) for the full-length receptor,[19] Moreover, the PKC feedback attenuation of EGFR is mediated by the phosphorylation of Thr654 within $P13^+$.[12]

The C-terminal domain has also a 13 amino acid-negative peptide ($P13^-$; D979–E991) containing the Mig 6 binding domain involved in the down regulation of the EGFR signal.[20] In addition, the key tyrosine residues of autophosphorylation are localized in the C-terminal domain.

We have previously shown that full-length EGFR lacking $P13^+$ fails to dimerize and autophosphorylate. Moreover, the intact intracellular domain of EGFR expressed in Cos7 cells was able to dimerize with the full-length endogenous receptor and was tyrosine phosphorylated. $P13^+$ deletion abolished all these abilities.[10]

These experimental findings lead us to propose an intracellular dimerization model based on an electrostatic interaction between $P13^+$ and $P13^-$, where the C-terminal tail of one receptor molecule is facing the TK domain of the other receptor molecule, allowing reciprocal autophosphorylation. Landau et al.[11] have presented a similar model based on theoretical considerations, in which they have suggested an electrostatic interaction between $P13^-$ and a positive region within the TK domain. Very recently, Bagossi et al.[21] have also presented a structural model for the dimer but without explaining the autophosphorylation mechanism.

In this article, we provide *in silico* arguments based on previous experimental results that show a high conservation of sequence and position of positive and negative peptides within all known vertebrate ErbB receptors and propose a structural model for the interaction of these two peptides based on Stamos structure of the EGFR kinase[22] (Fig. 1). Our data suggest that the proposed model is likely to be a general mechanism of intracellular dimerization of the EGF receptor family.

MATERIALS AND METHODS

Protein Sequence Analysis

We searched by December 15 (updated in April 15), 2005, Refseq and Swissprot databases for the presence of annotated ErbB proteins for which the full protein sequence is available and has been validated by the isolation of corresponding full-length mRNA. We found 23 sequences from which 21 were from 9 vertebrate species (Table I) and 2 from invertebrates. Representative of the four members of the ErbB family are found only for human, rat, and mouse, whereas other species have only two members. Similar protein size was found within the ErbB subfamily members except for the two EGFR homologs from *Drosophila* and *Caenorhabditis elegans,* having, respectively, 1426 and 1367 amino acids.

The position of the TK domain within each sequence was determined by comparison (using rps-blast) with the Conserved Domain Database (CDD) at the NCBI website.[23] Positions and sequences of the positive and negative peptides were searched using a Perl script implementing a charge cluster detection algorithm similar to that of Karlin et al.[24] This script (available on request), named ChargeClusterFinder (CCF), detects runs of residues of the same charge (+ or −) possibly interrupted by uncharged residues (but not mixture of + and − charged residues). It outputs short segments of 10 to 20 residues of high charge concentration, which are statistically significant, based on the criteria of Brendel et al.[25] According to these criteria, a charge cluster segment is considered significant if the value of a test statistics T is larger than 4.5 (for proteins of length 750 to 1500 residues) with $T = (c - Lf)/\sqrt{Lf(1-f)}$, where L is the length of the segment, c is the number of charged residues in this segment, and f is the fraction of charged residues in the protein.

A multiple sequence alignment of homologous peptides from different species was performed using Clustalw[26] and consensus motifs were represented as sequence logos[27] (http://weblogo.berkley.edu/) .

