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High free energy of lipid/protein interaction in biological membranes

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Abstract The free energy of lipid/protein interaction in biological membranes is still unknown although extensive partitioning and modelling studies have revealed many partial energetic increments. Multiple site binding kinetics are now applied to four well-studied functional membrane proteins, and mean free energy values (±S.D.) of −4.23±0.49 kcal/mol for single lipid binding sites and of −89.7±35.4 kcal/mol for complete lipid substitution are obtained. These high free energy values point to an important bioenergetic role of lipid/protein interaction in membrane functions.

Key words: Lipid/protein interaction; Membrane energetics; Na^+K^+-ATPase; Ca^{2+}-ATPase; Cytochrome oxidase; Nicotinic acetylcholine receptor

1. Introduction

The thermodynamics of lipid/protein interactions are ill understood in spite of their central role in membrane assembly and function. Partitioning and modelling studies have identified many partial free energy increments of membrane protein folding and assembly into lipid phases. These increments showed mutual compensation so that the overall lipid solvation free energy of functional membrane proteins is considered to be low [1–3]. A pioneering early study had calculated −15 kcal/mol for the transfer of a single hydrophobic 20 amino acid α-helix into a bilayer [4]. However, later re-calculations came to only −4 kcal/mol [1]. Transmembrane proteins tend to form helix bundles with the formation of protein/protein and lipid/lipid at the expense of lipid/protein interactions [2], thereby again documenting the mutual compensation of free energy increments [1,3]. A surprisingly high free energy value of −6.4 kcal/mol for lipid/protein interaction at a single binding site has recently been derived by kinetic analysis of enhanced agonist binding to the nicotinic acetylcholine receptor [5]. General anaesthetics and organic solvents appeared to act by competitive displacement of protein-bound lipids [5]. The present article applies Adair-type multiple binding site kinetics and electron-spin resonance spectroscopic binding data to four well-known functional membrane proteins. High free energy values are again obtained, and it is concluded that future mechanistic and bioenergetic studies of functional membrane proteins should take lipid/protein interaction free energies into account.

2. Thermodynamic framework

The current low estimates of overall lipid solvation free energy were largely derived from partitioning and modelling studies [1–3]. In contrast, the present study is based on a binding site approach [6] that utilizes the fixed stoichiometries of 23 to 60 lipid molecules per protein molecule that have been determined by electron-spin resonance spectroscopy [7,8]. The previous kinetic formalism [6] describes lipid solvation at a single lipid binding site as follows:

$$K_{\text{solv. }(0\rightarrow 1)}=\frac{[\mathrm{EL}_1]}{[\mathrm{E}]}=n\left(\frac{[\mathrm{L}]}{K_1}\right) \quad (1)$$

Here, [E] refers to the concentration of unsubstituted enzyme, $[EL_1]$ to the concentration of enzyme with a single lipid ligand and [L] to total lipid concentration. K_1 is the uniform microscopic lipid dissociation binding constant for each independent binding site, and n is the total number of lipid binding sites. The free energy of lipid solvation at a single binding site is obtained from:

$$\Delta G^0_{\text{solv. }(0\rightarrow 1)} = -1363\cdot \log K_{\text{solv. }(0\rightarrow 1)} \text{ (at 25°C)} \quad (2)$$

When considering the lipid solvation free energy of the completely lipid-substituted protein, EL_n, the previous kinetic formalism [6] leads to:

$$K_{\text{solv. }(0\rightarrow 1)} = \frac{[\mathrm{EL}_n]}{[\mathrm{E}]} = \left(\frac{[\mathrm{L}]}{K_1}\right)^n \quad (3)$$

The free energy of overall lipid solvation is obtained from:

$$\Delta G^0_{\text{solv. }(0\rightarrow n)} = -1363\cdot \log K_{\text{solv. }(0\rightarrow n)} \text{ (at 25°C)} \quad (4)$$

The microscopic lipid dissociation binding constants employed have previously been derived by non-linear regression analysis of lipid activation curves, as first reported in detail for Na^+K^+-ATPase [9]. The same method was subsequently applied to published lipid activation curves of Ca^{2+}-ATPase from muscle sarcoplasmic reticulum and cytochrome oxidase from inner mitochondrial membranes [10,11]. In addition, the same methods were more recently used to obtain the microscopic lipid dissociation binding constant for the nicotinic acetylcholine receptor of *Torpedo* electroplax membranes [5]. In analogy to extensive previous work [5,12,13] the concen-

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tration unit used here is the number of lipid molecules per protein molecule.

