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The Sigma-1 Receptor as a Pharmacologic Chaperone: Energetics

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Abstract

Initially thought to be an opioid receptor subtype, Sigma-1 receptors (S1R) are now known to be unique proteins that have chaperone-like properties. As such, they play critical roles in cellular signaling, homeostasis, and cell survival. These roles offer significant insight for understanding homeostasis of normal physiologic processes, and the pathophysiologic consequences of disruption of normal function. Because of the broad nature of chaperone action, S1R agonists and antagonists represent potential drug discovery goals for the pharmacotherapeutic treatment of a variety of disorders that result from dysfunctional proteins. The present study summarizes the S1R as a pharmacologic chaperone crucial for protein folding and cellular homeostasis. Through literature review and thermodynamic analysis, it explores how S1R stabilizes target proteins, influencing neuroprotection and potential drug therapies. The binding of chaperones to target proteins is thermodynamically favorable, offering insights into treating diseases linked to protein misfolding.

Keywords

Chaperone, Sigma-1 Receptor, Energetics, Thermodynamics, Isothermal Titration Microcalorimetry

1. Introduction

The Sigma-1 receptor (S1R) is a relatively small protein consisting of 223 amino acids, which in humans is encoded by the *SIGMAR1* gene located on chromosome 9. The receptor is modeled as having two transmembrane domains with *N*- and *C*-terminals both oriented inwards towards the cytosol. This is in distinction to traditional G protein-coupled receptors (GPCRs), which are members of the

largest family of human membrane receptors and a major target of drug therapies, so S1R does not share homology with GPCRs or other receptor families, explaining the unique nature of S1R [1]. A crystal structure was published in 2016 [2], and further crystallographic evidence shows that the receptor's binding domain can accommodate a variety of endogenous and exogenous ligands; this diversity contributes to functional versatility. Additionally, S1R can form oligomers, which have been proposed to be important for chaperone activity [2].

S1R functions as molecular chaperones at the endoplasmic reticulum, particularly at the mitochondrion-associated ER membrane. They modulate Ca^{2+} signaling between the ER and mitochondria, thereby influencing cellular bioenergetics and stress responses [1]. The chaperone activity of S1R is essential for stabilizing target proteins and maintaining proper protein folding, and cellular homeostasis. For example [1], S1R is typically in an inactive state bound to the endoplasmic reticulum (ER) chaperone BiP (Binding Immunoglobulin Protein), also known as GRP78 (Glucose-Regulated Protein 78), which is an important endoplasmic reticulum (ER) chaperone. BiP/GRP78 is a molecular chaperone that belongs to the heat shock protein 70 (Hsp70) family. It is predominantly located in the lumen of the ER and assists in the proper folding of newly synthesized polypeptides entering the ER. It binds to nascent and misfolded proteins, preventing their aggregation and facilitating correct folding. Misfolded or unassembled proteins are retained in the ER and eventually targeted for degradation via the ER-associated degradation (ERAD) pathway. BiP also contributes to the regulation of Ca^{2+} levels within the ER, which is critical for various cellular processes including protein folding. Dysfunction of BiP can lead to various diseases, particularly those associated with protein misfolding and ER stress. S1R are located in the ER, particularly at the mitochondrion-associated ER membrane. Under non-stress conditions, S1R are bound to BiP. Ligand binding to S1R induces a conformational change, leading to their dissociation from BiP and activation. This activation triggers a cascade of downstream effects resulting in the modulation of Ca^{2+} signaling, protein folding, and cellular stress responses that enhance the chaperone's ability to stabilize other proteins and modulate cellular stress responses.

At the cellular level, S1R modulates various ion channels and receptors, including voltage-gated Ca^{2+} channels, *N*-methyl-D-aspartate (NMDA) receptors, K^+ channels, and a range of signaling molecules, including kinases, phosphatases, and lipid signaling molecules. Through these interactions, S1R influences intracellular calcium levels, neuronal excitability, and synaptic plasticity [3]. S1R is strategically located at the MAM, a site of close contact between the ER and mitochondria. This location allows S1R to facilitate the transfer of Ca^{2+} from the ER to the mitochondria by interacting with S1R ligands to influence Ca^{2+} signaling between the ER and mitochondria. Upon activation by ligands, S1R interacts with the inositol 1,4,5-trisphosphate receptor (IP3R) and the voltage-dependent anion channel (VDAC) on the ER membrane, facilitating Ca^{2+} transfer to the mitochondria. This process is critical for maintaining cellular bioenergetics and