Models Construction of the Intracellular EGFR Domain

The 3D structure of the intracellular EGFR kinase domain was determined by Stomos et al. (pdb code 1M14). This structure lacks the $P13^+$ peptide and 18 amino acids (Asp960–Asp979) in the C-terminal $P13^-$ peptide. Automated modelling was used only to generate the missing loop in the C-terminal region (Asp960–Asp979). This led us to obtain a 3D structure of the intracellular EGFR domain with a complete C-terminal region. As far as the $P13^+$ peptide is concerned, we used a peptide homology comparative software (http://npsa-pbil.ibcp.fr) . The first hit corresponds to a peptide belonging to an Isomerase of *Saccharomyces cerevisiae* (pdb code 1P1F). This peptide shares 35.7% identity (64% similarity) with $P13^+$. A model of the $P13^+$ was constructed using the Deep View/Swiss-Pdb Viewer v 3.7 (http://www.expasy.org/spdbv) and the isomerase peptide (pdb code 1P1F) as template. The so obtained peptide was linked to the structure of the intracellular EGFR domain having a completed C-terminal region by introducing a peptidic bond between Ser697 and Gly698. The modelled structure containing both P13 peptides was then subjected to energy minimization using the Gromos software implemented to Swiss-Pdb viewer (http://iqc.ethz.ch/gromos). Five cycles of minimization were performed (500 steps of steepest descent, 500 steps of conjugated gradient, 500 steps of steepest descent, cutoff 50 Å)

TABLE I. Sequences and Positions of P13$^+$ and P13$^-$ Peptides Identified in the ErbB Proteins in Different Vertebrate Genomes

Genename	Organism	Accession[a]	Protein size[b]	Position and sequence of P13^{+}[b]	Position of the TyrKc domain[b,c]	Position and sequence of P13^{-}[b]
EGFR	Human	P00533	1210	669–681: RRRHIVRKRTLRR	704–971	1003–1015: DEEDMDDVVDADE
EGFR	Mouse	NP_997538	1210	670–682: RRRHIVRKRTLRR	706–973	1005–1017: DEEDMEDVVDADE
EGFR	Rat	NP_113695	1209	670–682: RRRQLVRKRTLRR	705–972	1004–1016: EEEDMEDVVDADE
EGFR	Zebrafish	NP_919405	1191	668–680: RRRHIRRKRTLRR	703–970	1004–1013: ELDEAVDADEYLV
EGFR	Chicken	TVCHLV	1223	678–690: RRRHIVRKRTLRR	713–980	1012–1024: EEEDMEDIVDADE
ErbB2	Human	P04626	1255	677–689: RRQQKIRKYTMRR	712–979	1010–1022: EDDDMGDLVDAEE
ErbB2	Chimps	XP_511461	1381	992–1003: RRQQKIRKYTMRR	1027–1294	1010–1022: EDDDMGDLVDAEE
ErbB2	Mouse	NP_001003817	1256	678–690: RRRQKIRKYTMRR	713–980	1011–1023: EDDDMGELVDAEE
ErbB2	Rat	P06494	1257	679–691: RRRQKIRKYTMRR	714–981	1012–1024: EDDDMGDLVDAEE
ErbB2	Dog	O18735	1259	676–688: RRRQKIRKYTMRR	711–978	1009–1021: EDDDMGDLVDAEE
ErbB2	Hamster	Q60553	1263	677–689: RRRQKIRKYTMRR	712–979	1325–1337: EDDDMGDLVDAEE
ErbB2[c]	Cow	XP_582040	1131	553–565: RRRQKIRKYTMRR	591–855	886–898: EDDDMGDLVDAEE
ErbB3	Human	P21860	1342	667–679: RGRRIQNKRAMRR	701–968	1008–1020: ELDLDLDLEAEED
ErbB3	Mouse	NP_034283	1339	665–677: RGRRIQNKRAMRR	702–966	1006–1018: ELDLDLDLEAEEE
ErbB3	Rat	Q62799	1339	665–677: RGRRIQNKRAMRR	699–966	1006–1018: DLDLDLDVEVEEE
ErbB3	Zebrafish	NP_001005320	1305	617–629: RSLSIRRKRAMRR	651–918	957–969: EDQDDEVLEDAID
ErbB4	Human	Q15303	1308	676–688: RRKSIKKKRALRR	710–977	1009–1021: DEEDLEDMMDAEE
ErbB4	Chimps	XP_516067	1293	677–689: RRKSIKKKRALRR	711–978	1010–1022: DEEDLEDMMDAEE
ErbB4	Mouse	XP_136682	1298	666–678: RRKSIKKKRALRR	700–967	999–1010: DEEDLEDMMDAEE
ErbB4	Rat	Q62956	1308	676–688: RRKSIKKKRALRR	710–977	1009–1021: DEEDLEDMMDAEE
ErbB4	Chicken	AAD3131764	1137	524–536: RRKSIKKKRALRR	558–825	857–869: DEEDLEDMMDAEE

