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Contribution of Electrostatics in RNA-Protein binding

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Abstract

Proteins interact with nucleic acids to control gene regulation and expression. In order to understand these control processes in atomic detail, the structural and energetic basis for the specificity and stability of binding must be elucidated. Various protein-DNA complexes were studied in detail previously. Protein-tRNA interactions are also very specific but have not been studied computationally. This chapter reports detailed study of electrostatic interactions between $tRNA^{gln}$ and GlnRS. At physiological pH, tRNA has regular (-) ve charges along their chains, which would produce electric field around them. Salt effect on tRNA binding to Aminoacyl tRNA synthetase has been computationally studied extensively with Poission Boltzmann equation. Values of various components of free energy term contributing to the total salt dependent electrostatic free energy are calculated e.g., coulombic energy, reaction field energy, ionic contribution, osmotic pressure term and rho-phi term. Finally total electrostatic energy is calculated at different salt concentrations. The log-log plot of association constants versus KCl concentration shows monotonic decrease in affinity with increasing KCl concentration. The slope of the straight line corresponds to a cation release stoichiometry of 1.7 for this complex. It is found here that tRNA has weaker electric field around it and the decrease in the association constant with increasing monovalent ion concentration is relatively small for cognate tRNA binding when compared to known DNA-protein interactions. Amount of ion-release is low. The electroneutral nature of tRNA binding domain may be responsible for this low ion release stoichiometry.

1. Introduction

To understand the physicochemical basis and specificity of protein-tRNA binding, electrostatic effects must be considered. The importance of electrostatic effects in macromolecules has been reviewed. Salt effects in nucleic acid systems are often treated with the 'standard' model in which the DNA is described as an isolated, infinitely long, uniformly charged cylinder in solvent modeled as a structure-less continuum (3-4). In this model, the binding of Z-valent ligand is described by the neutralization of Z charges on the cylindrical polyion (5-6). The neutralization of charges changes the interaction of DNA with the small ions resulting in the salt dependence of binding. Although work on DNA-protein interaction has been reported little is known about RNA-protein interaction.

The standard model can be developed in terms of counter ion condensation theory (7) and Poisson-Boltzmann (PB) theory formulated as the electroneutral cell model or the full NLPB model with the added salt (8). The essential difference in the description of salt effects between the CC and the PB theories is the description of the radial distribution of small ions around the nucleic acid. The CC theory models the counterion atmosphere around DNA as two distinct populations; a salt invariant condensed layer bound within a well-defined volume around the polyion; and a salt dependent Debye-Huckel layer which is treated as a classical ion atmosphere. In contrast the PB theory models the counterion atmosphere around a polyion as a single population described by a continuously distributed salt dependent ion atmosphere. These differences have been shown to result in very different thermodynamic

descriptions of salt dependent effects in nucleic acid equilibria. The CC model typically describes salt effects in terms of the cratic entropy of ion release, while the PB model emphasizes the role of long-range electrostatic interactions (1-3).

Although CC and cylindrical PB cell models have been remarkably successful in describing the interactions of small ions with rod-like polyions, these standard models do not explicitly account for the detailed molecular structures and charge distribution of the ligand-nucleic acid systems (2-4). Their applicability may then be limited in systems, which significantly deviate from simple cylindrical geometry. In systems with complicated shape and charge distributions, this simple model cannot make quantitative thermodynamic predictions. A theory that specifically relates a molecule's three dimensional shape and charge distribution to its free energy is clearly needed.

Accurate descriptions of the electrostatic properties of a variety of complex macromolecules are given by the finite difference solutions to the PB equation. Furthermore the electrostatic free energy for any system modeled with the full nonlinear PB equation has been unambiguously defined. Thus the electrostatic contribution to the binding energies can be calculated from finite difference solutions to the NLPB equation for detailed molecular geometries (2-3). We report here on the application of the finite difference PB method to evaluate the salt dependent contribution to the total electrostatic free energy of binding of glutaminyl-tRNA synthetase to cognate tRNA.

2 Materials and methods:

a) Theory

In classical electrostatic theory, materials are considered to be homogeneous dielectric media, which can be polarized by electrical charges. Therefore, a dielectric constant is used as a bulk measure of the polarizability of the media, rather than explicitly accounting for the polarization of each atom. This is therefore a continuum model.