preventing Ca^{2+} overload, which can trigger apoptosis [1]. This modulation affects processes such as pain perception, memory formation, and neuroprotection. S1R can also be activated by a variety of exogenous ligands of diverse chemical classes, such as benzomorphans, antipsychotics, and antidepressants [4]. Ligand binding induces conformational changes in S1R, which modulates their interaction with their target proteins and ion channels. This provides small-molecule drug development opportunities.

Given their role in modulating calcium homeostasis and proper protein folding, S1Rs are involved in neuroprotective mechanisms. Activation of S1R has been shown to protect neurons from apoptosis and excitotoxicity, making them potential therapeutic targets for neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases [5]. Due to their influence on neurotransmitter systems and synaptic plasticity, S1R agonists have potential utility for treating conditions such as depression, schizophrenia, and anxiety [1]. S1R is also involved in pain perception and modulation. S1R antagonists reduce neuronal excitability and pain signaling [6], and correspondingly, have been shown to reduce neuropathic pain in preclinical models, suggesting their potential use in clinical pain management [7].

The concept of molecular chaperones [8] is relatively new, but potential applications to drug discovery and development [9] to address a variety of medical disorders are already a burgeoning field [10]. The purpose of the present communication is to summarize the available information that answers the question "Why do chaperones do what they do". The answer, as with all biochemical processes, has to do with thermodynamics (change in free energy). We aim to present a reader-friendly introduction to the energetics of the processes involved in chaperone functioning and provide insight into the medical benefits that chaperones can provide, using S1R as an example.

2. Methods

A literature search was conducted using databases such as PubMed, Google Scholar, Ovid MEDLINE®, etc. for recent publications in the English language on the topic of S1R and the energetics of chaperone function. MeSH search terms such as "sigma-1 receptor", "chaperone", "energetics", and "thermodynamics", and various combinations of them were used. In addition to the content of the identified publications, citations within them were searched. Additionally, review articles on each of the broad topics were utilized to supplement background information. The material was reviewed for applicability to the specific target topic, namely, identification of publications that provided or discussed the role of chaperones, and the energetics (thermodynamics) of the processes, particularly as it informed an understanding of the underlying processes, and how, in turn, that relates to potential clinical utility. Due to the limited intersection of thermodynamic studies in pharmacology, sigma receptors, and chaperones, a limited number of studies were identified. Following the initial searches, we continued to try to find publications not already identified, but no additional ones

were found. So we believe that all were identified, and none were excluded. Any missed publication was unintentional and regretted.

3. Results

The S1R functions as part of a coordinated cellular "molecular chaperone" activity [11]. A "molecular chaperone" [12] assists in the proper folding, placement (e.g., translocation), functioning, and disposition (e.g., proteolysis) of large proteins or macromolecular complexes [13]. The specific activity can differ based on the physiological process and location. A subset of molecular chaperones is "pharmacological chaperones", which are small molecules that perform a chaperone function, *i.e.*, establishment and stabilization of proper functional conformation, transport to the cellular active site, and eventual disposition, the totality of which is termed "protein homeostasis" or "proteostasis" [14]. Aberrations in proteostasis can lead to serious disease conditions. Hence, pharmacological chaperones that stabilize the proper fold of proteins or protect them from abnormal degradation represent important potential therapies [15].

Several studies have helped elucidate energy and kinetic frameworks for understanding the function of chaperones. A sampling of these is summarized below.

Panse *et al.* (2000) [16] view the positive actions of chaperones on proteins (*i.e.*, assisting with their proper folding, assembly, and transport) as favorably counteractive to nonproductive processes that lead to problems of inappropriate protein aggregation. They measured the thermodynamics of the binding of unfolded polypeptides to the associated chaperone using isothermal titration calorimetry (ITC) [17] and fluorescence spectroscopy. ITC measures the exchange of heat on binding as a function of time, allowing direct or fitted determination of change in enthalpy (ΔH°), entropy (ΔS°), free energy (ΔG°), and heat capacity (ΔC_{p}). The results revealed valuable insight into the binding characteristics of the chaperone-substrate interaction.