[a]From Swissprot if available; otherwise from Refseq or Genpept databases at NCBI (Searched 15 April 2005).
[b]Full size of the precursor protein including signal peptide and positions relative to this full-size protein.
[c]TyrKc is the tyrosine kinase catalytic domain as in the Conserved Domain Database (CDD) of the NCBI (accession cd00192).
[d]Partial sequence predicted from the *Bos taurus* genome (not supported by experimental data).

using a harmonic constrain. To construct the dimer model, basic and acidic P13 peptides were manually placed in close contact to each other. P13$^-$ and P13$^+$ peptides were nicely interacting together without any need to change the structure of the monomer as it was reported.[22] This yielded two possible orientations: face to face (catalytic cavities facing each other) or back to back (catalytic cavities exposed to the solvent). The generated dimer models were subjected to energy minimization using five minimization cycles as described above.

RESULTS

Presence of Positive and Negative Peptides

All investigated ErbB proteins from vertebrate species have the P13$^+$ and P13$^-$ peptides with highly conserved sequence. No such peptides have been found in the two invertebrate sequences. Sequences of the charge clusters are shown in Table I. The peptide sequences are highly conserved, especially within ErbB subfamilies, where they show block-like pattern. The value of T for these peptides vary from 5.9 to 7.2, showing the high statistical significance of these charge clusters.

The consensus motifs are given as logo in Figure 2. We see that the most conserved residues are those located at the beginning and end of the peptides with the mandatory presence of a charged amino acid at the first and two last positions of each peptide. The distance between the two peptides and their position relative to the TK domain are also very well conserved (Table I). The distance between P13$^+$ and the beginning of the TK domain is 22 to 24 amino acids for all ErbBs, whereas the distance between the end of the TK domain and P13$^-$ is 31 to 32 amino acids (39–40 for ErbB3 proteins). The distance between the two peptides is thus 321 to 329 amino acids.

Model of the Intracellular Domain of EGFR

The crystal structure of the EGF receptor (pdb code 1M14) was used as template to model the structure of the intracellular domain of EGFR. The root mean squared deviation (rmsd) between the template and the model obtained was 1.51 Å (280 atoms involved) without taking into account the added N-terminal region. This N-terminal region was suggested to be helical.[10] A homology modelling showed this peptide to be composed of two small helices linked by a short turn and ending by an extended loop. This structure is in agreement with the secondary structure prediction using AnTheProt program (data not shown). The N-terminal peptide P13$^+$ seems to be very well placed when connected to the transmembrane segment. Apart from the peptide bond between the C-terminal amino acid of the transmembrane region and the N-terminal Met of P13$^+$ peptide, we do not have any indication of a direct interaction between the JM and the transmembrane domains. Besides, the positively charged content of the P13$^+$ is not in favor of a possible interaction with the hydrophobic transmembrane region. It is worth noting that we constructed a model for only the EGFR intracellular domain homodimer. The sequence homology

with the ErbB family suggests homologous 3D structure models of homodimers

Charge Complementary between $P13^+$ and $P13^-$ Peptides

Two highly charged regions flanking the tyrosine kinase domains of all the vertebrates ErbBs were identified. These regions are well conserved among vertebrates (Table I, Fig. 1) and the lack of the positively charged region ($P13^+$) impairs TK dimerization and activity.[10] This suggests the importance of the $P13^+/P13^-$ interaction in the activity of the EGFR family members. Electrostatic calculations show strong positive and negative potentials flanking the kinase domain (Fig. 2). This led us to suggest the formation of a head-to-tail dimer model. According to this model, the $P13^-$ peptide of one monomer is interacting with the $P13^+$ of the second monomer. Basic and acidic residues are nicely interacting when $P13^-$ and $P13^+$ peptides were placed close to each other.