3. Calculation of free energy values

Eqs. 1–4 were applied to four well-studied functional membrane proteins, as summarized in Table 1. The respective protein, its source, the lipid/protein ratio of its native membrane, the number n and the microscopic lipid dissociation binding constant, K_1, are listed. The latter had been obtained by non-linear regression analysis of lipid activation curves. In order to apply Eqs. 1 and 3, [L] needs to be known. The lipid/protein ratios of Table 1, the known protein molecular weight values [7,8] and the assumption that the purified membranes contained only these catalytic proteins led to concentration values of 63, 75, 140 and 200 lipid molecules per molecule of Ca^{2+}-ATPase, Na^+K^+-ATPase, cytochrome oxidase and the nicotinic acetylcholine receptor, respectively. However, the purified membranes also contain other proteins, so that Eqs. 1–4 were used to generate values of $\Delta G^0_{\text{solv. }(0\rightarrow 1)}$ and $\Delta G^0_{\text{solv. }(0\rightarrow n)}$ for a representative lipid concentration matrix of 50, 100, 200 and 300 lipid molecules per protein molecule (Table 1).

4. Free energy values for a single binding site

Considering first the free energy values for a single binding site, Table 1 leads to a mean value ($\pm$S.D.) of -4.23 ± 0.49 kcal/mol. This value is significantly lower than our previous estimate of -6.4 kcal/mol [5] and our preliminary estimate of -7.1 kcal/mol [11]. The previous estimates were derived from the competitive displacement of lipids from preformed lipid/protein complexes of the nicotinic acetylcholine receptor [5] and of synaptosomal Ca^{2+}-ATPase [11], respectively. In contrast, the present estimates were derived from functional lipid titrations of delipidated protein preparations. In addition to the different standard states considered, the previous and the present studies are limited by the rather crude assumptions made for the kinetic analyses. In any case, lipid solvation free energy at a single site appears to be much higher than apparent from the extensive previous literature that is dominated by the mutual compensation of free energy increments [1–3].

5. Free energy values for complete lipid substitution

A mean value ($\pm$S.D.) of $\Delta G^0_{\text{solv. }(0\rightarrow n)}$ of -89.7 ± 35.4 kcal/mol for complete lipid-substitution was obtained from Table 1. A similar free energy value (-86.7 kcal/mol) is obtained for a hypothetical membrane protein with the mean values of K_1, n and [L] of the four proteins of Table 1. These values indicate that the lipid/protein complexes of biological membranes can be considered as reservoirs of high free energy. Each free energy value obtained was significantly lower than the product of solvation free energy at a single binding site and the total number of binding sites. For example, overall lipid solvation free energy of the nicotinic acetylcholine receptor at its known value of [L] = 200 lipid molecules per protein molecule [7,14] was -94.2 kcal/mol. This is only 52% of the value expected from $n = 40$ and the free energy value of lipid solvation at a single site (-4.54 kcal/mol). A similar discrepancy existed for the other membrane proteins of Table 1.

