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Structural and thermodynamic insight into the process of "weak" dimerization of the ErbB4 transmembrane domain by solution NMR

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ABSTRACT

Specific helix–helix interactions between the single-span transmembrane domains of receptor tyrosine kinases are believed to be important for their lateral dimerization and signal transduction. Establishing structure–function relationships requires precise structural-dynamic information about this class of biologically significant bitopic membrane proteins. ErbB4 is a ubiquitously expressed member of the HER/ErbB family of growth factor receptor tyrosine kinases that is essential for the normal development of various adult and fetal human tissues and plays a role in the pathobiology of the organism. The dimerization of the ErbB4 transmembrane domain in membrane-mimicking lipid bicelles was investigated by solution NMR. In a bicellar DMPC/DHPC environment, the ErbB4 membrane-spanning α-helices $(651–678)_2$ form a right-handed parallel dimer through the N-terminal double GG4-like motif $A^{655}GxxGG^{660}$ in a fashion that is believed to permit proper kinase domain activation. During helix association, the dimer subunits undergo a structural adjustment (slight bending) with the formation of a network of inter-monomeric polar contacts. The quantitative analysis of the observed monomer–dimer equilibrium provides insights into the kinetics and thermodynamics of the folding process of the helical transmembrane domain in the model environment that may be directly relevant to the process that occurs in biological membranes. The lipid bicelles occupied by a single ErbB4 transmembrane domain behave as a true ("ideal") solvent for the peptide, while multiply occupied bicelles are more similar to the ordered lipid microdomains of cellular membranes and appear to provide substantial entropic enhancement of the weak helix–helix interactions, which may be critical for membrane protein activity.

1. Introduction

Despite their involvement in a large variety of processes in living organisms, membrane proteins are a challenging subject for modern structural biology and are still poorly explored. Most membrane proteins are composed of a number of transmembrane (TM) α-helices, which interact with each other and thus form a tertiary structure inside the lipid bilayer. The folding of helical segments into a TM domain as well as the functional mobility of certain helices often determine the functional characteristics of the full-size membrane protein. Thus, it is necessary to study the principles underlying TM helix–helix interactions.

Bitopic proteins, which have only a single α-helical TM domain separating the ecto- and cytoplasmic domains, are a class of biologically significant membrane proteins that provide a convenient means to study helix–helix interactions in the membrane. The regulation of the activity of these proteins is mostly associated with their lateral dimerization in cell membranes. Homo- and heterodimerization of bitopic proteins was once thought to primarily involve extracellular and cytoplasmic domains, but recent studies have made it increasingly clear that the TM domains are also critical for dimerization and modulate the biological function of the proteins [1,2]. Furthermore, some polymorphisms and mutations in the TM domains of bitopic proteins have been implicated in numerous human diseases [3]. Upon bitopic protein activation, which can be ligand-dependent or -independent, significant intra-molecular conformational transitions result in the rearrangement of the receptor domains and subsequent receptor dimerization or switching from one dimerization state to another, e.g., a ligand-dependent transition from the preformed inactive dimeric state to the active dimer, as has been proposed for the ErbB receptor tyrosine kinases [4–8].

Proteins from the epidermal growth factor receptor family, also known as HER or ErbB, which transmit biochemical signals across the plasma membrane, play an important role in cell growth and differentiation events in embryonic and adult tissues, whereas inappropriate ErbB activity is implicated in human diseases, including several cancers [9,10]. The single-span α-helical TM domains of ErbB can

Abbreviations: ErbB, epidermal growth factor receptor; TM, transmembrane; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; MHP, molecular hydrophobicity potential; DMPC, dimyristoylphosphatidylcholine; DHPC, dihexanoylphosphatidylcholine

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homo- and heterodimerize in the absence of the extracellular ligand-binding and cytoplasmic kinase domains [5,11]. Mutagenesis studies have shown that the ErbB TM domains interact through the so-called GG4 motifs [5,12,13], a small-X_3-small tetrad repeat of small side chain-containing residues separated by three other residues [14]. The TM segments of all four human ErbB receptors have at least one such motif, and all except ErbB3 have two motifs, which are located in the N- and C-terminal portions of the TM helices. The ErbB3 TM domain can also self-associate through a non-standard heptad repeat motif [15]. The switching of helices between two possible conformations of the dimeric TM domain is thought to be one of the key stages of ErbB signal transduction [5-8]. The measured dissociation constants of various TM helical homo- and heterodimers formed by different members of the ErbB family have permitted the establishment of the structure–energy relationship for "weak" TM helix–helix associations [11,16].

ErbB4 is a ubiquitously expressed member of the HER/ErbB family of receptor tyrosine kinases, which are essential for normal development of different adult and fetal human tissues [17]. Neuregulins and certain ligands of the EGF family, which trigger the activation of the ErbB4 receptor and subsequent signaling, induce a spectrum of cellular responses such as mitogenesis, differentiation, growth inhibition, and survival [17]. The ligand-induced translocation of ErbB4 and associated signaling molecules into lipid rafts is critical for signal transduction [18]. Additionally, ErbB4 is a unique member of the ErbB family because its TM domain undergoes proteolytic processing by the presenilin-dependent γ-secretase within the cell membrane to release the intracellular domain, a critical event that regulates multiple receptor signaling activities, including proapoptotic activity [19]. Recent clinical studies have revealed that tumoral expression of ErbB4 improves the overall survival of breast cancer patients, and thus ErbB4 signaling is believed to play a significant role in cancer pathobiology [20,21]. Therefore, a structural-thermodynamic investigation of the dimerization process of the ErbB4 TM domain would aid in the elucidation of the underlying mechanisms of TM helix–helix association and signal transduction and provide a basis for the molecular design of pharmaceutical compounds that affect the specific helix–helix interaction in the cell membrane for the appropriate control of receptor kinase activity. In the present work, we have studied the self-association of the ErbB4 TM domain; solution NMR spectroscopy was used to obtain its homodimeric spatial structure and describe the monomer–dimer transition under experimental conditions in membrane-mimicking lipid bicelles. The processes observed during homodimer formation reveal some aspects of the kinetics and thermodynamics of helix–helix association that may be relevant to the process that occurs in biological membranes, providing a better understanding of membrane protein function.