The simplest model is described by Coulomb's law, where the electrostatic energy G between two charges i and j is given by:

$$G_{ij}=q_iq_j/\varepsilon r_{ij}$$

And the potential at atom I due to a charge at atom j is:

$$\phi_i= q_j/\varepsilon r_{ij}$$

where q_j = charge on atom j

ε = dielectric constant

r_{ij} = distance between atoms i and j

This model, and variations such as using a distance –dependent dielectric , is currently used in modeling software because of their simplicity. However, this model is valid only for an infinite medium of a uniform dielectric, which is not the case for biological molecules.

Poisson-Boltzmann equation:

A molecule such as protein has a low dielectric constant since its dipolar groups are frozen into a hydrogen bonded lattice and can not reorient in an external electrostatic field. A value near 2 measures its electronic polarization response while a value near 4 includes some contribution from dipole reorientations. Water on the other hand , has a very high dielectric constant(80) since its dipole reorient freely. Therefore , a molecule in aqueous solution yields a system with two very different dielectric media. The effect of this large difference can be considerable and should be accounted for by the electrostatic model. The Poisson –Boltzmann equation is such a model.

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The electrostatic contribution to the protein-RNA binding free energy can be determined using the nonlinear Poisson –Boltzmann equation . The PB equation is a nonlinear partial differential equation relating the electrostatic potential to the fixed and the mobile charge densities, the former coming from the atomic partial charges on the DNA, tRNA, RNA and protein macromolecules and the later from the salt ions floating in the water medium around the macromolecules. For a biological macromolecule in a monovalent salt solution the nonlinear PB equation is defined as

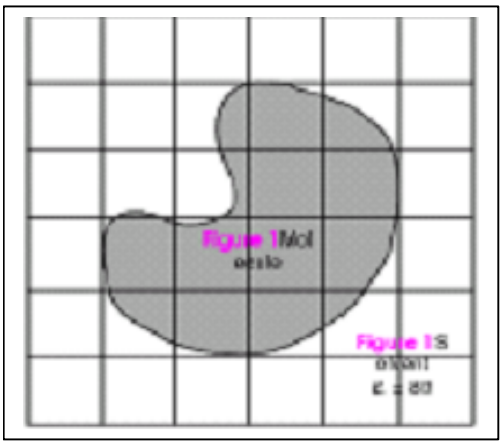
$$\nabla [\varepsilon(r)\nabla.\phi (r)] - \varepsilon\kappa^2 \sinh[\phi(r)] + 4\pi e\rho^f (r) /kt = 0 \quad \text{.....(1)}$$

Where $\phi(r)$ is the dimensionless electrostatic potential in units of kt/e in which k is Boltzmann constant .T is the absolute temperature, e is the proton charge. In addition ε is the dielectric constant and ρ^f is the fixed charge density. The Debye length λ is inversely proportional to κ which is given by $\kappa^2=1/\lambda^2 = 8\pi e^2 I/\varepsilon KT$ where I is the ionic strength of the bulk solution . The quantities ϕ, ε,κ and ρ are all functions of the position vector **r** in the reference frame centered on a fixed macromolecule. This equation appears to be a good model, because it accounts for both the effect of dielectrics and ionic strength. Unfortunately , this equation can be solved analytically only for systems with simple dielectric boundary shape such as planes and spheres. Most molecules have complex shapes. So the alternative to analytical solution is to use numerical techniques to find an approximate solution.

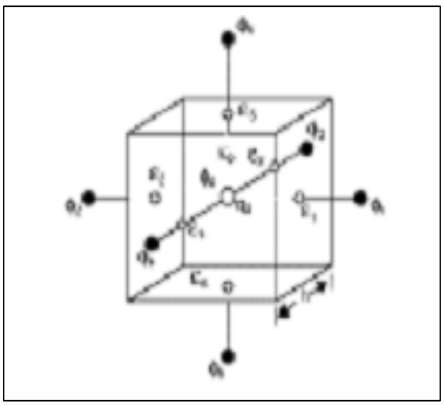
Numerical solutions to the Poisson-Boltzmann equation have found multiple applications in biology and chemistry. Different numerical techniques, including finite difference , boundary element, and finite-element methods have been used to solve the equation. Programmes such as Delphi, UHBD, MEAD, ITPACT and manifold code are widely used.