In order to further examine the discrepancy, hyperbolic hemoglobin, i.e. a hemoglobin analogue containing four identical non-interacting binding sites, was considered. Hyperbolic hemoglobin has previously been analysed in order to derive the interaction free energy values of hemoglobin [15] and to verify the occurrence of positive kinetic cooperativity in the absence of allosteric interaction [16]. When the typical oxygen partial pressure of arterial blood (100 Torr) and the known 'hyperbolic' microscopic dissociation binding constant of 8.8 Torr [15] are inserted as [L] and K_1 into Eqs. 2 and 4, free

Table 1
Calculation of lipid solvation free energies

Enzyme, source, lipid/protein ratio (by weight)[a]	n[b]	K_1-value[c]	$\Delta G^0_{\text{solv.}}$ (kcal/mol)[e] lipid concentration, [L][f]				
			case[d]	50	100	200	300
Na^+K^+-ATPase, dogfish salt-gland microsomes; 0.36 [21]	60	5.59 [9,10]	$(0\rightarrow 1)$	−3.72	−4.13	−4.54	−4.78
			$(0\rightarrow n)$	−77.8	−102.4	−127.1	−141.5
Ca^{2+}-ATPase, muscle sarcoplasmic reticulum microsomes; 0.68 [7]	23	4.75 [10]	$(0\rightarrow 1)$	−3.25	−3.66	−4.07	−4.31
			$(0\rightarrow n)$	−32.1	−41.5	−50.9	−56.4
Cytochrome oxidase, inner mitochondrial membranes, 0.32 [7,8]	55	3.60 [10]	$(0\rightarrow 1)$	−3.93	−4.34	−4.75	−4.99
			$(0\rightarrow n)$	−85.7	−108.2	−130.8	−144.0
Nicotinic acetylcholine receptor, *Torpedo* electroplax membranes, 0.7 [7,14]	40	3.75 [5]	$(0\rightarrow 1)$	−3.72	−4.12	−4.54	−4.78
			$(0\rightarrow n)$	−61.3	−77.7	−94.2	−103.8

[a]References for the indicated membrane lipid/protein ratios (by weight) are given. A uniform lipid molecular weight value of 800 Da was used for calculation.
[b]Number of lipid solvation sites as determined by electron-spin resonance spectroscopy. Values adopted from [7,8].
[c]Microscopic lipid dissociation binding constants adopted from the indicated references. In analogy to previous work [5,12,13] the concentration unit used is number of lipid molecules per protein molecule.
[d]The two cases considered were lipid solvation at a single site, symbolised by $(0\rightarrow 1)$, and complete lipid-substitution of membrane proteins, symbolised by $(0\rightarrow n)$.
[e]Lipid solvation free energy values obtained with Eqs. 2 and 4 of text. Lipid concentration-values of 50, 100, 200 and 300 lipid molecules per protein molecule were used for calculation.
[f]Dimension: lipid molecules per protein molecule.

binding energies of $\Delta G^0_{\text{solv. }(0\rightarrow 1)} = -2.26$ kcal/mol and $\Delta G^0_{\text{solv. }(0\rightarrow 4)} = -5.75$ kcal/mol are obtained. A free energy ratio of 2.55 rather than the intuitively expected ratio of 4.0 is obtained. This confirms the result of Table 1. Remarkably, this calculation reveals that the free energy of oxygen binding at a single site is close to the known overall free energy of allosteric interaction between the first and the fourth binding site of hemoglobin A (−2.08 kcal/mol, [15]).

6. Functional aspects

Recent years have seen enormous progress in extending the knowledge of high-resolution structures of membrane proteins to detailed analyses of assembly and mechanism of bacteriorhodopsin [17], cytochrome oxidase [18], H^+-ATPases [19] and further functional membrane proteins. Conformational changes always played a central role, but changes in lipid/protein interactions were generally not discussed although the membrane proteins are lipid-embedded. Only recently, a reorganization of free and protein-bound phospholipids was demonstrated in response to the light-induced conformational change of rhodopsin [20]. The high free energy values obtained in the present study suggest that changes in lipid/protein interaction should be considered in future mechanistic and bioenergetic studies of functional membrane proteins. In principle, the present high free-energy values should consist of partial energetic increments of the type thoroughly studied in the literature [1–3]. However, the presently available data base appears insufficient to reproduce the high overall free energy values from energetic increments.

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