Enthalpy–entropy compensation in the binding of peptide ligands to human Arc

Hallin et al. characterized the binding of peptides to the activity-regulated cytoskeleton-associated protein (Arc) in a recent paper [1]. The research is particularly interesting since it provides mechanistic insight into the molecular basis of the function of the Arc protein in neural plasticity [2,3]. The authors of the original paper [1] employed three forms of human Arc in the peptide binding assay; hArc-NL (residues 207–277), hArc-CL (residues 278–370), and hArc-CT (residues 206–396). The peptide sequences used in the binding assay represent the parts of proteins, guanylate kinase-associated protein and stargazin, which are considered to play a role in synaptic plasticity [4,5]. Therefore, the peptide-protein interactions that they examined may characterize the binding of hArc to its target proteins in postsynaptic neurons.

The authors measured peptide-binding capacity of each form of the Arc protein (hArc-NL, hArc-CL, and hArc-CT) at 25 °C by isothermal titration calorimetry. The primary finding of the experiment is that the peptide-binding capacity is present only in hArc-NL, not in hArc-CL, despite the structural similarity between the two domains. In addition, they confirmed that hArc-CT binds peptide ligands as expected since it contains hArc-NL. While their research provided valuable information for the binding thermodynamics, we noticed that one of their interpretations in their paper [1] needs a clarification. In this paper, we report our analysis of the thermodynamics of hArc-peptide interactions, which we hope clarifies the ambiguity in the original paper [1]. We also propose the role of hArc-CT in the recognition of target peptides of hArc protein.

The authors in the original paper [1] stated that all peptides exhibited similar enthalpy–entropy compensation in binding to hArc-NL, as alone or as a part of hArc-CT, based on the binding thermodynamics obtained from isothermal titration calorimetry. To examine the apparent similarity of enthalpy–entropy compensations, we statistically examined the thermodynamic data reported in the original paper [1]. The relationship between enthalpy (ΔH) and entropy (ΔS) in the binding of peptides to the two different forms of hArc-NL, as alone or as a part of hArc-CT, is shown in Fig. 1A. The plot shows a wide variation of ΔH and ΔS, as well as a significant correlation between ΔH and ΔS in both hArc-NL and hArc-CT. Numerical relationship of the correlation, which represents enthalpy–entropy compensation, can be described by Eq. (1):

$$\Delta H = T_C \times \Delta S + \beta \tag{1}$$

where T_C, called compensation temperature [6,7], is the slope of the fitting line (Fig. 1A). The values of T_C and its standard errors for hArc-NL and hArc-CT are 308.4 ± 6.6 K and 343.0 ± 11.7 K, respectively. A *t*-test indicates that the difference of T_C between hArc-NL and hArc-CT is statistically significant ($t = 2.769$, $df = 5$, $P = 0.039$). In the *t*-test, the value of degree of freedom (*df*) [8] was calculated as $df = (n_1 - 2) + (n_2 - 2)$, where n_1 and n_2 are the number of data points of hArc-NL and hArc-CT, respectively: $n_1 = 5$ and $n_2 = 4$ (Fig. 1A). It is known that T_C can be a quantitative measure of the degree of compensation between enthalpy and entropy [7]. The statistical difference of T_C between hArc-NL and hArc-CT strongly suggests that hArc-NL and hArc-CT have distinct peptide binding properties.

According to the theory of enthalpy–entropy compensation [6], the deviation of binding free energy (ΔG) among related reactions becomes minimized at the compensation (iso-equilibrium) temperature (Fig. 1B). To visualize the theory in the interactions of hArc and peptides, we calculated the standard deviation (*SD*) of ΔG in the bindings at temperature T using Eq. (2):

$$SD = \sqrt{\sum_i (\Delta G_i - <\Delta G>)^2 \Big/ (n - 1)} \tag{2}$$

where ΔG_i is the free energy in the binding of peptide i to either hArc-NL or hArc-CT, and $<\Delta G_i>$ is the average of the binding free energy of all species to hArc-NL or hArc-CT at temperature T, and n is 5 and 4 for hArc-NL and hArc-CT, respectively (Fig. 1A). The procedure of *SD* calculations is available in Supplementary material that contains ΔG_i and *SD* values in each binding at a temperature. The plot confirms that the standard deviation is minimum at the corresponding compensation temperature in each case (Fig. 1B). According to our statistical analysis, hArc-CT has a lower standard deviation of ΔG than hArc-NL in the range of physiologically-relevant temperature (Fig. 1B). This means that hArc-NL in the context of hArc-CT has a broader specificity than hArc-NL alone. Therefore, we can postulate that the role of hArc-CL is broadening the binding specificity in hArc-NL (Fig. 1C).