Finite Difference Approximation:

Delphi uses the finite difference method which involves mapping the molecule on to a three dimensional cubical grid, with spacing between the grid points of size h. The interior of the solvent–accessible surface is assigned one dielectric, and the exterior is assigned another.



Two dimensonsal mapping of the molecule



cube of side h surrounding the grid point

The Poisson-Boltzmann equation must be satisfied everywhere in the grid, and in particular, at each grid point. If the cube of side h surrounding a grid point is considered as shown in the above fig the derivatives in the equation can be replaced by finite differences over this cube and the continuous functions ϕ, ρ and ε can be replaced by their values at the points. Using this strategy, a finite difference formula can be obtained in which the potential at any grid point depends on the charge at the grid point,

the value k at the grid point, the grid spacing h and the potential and dielectric values of the six neighboring grid points.

$$\Phi_0 = \Sigma\varepsilon_\iota\phi_\iota + 4\pi\theta_0 / \eta \ / \Sigma\varepsilon_I + \kappa_o^2\eta^2 N$$

where N = 1 for linear equation, N = $(1+ \phi_0^2/3! + \phi_0^4/5! + \ldots\ldots)$ for non linear equation. This equation is equivalent to the model with charge density ρ_0 obtained by the uniform smearing of the charge q_0 within the cube :

$$\rho_0 = q_0 / h^3$$

The potential at each grid point can therefore be calculated and will change as its neighbor's potentials change. If this calculation is repeated iteratively, the potentials will be more accurate with each iteration until they meet the convergence criteria.

Although most applications of the PB equation have been limited to the linearized form, the nonlinear PB equation is more accurate for highly charged systems such as DNA and RNA or the surface of the many biological membranes. Solutions to the nonlinear PB equation are also available as in an expression for the electrostatic free energy consistent with this form of the equation.

Free energies: Given the complexity of the system, it is necessary to partition the total energy into an easily and accurately computable form. The total electrostatic free energy of a system consisting of fixed charges and mobile ions is

$$\Delta G_{el} = \int_R^3 (\rho^{fix}\phi - \Delta\pi - \tfrac{1}{2}\,\mathbf{E.D}\,)\, dV \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where ρ^{fixed} is the fixed charge distribution of the polyelectrolyte. Φ is the electrostatic potential., E is electrostatic field. D is the displacement vector in solution, and

$$\Delta\pi_i = C_i[\exp(-z_i e\phi / k_B T) - 1] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

is the local difference in concentration of the i-th ion compared to the bulk. Equation 3 accounts for the osmotic work of introducing the excess (or deficit) ions into the solution. Using the well –known expression,

The first term in the integral can be calculated as

$$\int_R^3 \tfrac{1}{2}\rho^{free}\phi\, dV = \int_R^3 \tfrac{1}{2}\,\mathbf{E.D}\, dv \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

and the definition that free (non polarizable)charge is the sum of the fixed charge on the molecule and the ionic charge in the solvent, $\rho^{free} = \rho^{fixed} + \rho^{mobile}$ one can rewrite the energy density expression as

$$\Delta g = \tfrac{1}{2}\rho^{fixed}\phi - \Sigma\,\Delta\pi_i - \tfrac{1}{2}\rho^{mobile}\phi$$

In the above equation , the second term is an osmotic pressure and the third is an electrostatic stress. It can be shown that in the case of the linearized PB equation the last two terms cancel so that the free energy is given by the first term. This can be used as a criterion for the nonlinearity of the system, i.e., in a system with weak nonlinearity these two term should be small and have similar magnitudes.

In the finite –difference method, the system is discretized and the free energy term, $\tfrac{1}{2}\rho^{\phi\iota\xi\varepsilon\delta}\phi$ can be rewritten and expanded in the form

$$\tfrac{1}{2}\rho^{fixed}\phi = \tfrac{1}{2}\Sigma q_j\phi(r_j) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

where the potential is the one generated by all the charges , except for one located at r_j.