Kriechbaumer *et al.* (2011) [18] used the optical technique of spectroscopic ellipsometry (a technique that measures the change in polarization of light reflecting or transmitting from a material surface) to study the role that different chaperone types and isoforms (members of a set of highly similar proteins that originate from a single gene or gene family and perform the same general biochemical function) play in the targeting specificity of chaperone-receptor interactions. They modeled the chaperone-receptor interaction between chaperone (C) to single binding sites on the surface according to $\mathrm{d}n/\mathrm{d}t = k_{\mathrm{a}}C\left(N-n\right)-k_{\mathrm{d}}n$, where k_{a} and k_{d} are the adsorption and desorption rate constants, respectively, N is the total receptor concentration, and n is the concentration of chaperone-bound receptor. This proved to be suitable for highly specific interactions between chaperone and surface-immobilized receptors (protein receptors that are immobilized on a biosensor surface to allow monitoring of their interaction with a second molecule in solution).

Hartl *et al.* (2011) [19] envisioned a free-energy landscape (funnel-shaped energy well) of competing processes of protein folding and protein aggregation. The model incorporates intra- and inter-molecular contacts and transitions from states of native conformation, folding intermediates, partially-folded conformations, and amorphous and toxic aggregates. Certain states can be conditionally stable although not at the lowest energy level. Chaperones can assist trapped conformations traverse free-energy hurdles to transition to another (lower free-energy) state. Each of the steps can be characterized by equilibrium reactions and forward and reverse rate constants (e.g., unfolded ⇔ partially-folded ⇔ native-conformation) or irreversible (large decrease in free-energy) reactions leading to aggregation.

Taipale *et al.* (2013) [20] describe the use of chaperones as "thermodynamic sensors" (the use of measurement of the thermal stability of protein-protein interaction in vivo) of drug-target interactions for drug discovery. Basically, the detection of the binding of small-molecule test compounds that shift the equilibrium to the properly folded shape of the target protein (thermodynamic stabilization) [21] results in decreased interaction with a chaperone (whose assistance is no longer needed). The concentration of free (non-bound) chaperone provides a measure of the desired activity of the test compound.

Wang *et al.* (2014) [22] provide a valuable review of protein folding dynamics and the energy transitions associated with the processes. Proteins consist of chains of a large number of amino acids, so a large number of possible conformations are possible, making folding to the proper conformation complex critical to the attainment of effective functionality [23]. The process requires the sequential and coordinated influence of many weak, non-covalent (e.g., van der Waals) interactions [24]. For soluble proteins, hydrophobic interactions are particularly important. In this regard, it is helpful to visualize the processes of proper folding and maladaptive folding as occurring along funnel-shaped potential energy surfaces (**Figure 1**).

The existence of energy wells of partial, but inappropriate, stability allows for entrapment of proteins in suboptimal, or even deleterious, conformations. Or, more technically, non-optimal or intermediate states can become trapped in energetically favorable, but not physiologically desirable or even deleterious (e.g., aggregation), states (energy wells). The relatively high temperature of the body promotes sampling of the energy landscape by molecules, but chaperones provide an efficient mechanism for the attainment of the optimal conformation. The authors envision a "proteostasis network" that maintains the integrity of the proteome to achieve optimal functioning.

Hingorani *et al.* (2016) [25] propose that pharmacologic chaperones improve the folding of destabilized proteins by "biasing" (directed to favor a particular biochemical path over another) the kinetic partitioning between desirable folding vs. undesirable alternatives (e.g., aggregation or degradation). In the model, protein can reversibly fold to, or unfold from, the desired state with forward and reverse rate constants k_f and k_u, respectively, or irreversibly aggregate or degrade