Model of the Dimer of the Intracellular Domain of EGFR

When placing $P13^-$ of one monomer close to $P13^+$ of the second monomer, two possible orientations of the kinase domains were generated. In the first orientation, the two catalytic cavities are buried in the dimer interface (face to face). This corresponds to an inactive dimer. Therefore, we focused on the orientation in which the catalytic cavities are exposed to the solvent (back to back) that corresponds to an active state of the dimer. The N-terminal ends of both monomers are facing in the same direction and are well positioned to interact with the transmembrane region, without any further modification of their secondary structures [Fig. 3(A)].

The $P13^-$ peptide is linked to the C-terminal region containing autophosphorylation sites (Tyr 992, Tyr 1086, and Tyr 1173) is well placed to be transphosphorylated by the kinase domain of the second monomer.

The Dimer Interface

We carried out calculations of the solvent accessible surface of the monomer alone and of the homodimer. The interaction surface of the homodimer is 2845 $Å^2$. This surface corresponds to two interfaces between the P13 peptides (2540 $Å^2$) and between two helical regions (305 $Å^2$). The $P13^-/P13^+$ interaction is mainly electrostatic [Fig. 3(C)]. These interactions involve 11 ion pairs between acidic residues belonging to $P13^-$ and basic residues belonging to $P13^+$ (Fig. 4). Nine additional hydrogen bonds are further stabilizing this interaction. The helical region (residues 937–948) is involving two electrostatic interactions as well as Van der Waals and hydrogen bonding interactions [Fig. 3(D)].

DISCUSSION

Two highly charged 13 amino acid peptides flanking the tyrosine kinase domain of the EGFR family receptors show a high degree of sequence and position conservation in vertebrates. These two peptides have already been reported as conserved within the human ErbB proteins and have been identified as calmodulin binding and calmodulin-like binding domains.[28] Landau et al.[11] gave the sequence of the negative peptide for the four human ErbB but extended the peptides to a length of 18 amino acids to accommodate for the ErbB3 peptide. All other three peptides have 13 residues in length. The peptide found by our program for ErbB3 starts 7 residues downstream to that of Landau et al.,[11] is shorter and has a higher statistical significance.

Conservation of the peptides go further to the genomic level; the alignment of genomic and protein sequences of ErbB genes in human showed that $P13^+$ and $P13^-$ are invariably encoded by exons 17 and 25, respectively (data not shown). On the other hand the conservation of the distance between the peptides and the TK domain might be important for the appropriate folding of the protein in such a way that interaction between peptides (within or among monomers) occurs. The positively charged peptide $P13^+$ was shown to play a role in EGFR intracellular dimerization and autophosphorylation.[10] We have previously suggested an electrostatic interaction of $P13^+$ with the C-terminal negatively charged P13 peptide ($P13^-$), belonging to a different monomer, to mediate intracellular dimerization. In the present work, in addition to the P13 peptides conservation we show new data strengthen our *in silico* model. The conservation of the P13 sequences among vertebrate ErbBs, including ErbBs2, supports the idea that these regions are involved in homodimerization and activity.

We constructed by sequence homology models of homodimers of the four ErbBs members and showed similar $P13^+/P13^-$ interactions (data not shown). In fact, intracellular dimerization of tyrosine kinase domains is more likely to be back to back, to obtain autophosphorylation. Moreover there is a network of electrostatic interactions

Fig. 3. Electrostatic analysis of two EGFR kinase monomers. This represents a projection of the electrostatic potential (ϕ) onto the molecular surface; $\phi \leq 74$ kT/e is dark red, $\phi = 0$ is white, and $\phi > 74$ kT/e is dark blue. (**A**) The two monomers are positioned back to back in the same orientations as in the homodimer model. The positively charged $P13^+$ and the negatively charged $P13^-$ are circled. This shows the geometric and electrostatic complementarity between the negative charge of $P13^-$ and the positive charge of $P13^+$. (**B**) View of the internal surfaces of the two monomers facing each other. Each monomer is rotated 90° backward.