The standard way used to extract free energies form equation 5 is to multiply the charge that is located at a grid point by the potential at that point. The grid charge is obtained with some method that distributes point charges located at atomic nuclei at neighboring grid points. Because the grid charge is sensitive to this details of algorithm , and to the grid spacing , this procedure results in a loss of precision in the free energy calculation. An alternative grid –independent procedure is now introduced. The potential at any given point arises from the direct effect of real charges, from surface polarization charges (the reaction field term) and from mobile ions in solution. Thus, the potential at the position of charge j can be written as

$$\phi(r_f) = \phi_{coul}(r_f) + \phi_{reactn}(r_f) + \phi_{ion}(r_f)$$

The electrostatic potential at j th site has got contributions from : arising due to the all other fixed charges (coulombic), that arising due to the polarization of water media because of the molecular charges (reaction field) and that arising due to the mobile salt ions (ionic).

Hence

ΔG_{el} =(**coulombic energy + reactn. Field energy) + ionic contribution**

- osmotic pressure term –1/2 rho⊛ phi term in solution

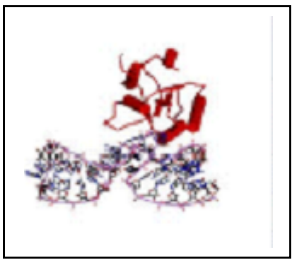
Coulombic term can directly be calculated from the given atomic partial charges while all the other terms are related to the electrostatic potential, which is obtained as the solution of the PB equation. The total electrostatic free energy of a biological macromolecule can be decomposed into a salt independent (ΔG_{ns}) and a salt dependent (ΔG_s) part. The first two terms above are salt independent and the last three terms constitute the salt dependent energy.

$$\Delta G_{el} = \textit{Salt independent energy + salt dependent energy}$$

In the protein tRNA association process each biomolecule , the free protein and free tRNA undergo structural changes respectively to obtain the conformation in which the biomolecule has in the bound complex. These processes are not explicitly considered because the crystal structures do not exist of the free tRNA. In the next step of association process the protein –tRNA complex is formed. The total electrostatic free energy of binding of the protein-tRNA complex is determined from

$$\Delta G_{el} = \Delta G_{el}^{complex} - \Delta G_{el}^{protein} - \Delta G_{el}^{tRNA} \quad \text{.....(6)}$$

where the superscripts indicate the complex the isolated proteins and isolated tRNA s.



To understand the thermodynamic process of protein tRNA association the salt independent part of the total electrostatic free energy can be physico-chemically interpreted. In the initial process the fully charged and solvated protein and tRNA are infinitely separated. There after each biomolecule is partially solvated by a low dielectric medium, which represents the region that the other biomolecule will occupy in the complex. The electrostatic free energy is the desolvation free energy of the protein and the tRNA.

In the next step the fully charged protein and the tRNA are transferred into the low dielectric medium cavity and the complex are formed.

The salt dependent free energy ΔGs can be described as the change in solvation free energy of each biomolecule in aqueous salt solution upon binding.

3. Material:

In this study we have examined Glutaminyl-tRNAsynthetase-tRNA^{gln} in detail. The crystal structure of the protein-tRNA complex was obtained from PDB as 1QTQ.

The hydrogen atoms and the lacking 5' and 3'-terminal hydroxy groups of the tRNA and N and C terminal groups of GlnRS were added.

Energy Minimization: The structure 1QTQ was energy minimized using TINKER software. Hydrogen atoms to the structure were added and then energy minimization done.

Charge assignment: Partial charge assignment and radii assignment done using Amber force field parameters. The following residues are considered ionizable, Arg+, Asp-, Glu- and all the histidines are charged zero. The charge distribution of the GlnRS-tRNA complex investigated is displayed below.