2. Material and methods

2.1. NMR sample preparation

The recombinant isotopically labeled TM fragment $ErbB4_{642–685}$, ErbB4tm, was produced in bacteria and purified as described in Ref. [22]. Three ErbB4tm samples were prepared: uniformly ^{15}N-labeled, $^{15}N,^{13}C$-labeled, and a 1:1 mixture of uniformly $^{15}N,^{13}C$-labeled and unlabeled proteins ("isotopic-heterodimer" sample). The ErbB4tm samples were incorporated into small dimyristoylphosphatidylcholine/dihexanoylphosphatidylcholine (DMPC/DHPC) lipid bicelles with an effective lipid/protein molar ratio (L/P) of 35 to 230 at a total lipid concentration of 50 or 110 mM in a buffer solution containing 20 mM deuterated sodium acetate, 0.15 μM sodium azide, 1 mM EDTA, and 5% D_2O or 99.9% D_2O (v/v), pH 5.0. The effective molar ratio q of long- and short-chain lipids in the bicelle was 0.27, assuming a free DHPC concentration of 7 mM in the bicellar suspension [23]. Unless otherwise specified, lipids with deuterated hydrophobic tails were used. Deuterated 1,2-di-[$^2H_{27}$]-myristoyl-*sn*-glycero-3-phosphocholine (d_{54}-DMPC) and 1,2-di-[$^2H_{11}$]-myristoyl-*sn*-glycero-3-phosphocholine (d_{22}-DHPC) were synthesized from *sn*-glycero-3-phosphocholine by acylation with anhydrides of d_{27}-myristic acid and d_{11}-hexanoic acid, respectively, as described in [24].

First, ErbB4tm was dissolved in 1:1 water/trifluoroethanol at room temperature, supplemented with DMPC/DHPC (also in water/trifluoroethanol mixture), lyophilized and dissolved in the water buffer. Before NMR studies, the samples were subjected to several freeze/thaw cycles to ensure a uniform protein distribution among the lipid bicelles. An Eppendorf vial containing the NMR sample was frozen in a liquid nitrogen bath and kept at room temperature for ~10 min. The 5 freeze/thaw cycles were usually performed with slightly sonication of the samples at each cycle to obtain a clear solution and good NMR spectra. To verify the validity of experimental conditions, circular dichroism (JASCO-810 spectropolarimeter, Jasco, Tokyo, Japan) measurements were performed. The circular dichroism spectra were virtually identical for ErbB4tm incorporated into DMPC/DHPC bicelles and DMPC unilamellar liposomes, revealing approximately 62% α-helical structure in both cases.

2.2. NMR chemical shift assignments and structure determination

NMR spectra were acquired at 313 K on a 700-MHz Avance spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a pulsed-field gradient triple-resonance cryoprobe. The ^1H, ^{13}C, and ^{15}N resonances of ErbB4tm were assigned with CARA software [25] with triple-resonance techniques [26,27] as described in Appendix A: Supplementary data, Section A.1.

The local effective rotation correlation times (τ_R) for the individual ^{15}NH groups of ErbB4tm were calculated with DASHA software [28] from the ratio of the ^{15}N longitudinal and transverse relaxation times obtained at L/P ≈ 120 as described in Ref. [29].

The spatial NMR structure of the ErbB4tm homodimer was calculated with the CYANA program [30] based on intra- and inter-monomeric NOE distance restraints derived from the analysis of three-dimensional ^{15}N- and ^{13}C-edited NOESY and $^{15}N,^{13}C$-F1-filtered/F3-edited-NOESY spectra [26,31] acquired for the ^{15}N- and $^{15}N,^{13}C$-labeled ErbB4tm and $^{15}N,^{13}C$-ErbB4tm/ErbB4tm "isotopic-heterodimer" samples (at L/P ≈ 50). The backbone dihedral angle restraints for φ and ψ were estimated basing on the assigned chemical shifts with the program PREDITOR [32]. The spatial structure calculation procedure is described in Appendix A: Supplementary data, Section A.1.

The hydrophobic properties of the α-helices in the ErbB4tm dimer were calculated with the molecular hydrophobicity potential (MHP) approach [33] as described in Appendix A: Supplementary data, Section A.2. The contact area between the dimer subunits was calculated with the DSSP program [34] as the difference between the accessible surface areas of ErbB4tm residues in the monomer and dimer. The ErbB4tm dimer structures were visualized with the MOLMOL program [35].

2.3. NMR measurements of kinetics and thermodynamics

The dependence of the dimer association constants of ErbB4tm on the lipid concentration was treated according to the protein–bicelle complex model (similar to the protein–micelle model proposed in [36–39]), assuming that the apparent free energy of association:

$$\Delta G_{app} = \Delta G_0 + \gamma RT \ln[Lip]_B, \quad (1)$$

where ΔG_0 is the standard free energy of association, γ is a measure of the ideality of a bicellar system (formally the reaction order on the lipid) [15,38], R is the universal gas constant, T is the temperature in K, and $[Lip]_B$ is the concentration of the lipid in bicellar form, which corresponds to the overall lipid concentration excluding the free