Fig. 4. Network of ion pairs and hydrogen bonds across the interface of the EGFR kinase dimer. Kinase domains are displayed as ribbons and colored blue and yellow. Key residues involved in the dimer interface are displayed as sticks. (**A**) Front view of the EGFR kinase domain homodimeric complex showing the juxtamembrane N-terminal (Nt) region directed toward the membrane plan (dotted line). The C-terminal end (Ct) is also indicated. (**B**) Top view of the EGFR homodimeric complex [rotated 90° compared with (**A**)]. (**C**) A close view, in the same orientation as in (**A**), of the main electrostatic interactions between the $P13^+$ of one monomer (side chains depicted in blue) and the $P13^-$ of the second monomer (side chains depicted in red). Residues H648, R651, K652, and R653 from $P13^+$ are interacting with residues D984, E980/D982/E981, E980/D982, and D982/E991 from $P13^-$, respectively. (**D**) Close view, in the same orientation as in (**B**), of the second interaction interface between the two kinase monomers. These interactions are mainly polar and involve two α-helices. Residues R938, I941/ I942, and R949 from one monomer are interacting with residues R949/D950, R949, and I941/I942 from the second monomer, respectively.

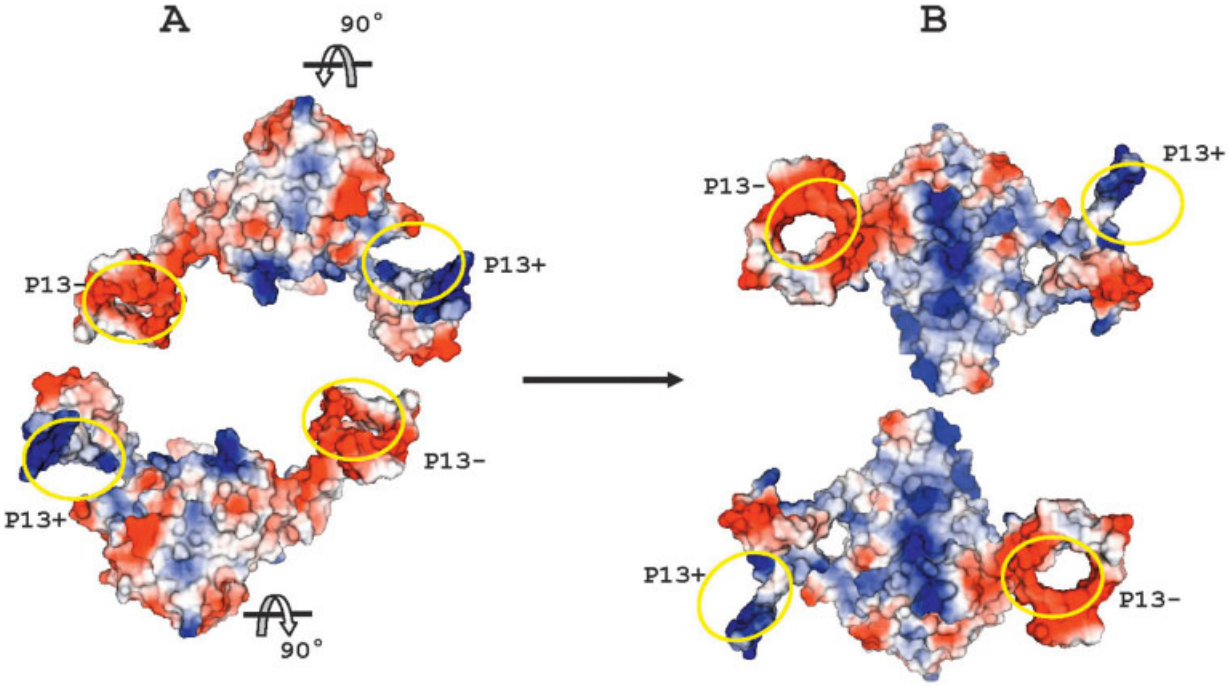

Figure 3.