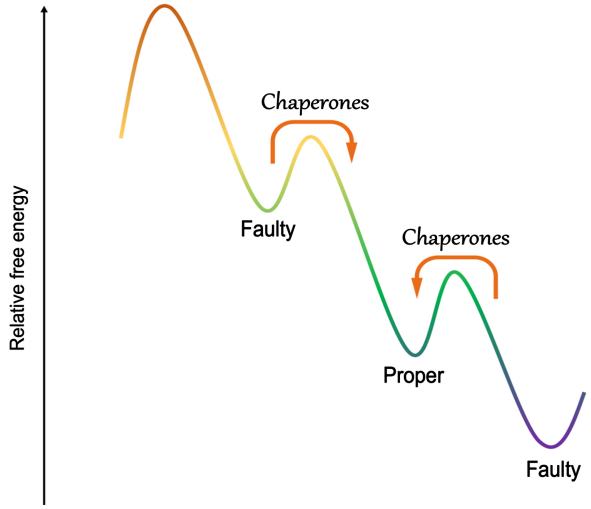

Figure 1. Based on [19] and [22], a model depicting the free-energy surface that proteins "explore" due to the thermal influence of body temperature. Ideally, inter- and intra-molecular interactions occur in the coordinated and sequential manner needed to attain the conformation needed for proper physiological functioning. However, intermediates, or partially or improperly-folded forms can get "trapped" in free-energy wells other than the optimal. Chaperones assist the protein reach and maintaining the proper free-energy well (note: this is a structural assist, the chaperone does not provide a template, or catalyze a chemical reaction).

with rate constant k_{agg} and k_{deg}, such that available protein (F_r) in the presence of chaperone attains $F_r = 1 - 1/(1+\beta)$, where $\beta = k_f/(k_{agg} + k_{deg})$. They thus suggest that at high chaperone concentrations, the balance between active and inactive forms of the protein depends on folding kinetics rather than folding thermodynamics.

Bagdany *et al.* (2017) [26] provide evidence that chaperones provide both thermodynamic and kinetic stabilization of a mutant integral plasma membrane protein (ΔF508-CFTR). Based on their and others' results, the authors postulate that the stability of plasma membrane proteins is influenced by molecular chaperone "networks" on the cell surface. That is, chaperones coordinate with "co-chaperones" and other factors that contribute to the attainment and maintenance of proper protein folding. They discuss the influence of the proteostasis network construct on the phenotypic presentation of protein conformational diseases.

Goloubinoff *et al.* (2018) [27] examined the questions of why some molecular chaperones require ATP hydrolysis to assist in favoring the native folding and avoid aggregation, of their substrates, and what the consequences of the energy contributed by the ATPase cycle to the processes. They used biochemical assays and physical modeling to show that for at least one system chaperones use part of the energy generated from the hydrolysis of ATP to stabilize the native state of their substrates even under thermodynamically unstable conditions, then upon the exhaustion of ATP, the metastable native chaperone products spontaneously revert to their equilibrium states, consistent with thermodynamics.

Fauvet *et al.* (2021) [28] present a fascinating viewpoint that also unites the

thermodynamic, kinetic, and other analyses of chaperone action. They opine that: molecular chaperones' actively counteracting the misfolding of proteins is dictated by thermodynamic considerations, and that the energy from ATP hydrolysis can unfold stable misfolded proteins and allow them to collapse into the native conformation under otherwise unfavorable conditions. But this proteostasis network presents a "thermodynamic dilemma", namely, the greater energy cost of degrading and replacing a protein vs the cost of its chaperone-mediated repair. It would seem that an easily repaired protein should be processed by a chaperone using energy from ATP, whereas it would be energetically wasteful to repair overly-compromised proteins, and their degradation and replacement would be energetically preferable. They colorfully conclude with the analogy "… proteins are like used cars: there always comes a point where the cost of cumulative repairs exceeds that of buying a new car".

Isothermal Titration Microcalorimetry (ITC)

Lest it be thought that thermodynamics is too esoteric to have practical application to studying pharmacotherapeutic applications, there are many instructive examples of such applications [29]. Likewise, the measurement of thermodynamic quantities of biochemical reactions is now standard with readily available equipment. A clear explanation of one of these procedures, the aptly-named isothermal titration microcalorimetry, is provided in [29] by Haq *et al.* (Chapter 5) quoted here: "To gain further insight into [a biochemical] interaction the temperature dependence of the equilibrium must be examined; the temperature dependence of the free energy of a process is reflected in the enthalpy change for that process. This parameter is best obtained by direct measurement using isothermal titration calorimetry (ITC) … ITC measures the energetics of biochemical/molecular interactions at a constant temperature [isothermal]. This methodology relies upon a differential cell system within the calorimeter [microcalorimeter because of the small amount of sample needed] assembly. The reference cell contains only water or buffer and it may be sealed within the instrument. The sample cell contains the macromolecule or ligand as well as a stirring device. Injection [titration] of the second component into the sample cell produces heat effects that are due to injection and dilution of ligand, dilution of the macromolecule and the heat of the interaction. The amount of power that must be applied to actively compensate for the heat produced in the sample cell after an injection is measured directly. The applied thermal power as a function of time that is required to return the calorimeter to its steady state, following an injection, is directly proportional to the heat of reaction. A typical binding isotherm produced from an ITC experiment is defined in terms of the amount of heat that is released (exothermic) or absorbed (endothermic) as a function of the total concentration of the ligand. Enthalpy values are determined directly and binding constants and stoichiometry are estimated by non-linear least-squares analysis using an appropriate model. ITC is also a convenient method for determining the heat capacity change associated with a binding interaction. This is achieved