PDB	Arg	Asp	Glu	His(neutral)	Lys	GlnRS	tRNA	Complex
1QTQ	40	37	46	16	32		74	
charge	+40	-37	-46	0	+32	-11	-74	-85

Molecular model: For the complex the total electrostatic free energy of protein-tRNA binding was obtained using the finite difference nonlinear PB equation. In all the calculations the all-atom AMBER parameters for proteins and nucleic acids were applied. The GlnRS-tRNA^{gln} complex was treated as a low dielectric medium with a dielectric constant of 2.0 in all the calculations. This value describes each complex as a rigid body association and accounts only for polarizability and not for the small dipolar fluctuations, which may occur during structural changes. A dielectric constant of 80.0 was assigned to the surrounding solvent, which was treated as continuum. A radius of 2.0 A^0 was applied to exclude hydrated sodium ions from the surface of protein-tRNA complex. The cavity of the complex was defined by the molecular surface which was determined using a probe radius of 1.4A^0. To achieve sufficient accuracy the potentials were iterated to a change less than 0.00001 kT/e and the free energies were checked to not change during the last focusing step. A physiological ionic strength of 0.145 M NaCl was used for the GlnRS-tRNA binding free energies calculations. In the salt dependent analysis the following ranges of ionic concentrations were applied 0.0, 0.025, 0.05, 0.1, 0.145, 0.2, 0.45, 1.45 M. An experimental study has shown that the dielectric constant of NaCl solution changes due to high salt concentrations. At 0.45 and 1.45 M concentrations the dielectric constants used were 73.8 and 64.2, respectively.

Delphi setup: Electrostatic potentials were calculated with the finite difference NLPB equation implemented in the Delphi software package. The macromolecule was mapped onto a 291^3 cube grid lattice. The largest source of error in the finite difference method arises from the limited resolution of the lattice and the resulting error in the representation of the molecular surface. Therefore, the potentials were calculated using a 4-step focusing technique. In the initial calculation, the largest dimension of the complex molecule fills 36% (scale 1grid /A^0) of the grid, in the 2^{nd} step the molecule fills 54% (scale 2

grid /A^0) of the grid , in the 3rd step the molecule fills 72% (scale 2 grid /A^0) of the grid and in the last step the molecule fills 90% (scale 2.5 grid /A^0) of the grid with the boundary conditions interpolated from the previous step. The final resolution was good enough, to reach 2.5 grid /A^0. At this resolution, salt dependent electrostatic free energies vary by less than 1% with respect to grid placement and resolution.

4. Results:

Salt effect on binding has been treated extensively with PB equation.

Table 4.I

Value of various components of free energy term contributing to the total salt dependent electrostatic free energy

Salt	0.01	0.025	0.05	0.1	0.145	0.2	0.45	1.45
ΔG_i	-107.9	-109.6	-108.7	-108.1	-102.2	-98.4	-96.5	-99.6
ΔG_{osm}	-0.152	-3.182	-5.566	-7.353	-4.77	-5.091	-5.007	-6.425
$\Delta G_{\rho\phi/2}$	-4.63	1.522	6.177	9.014	3.628	4.311	4.421	6.778
ΔG_s	-103.12	-107.9	-109.31	-109.76	-101.06	-97.62	-95.91	-99.95

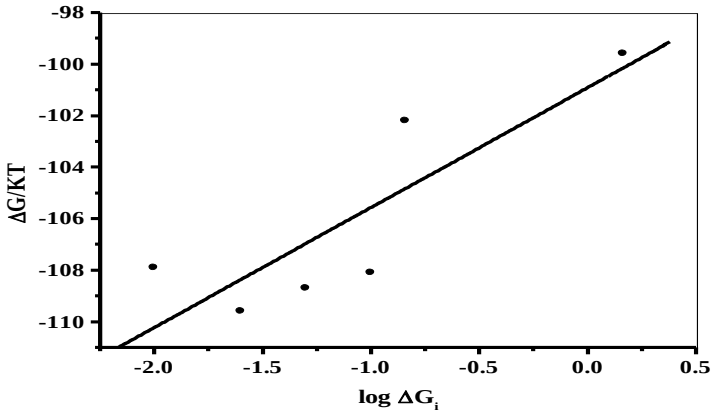


Fig 4.1: The salt dependency of the free energy term ΔG_i for the complex.