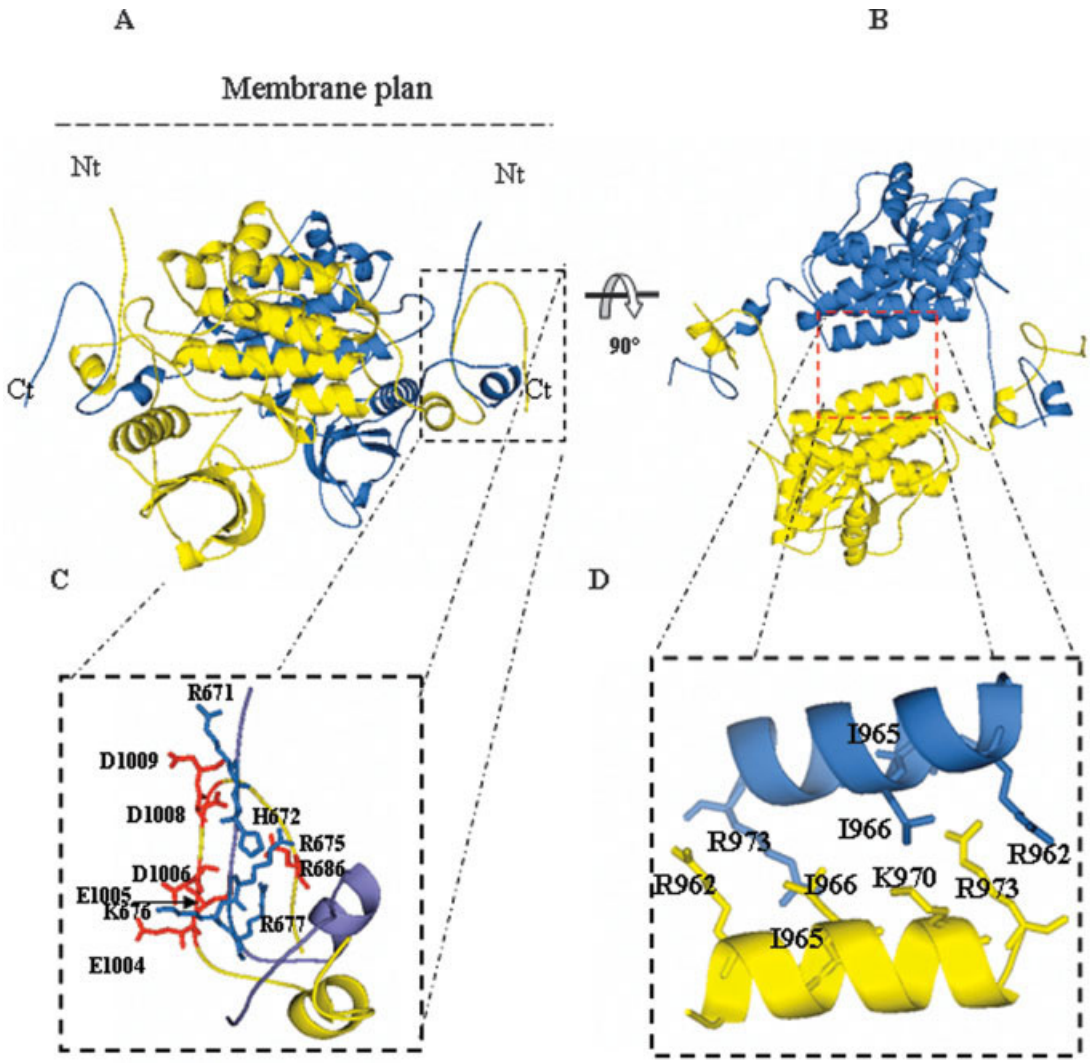

Figure 4.