simply by determining the binding enthalpy over a range of temperatures. The slope of a plot of enthalpy versus temperature will equal the heat capacity change." See Figure 2 for an example of application to a chaperone, from Panse *et al.* [16].

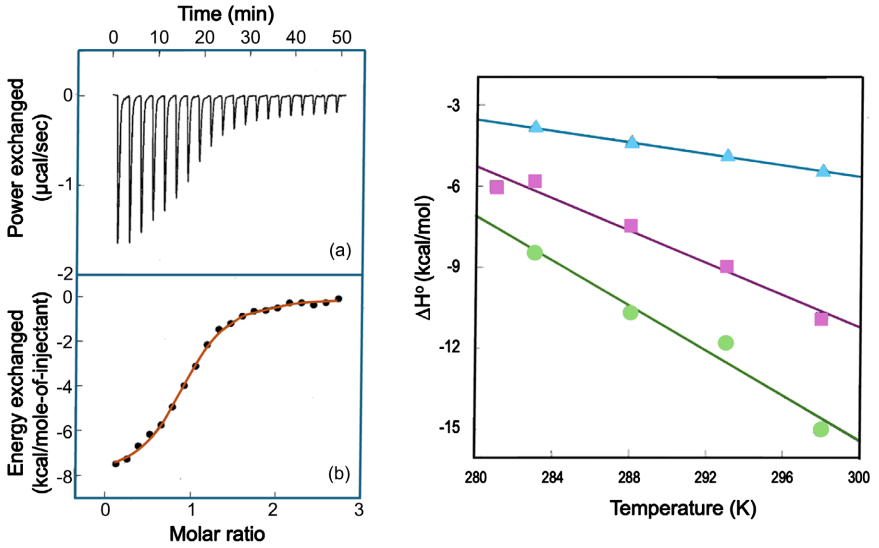

Figure 2. *Left:* Exchange of heat on binding of SecB (a chaperone) to CAM-BPTI (target protein): (a) Representative isothermal calorimetric titration of SecB into CAM-BPTI. (b) Exothermic heats exchanged per mole of injectant as a function of the ratio of SecB to CAM-BPTI. The data were fitted to a single site-binding model to obtain ΔH° and K of the interaction. *Right:* Temperature-dependence of the calorimetric enthalpy of the binding reaction between SecB chaperone and targets. The lines represent fits of the data to $DH(T) = DH^{\circ}(T^{\circ}) + Dc_{p}(T - T^{\circ})$. From Panse *et al.* [16] with permission.

4. Conclusions

The Sigma-1 receptor functions as a pharmacologic chaperone to help maintain proper protein folding and prevent diseases that are caused by misfolded proteins. The energetics (thermodynamics) of the binding of chaperone to target protein explains the advantage of having chaperones monitor protein folding, since the free energy of the chaperone complex is lower than the unfolded or improperly-folded states of the target protein. Diseases that have been implicated in improper protein folding disorders includes neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and Amyotrophic Lateral Sclerosis (ALS); prion diseases such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy (mad cow disease); systemic amyloidosis diseases such as primary, secondary, and hereditary amyloidosis; cystic fibrosis; inherited myopathies such as Duchenne muscular dystrophy and myotonic dystrophy; retinitis pigmentosa; type-2 diabetes; and familial mediterranean fever, among others. The fact that small molecule ligands can bind S1R and modify their chaperone activity suggests that S1R-directed drugs might offer therapeutic treatment op-

tions for these disorders.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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