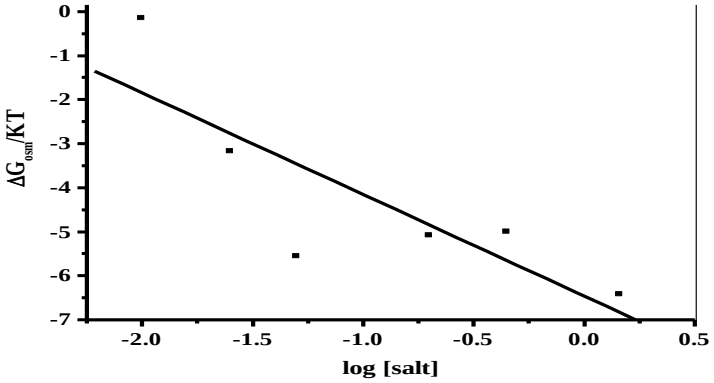


Fig 4.2: The salt dependency of the free energy term ΔG_{osm} for the complex.

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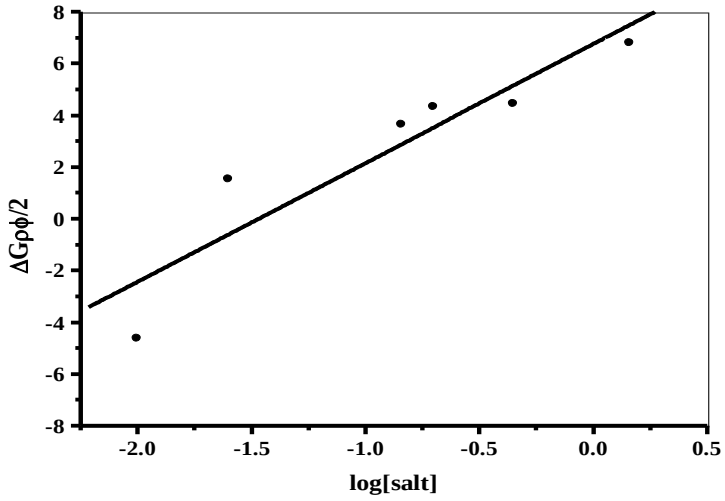

Fig 4.3: The salt dependency of the free energy term ΔGρϕ for the complex

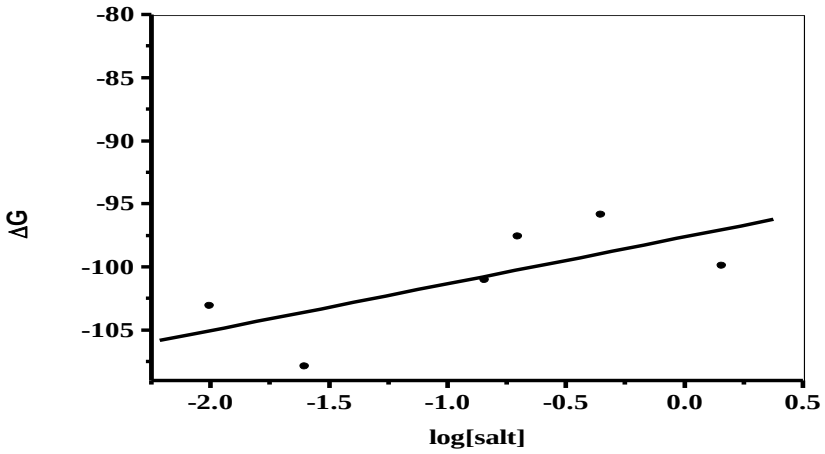

Fig 4.4: The salt dependency of the free energy term ΔGelec for the complex.

Table 4.II

The salt dependence of the free energy terms for the AARS-tRNA complex

δΔGi / δlog [M+]	4.64
δΔGosm/δ log [M+]	-2.3
δΔGρϕ / δlog [M+]	4.58
δΔGS / δlog [M+]	3.70

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Table 4.III

Total electrostatic energy at various salt concentrations

Salt [M]	ΔGelec /kT
0.01	81.30
0.025	76.48
0.05	75.11
0.1	74.66
0.145	82.94
0.2	86.80
0.45	88.51
1.45	84.46

Table 4.IV

Log [salt]	Log Ka
-2.0	-35.30
-1.60	-33.21
-0.84	-36.01
-0.69	-37.69
-0.35	-38.43
-0.16	-36.67