between the two monomers within a helical region other than the P13 peptides. Landau et al.[11] have suggested an analogous dimerization model but they have missed the $P13^+$ and searched for positive charges interacting with $P13^-$ within the tyrosine kinase domain. Our model is based on our previous finding that the $P13^+$ deletion abrogates dimerization and autophosphorylation incapability of EGFR. Martin-Nieto and Villalobo[28] designated $P13^+$ and $P13^-$ calmodulin binding and calmodulin-like domains, respectively, without assigning their function in dimerization. These authors have shown that calmodulin binding to $P13^+$ is impaired by the introduction of a negative charge within this peptide via phosphorylation or Glu substitution of Thr654.[14] We have shown the same results by Biacore analysis, abolishing calmodulin binding upon deletion of $P13^+$[15]. Replacement of Thr654 or Thr669 *in silico* by a negative charge (phosphothreonine or Glu) show that $P13^+/P13^-$ interaction is disrupted only by Thr654-P or Glu654 (data not shown).

The model proposed by Landau et al.[11] is in agreement with our model with regards to the back-to-back orientation and the relative placement of the EGFR kinase domain to the autophosphorylation C-terminal domain. However, the $P13^+$ of the two EGFR monomers are in close contact, which can be an obstacle toward dimerization according to the model of Landau et al., because of the repulsive electrostatic effect. In its inactive state, the C-terminal end of the intracellular domain could interact with its own kinase domain via $P13^+/P13^-$ interaction (named CaM-BD and CaM-LD, respectively), as it was proposed previously.[28] To allow dimerization then transphosphorylation, the C-terminal end wrap around the juxtamembrane region containing $P13^+$ peptide, which comes in close contact with the $P13^-$ peptide. This can be considered as domain swapping phenomenon.

The model we propose corresponds to an active state of the EGFR kinase dimer. The C-terminal region containing transphosphorylation sites of one monomer is brought into the catalytic site of the second monomer. Upon phosphorylation, the C-terminal tail of one monomer has to move away from the catalytic site of the second monomer and can thus play its physiological role in signal transduction binding SH2-containing intracellular signalling proteins allowing their phosphorylation. This phosphorylation can be carried out by a catalytic domain belonging to the counterpart monomer or to the same monomer involved in the SH2-binding. In the last case, the C-terminal autophosphorylation site has to come close to the kinase domain. It is not excluded that the dimer interaction undergoes a large change after transphosphorylation. Recently, Bagossi et al.[21] have presented a back-to-back model of dimerization according to FRET data. Meanwhile their model does not explain how autophosphorylation occurs. The back-to-back picture proposed by Bagossi et al.[21] for the intracellular EGFR domain could correspond to the activated dimer upon transphosphorylation.

When constructing the EGFR kinase homodimer model by placing in close contact $P13^+$ of one monomer and $P13^-$ of the second monomer, we obtained two possible relative orientations of the two monomers: the face to face orientation (catalytic sites facing each other and buried by the dimer interface) and the back-to-back orientation (catalytic sites exposed to the solvent). The back-to-back orientation of the dimer is developed in this work, whereas the face to face orientation (data not shown) can correspond to an inactive state of the EGFR kinase domain. The LVI motif conserved among mammalian EGFR kinases was shown to play a critical role in the dimerization and activity of the EGFR. The heterodimer ErbB3/ErbB2 activation is disrupted upon deletion of this motif.[29] Interestingly, this motif occupies a key position allowing the $P13^+/P13^-$ interaction. Taken together, our data support a general model of intracellular receptor dimerization of the EGFR family based on $P13^+/P13^-$ interaction and explain the occurrence of autophosphorylation. Furthermore, the *in silico* model suggests a new therapeutic strategy to noncompetitively interfere with EGFR activity.

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