The broad specificity is also observed in the immune system. For
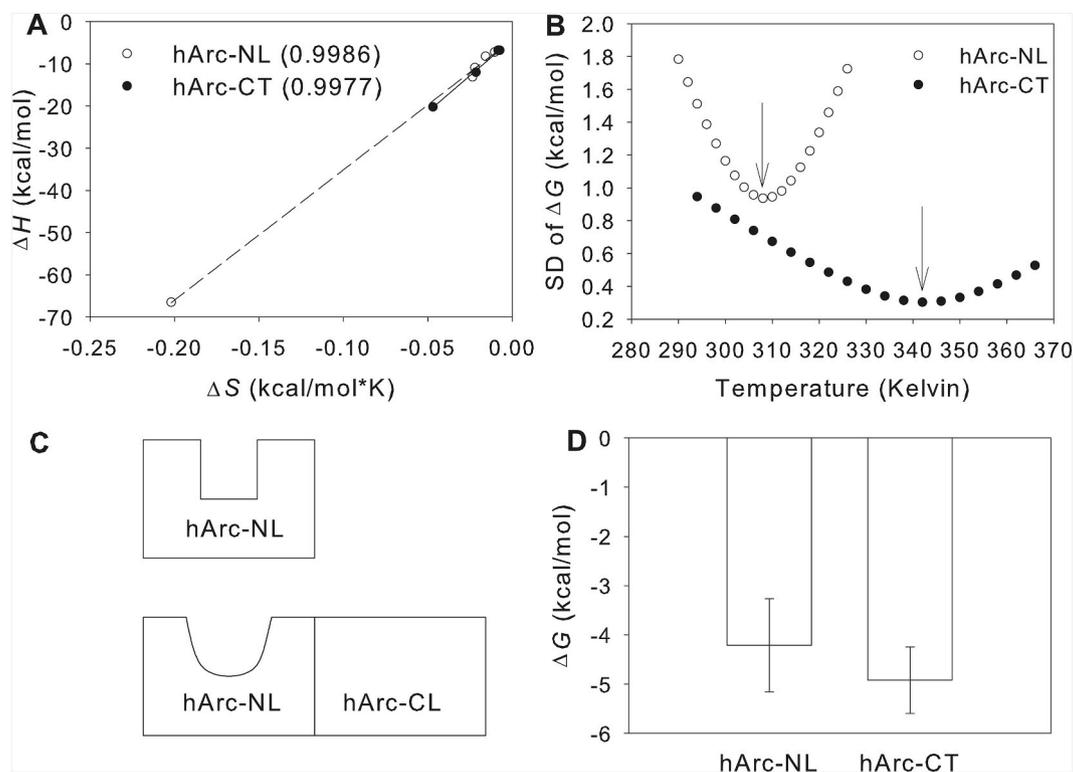
Fig. 1. Thermodynamic analysis of the interactions between Arc and peptide ligands. **A.** Enthalpy–entropy compensation in the binding of peptide ligands to two different forms of hArc. Each line represents linear fits using Eq. (1). Linear regression indicates a significant correlation between ΔH and ΔS as the R^2 values are 0.9986 and 0.9977 for hArc-NL and hArc-CT, respectively. Two data points of hArc-CT at the upper right are almost overlapped, not visually distinguishable. **B.** Standard deviation of ΔG in the binding of peptide ligands to hArc in a range of temperature. The arrows indicate the compensation temperature of each case: 308.4 K and 343.0 K for hArc-NL and hArc-CT, respectively. **C.** Schematic model of the role of hArc-CL in the modulation of the binding site in hArc-NL. The square or curved well represent a rigid or flexible peptide binding site, respectively. **D.** ΔG of each form of hArc at 37 °C. The error bars represent the standard deviation. Graph preparation and fitting was performed using SigmaPlot (version 11, Systat Software Inc., San Jose, CA).

example, major histocompatibility complex (MHC) molecules, which are antigen-binding proteins, bind many different peptides [9,10]. The high promiscuity of MHC molecules in binding target antigen peptides is essential for their antigen presentation function in order to recognize various peptide antigens derived from diverse pathogen proteins [11]. Based on our statistical and thermodynamic analysis, we propose that the function of hArc-CL is an enhancement of binding promiscuity of hArc-NL in its recognition of target proteins (Fig. 1C). This feature should be favorable for the role of hArc as a flexible hub protein for synaptic plasticity by binding various neuronal postsynaptic proteins [3].

While our analysis suggests that hArc-CL may act to broaden binding specificity toward target peptides, another aspect of binding worth discussion is its effect on the binding affinity of hArc-NL. Fig. 1D shows the average binding affinity represented by ΔG of hArc-NL and hArc-CL based on the data in the original paper [1]. It clearly shows that hArc-CT has a higher affinity (lower ΔG) than hArc-NL. Its statistical significance was examined by a *t*-test, which indicated that the difference in the mean values of the two groups was not great enough to reject the possibility that the difference is due to random sampling variability ($t = 1.259$, $df = 7$, $P = 0.248$). This means that there is not a statistically significant difference between the input groups. However, one should be cautious in accepting the result, since the power of the performed *t*-test (0.098) is much below the desired power of 0.8 [12], implying that it is less likely to detect a difference even if one actually exists. One reason for the lower power of the test can be attributed to the small number of data points (n total = 9). Therefore, a binding assay with more sample peptides may elucidate the effect of hArc-CL on the peptide binding affinity in hArc-NL.