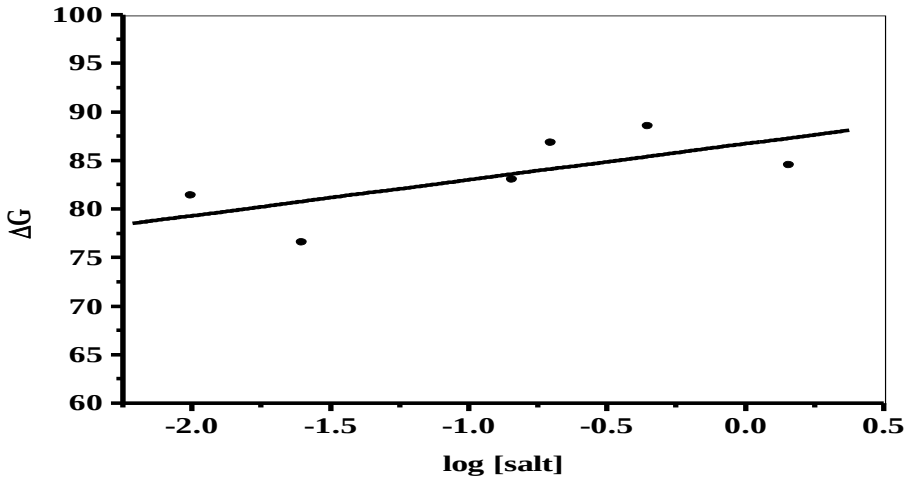

Fig 4.5The salt dependency of the total electrostatic energy ΔG for the complex

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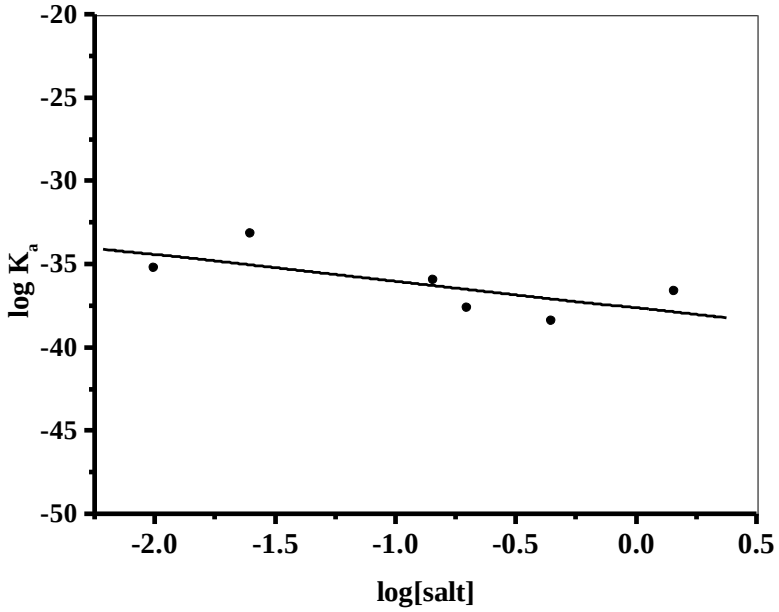

Fig 4. 6: Ionic strength dependency of AARS-tRNA interaction. Log-log plot of association constant versus monovalent ion concentration of GlnRS/$tRNA^{gln}$.

5. Discussion:

a) The *interpretation of salt effects on protein-tRNA interactions in NLPB model*

The calculated values of the salt dependent binding energies, ΔG_S0 increases linearly with ln $[M^+]$. ΔG_S0 has been interpreted in terms of changes in three salt dependent free energy contributions. Electrostatic energy opposes binding at all salt concentrations. The destabilization is more with increasing salt concentration.

The finite difference PB model provides an accurate theoretical framework for understanding nonspecific salt dependent effects in AARS–tRNA systems. PB theory describes the interaction of a continuously distributed ion atmosphere with each macromolecule. The redistribution of ions upon binding reflects both differential cation and anion interactions with the protein and the tRNA. Cations accumulate around highly negative tRNA while anions accumulate around the tRNA binding domain of the aaRS. The interaction of the positively charged tRNA binding domain of the AARS with the tRNA reduces the magnitude of the negative electrostatic potential around tRNA so that fewer cations accumulate. At the same time large negative electrostatic potential around the tRNA diminishes the anion and enriches the cations nearby the protein.

In addition the large protein molecules physically exclude cations from a high potential region near the tRNA while the tRNA excludes anions from near the positively charged protein surface. This redistribution effect increases with bulk salt concentration as proportionally more ions are displaced upon binding. Thus at salt concentration ligand binding to tRNA disrupts the large favorable electrostatic interaction of the highly organized ion atmosphere with the free polyion. The resulting unfavorable

change in ΔG_s^0 upon binding indicates that the molecules are better solvated by the salt in the separated state than in the bound state.

The univalent salt dependence of protein-tRNA binding can be a function of several other important effects: differential cation and anion binding to specific sites both on the AARS and the tRNA, differential protonation and hydration. Because of the continuum representation of the salt effects in the PB model, our analysis must assume that specific ion effects, if they are present do not substantially alter the salt dependence of binding in the system studied.

b)The AARS-tRNA interaction

Experimentally ion release resulting from the protein-DNA interaction has been studied from the dependence of binding equilibrium on ionic strength. Similar technique has been used for tRNA-AARS interaction. The log-log plot of association constants versus KCl concentration in the absence of $MgCl_2$ shows a monotonic decrease in affinity with increasing KCl concentration. The slope of the straight line corresponds to a cation release stoichiometry of 1.7 for $tRNA^{gln}$-GlnRS complex (15).

The calculated salt dependence of AARS-tRNA interaction shows cation release stoichiometry of 1.6 in magnitude.

The log-log plot shows similar trend of monotonic decrease in affinity with increasing KCl concentration.The slope of the line corresponds to a cation release of 1.60 which has a good agreement with the experimental result (15).

The AARS-tRNA binding reaction must be analyzed as a two-step process.

$$\text{tRNA} \xrightarrow{K_1} \text{tRNA}^*$$

$$\text{AARS} + \text{tRNA}^* \xrightarrow{K_2} \text{AARS-tRNA}^*$$

where tRNA is unbound or free tRNA and tRNA* is the conformation of the tRNA in the AARS-tRNA complex and AARS is GlnRS. Conformational change in tRNA due to AARS binding is less well documented since the structure of the free tRNA is not known. We have not taken this step in our consideration. However due to relatively small changes in the tRNA and AARS structure upon complex formation it is unlikely that a coupled folding event could entirely account for the low ion release stoichiometry.

The magnitude of the ionic strength effects is highly dependent on the electrostatic interactions between negatively charged phosphates and the charged groups of the protein. The best characterized protein-nucleic acid interaction with respect to ion and water release is perhaps the lac repressor/operator complex. Dependence of binding constant as a function of monovalent salt is relatively large and can be interpreted as equivalent to release of eight cations upon lac repressor dimer binding to lac operator. It can be easily seen from the crystal structure of the complex that groups from the lac repressor protein closely approach approximately eight phosphates (1). This is close to the number of cations released obtained from the ionic strength dependence of the association constant. Eight phosphates are closely approached by atoms from GlnRS (15). Six of these groups are positively charged lysine and arginine (15). This number is expected to contribute significantly to the ionic strength dependence of the association constant. Thus one may expect relatively high salt dependence of interaction energy in the tRNA-AARS system.

However the dependence of the tRNA-AARS system upon addition of monovalent salt is very low. A significant part of the explanation for lower salt dependence of the binding free energy lies in the overall charge distribution of the interacting surfaces of the protein. In the lac repressor, there are overall 14 positively charged groups and three negatively charged groups within 10 A^0 of the phosphate, giving the DNA binding domain a highly positively charged character. All 14 positively charged groups are within $8A^0$ of the phosphate, whereas the three negatively charged groups are within 8-10 $A^0$of the phosphate. In contrast, in the GlnRS/tRNA complex, 21 positively charged groups and 17 negatively charged groups are within 10 $A^0$of the phosphate and 17 positively charged and 14 negatively charged groups are within 8 $A^0$of the phosphates (15). This gives the tRNA binding interface a much more electroneutral character and probably much lower salt dependence of the binding free energy.

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