In this paper, we show our statistical analysis of thermodynamic data

in the binding of various peptides to three different forms of hArc. However, one should note that analysis based on purely thermodynamics has an intrinsic limitation, since classical equilibrium thermodynamics barely provides mechanistic descriptions of the system [13]. Further research will decipher the findings in this paper. For example, any potential role of solvent water molecules in modulating binding property of hArc-NL by hArc-CL requires investigation using alternative methods such as molecular dynamics of the system [14–16].

In conclusion, each of hArc-NL and hArc-CL exhibits a unique enthalpy–entropy compensation with a statistically different compensation temperature in binding peptides. Statistical analysis suggests that hArc-CL enhances binding promiscuity of hArc-NL.

Author statement

Funding

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101088.

References

[1] E.I. Hallin, C.R. Bramham, P. Kursula, Structural properties and peptide ligand binding of the capsid homology domains of human Arc, Biochem. Biophys. Rep. 26 (2021) 100975, https://doi.org/10.1016/j.bbrep.2021.100975.

[2] Y. Li, A.L. Pehrson, J.A. Waller, E. Dale, C. Sanchez, M. Gulinello, A critical evaluation of the activity-regulated cytoskeleton-associated protein (Arc/Arg3.1)'s putative role in regulating dendritic plasticity, cognitive processes, and mood in animal models of depression, Front. Neurosci. 9 (2015) 279, https://doi.org/10.3389/fnins.2015.00279.

[3] O. Nikolaienko, S. Patil, M.S. Eriksen, C.R. Bramham, Arc protein: a flexible hub for synaptic plasticity and cognition, Semin. Cell Dev. Biol. 77 (2018) 33–42, https://doi.org/10.1016/j.semcdb.2017.09.006.

[4] S.M. Shin, N. Zhang, J. Hansen, N.Z. Gerges, D.T. Pak, M. Sheng, S.H. Lee, GKAP orchestrates activity-dependent postsynaptic protein remodeling and homeostatic scaling, Nat. Neurosci. 15 (2012) 1655–1666, https://doi.org/10.1038/nn.3259.

[5] A. Kamalova, T. Nakagawa, AMPA receptor structure and auxiliary subunits, J. Physiol. 599 (2021) 453–469, https://doi.org/10.1113/JP278701.

[6] M. Daune, Molecular Biophysics: Structures in Motion, Oxford University Press, New York, 1999.

[7] R. Griessen, B. Dam, Simple accurate verification of enthalpy–entropy compensation and isoequilibrium relationship, ChemPhysChem (2021), https://doi.org/10.1002/cphc.202100431.

[8] J. Fowler, L. Cohen, P. Jarvis, Practical Statistics for Field Biology, John Wiley & Sons, Chichester, England, 2008.

[9] H.N. Eisen, X.H. Hou, C. Shen, K. Wang, V.K. Tanguturi, C. Smith, K. Kozyrytska, L. Nambiar, C.A. McKinley, J. Chen, R.J. Cohen, Promiscuous binding of extracellular peptides to cell surface class I MHC protein, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 4580–4585, https://doi.org/10.1073/pnas.1201586109.

[10] J. Kang, J.D. Auerbach, Thermodynamic characterization of dissociation rate variations of human leukocyte antigen and peptide complexes, Mol. Immunol. 46 (2009) 2873–2875, https://doi.org/10.1016/j.molimm.2009.05.184.

[11] A. Abbas, A.H. Lichtman, S. Pillai, Basic Immunology: Functions and Disorders of the Immune System, sixth ed., Elsevier, Philadelphia, PA, 2019.

[12] P.D. Ellis, The Essential Guide to Effect Sizes: Statistical Power, Meta-Analysis, and the Interpretation of Research Results, Cambridge University Press, New York, 2010.

[13] R. Chang, Physical Chemistry for the Chemical and Biological Sciences, University Science Books, Sausalito, CA, 2000.

[14] J.E. Ladbury, Just add water! the effect of water on the specificity of protein-ligand binding sites and its potential application to drug design, Chem. Biol. 3 (1996) 973–980, https://doi.org/10.1016/S1074-5521(96)90164-7.

[15] P. Matricon, R.R. Suresh, Z.G. Gao, N. Panel, K.A. Jacobson, J. Carlsson, Ligand design by targeting a binding site water, Chem. Sci. 12 (2020) 960–968, https://doi.org/10.1039/D0SC04938G.

[16] C. Päslack, C.K. Das, J. Schlitter, L.V. Schäfer, Spectrally resolved estimation of water entropy in the active site of human carbonic anhydrase II, J. Chem. Theor. Comput. (2021), https://doi.org/10.1021/acs.jctc.1c00554.

Jonghoon Kang[*], Lukas Smidtas

Department of Biology, Valdosta State University, Valdosta, GA, 31698, USA

[*] Corresponding author.

E-mail address: jkang@valdosta.edu (J. Kang).