Published in final edited form as:

Curr Protein Pept Sci. 2018 ; 19(11): 1088–1091. doi:10.2174/1389203719666180521092615.

The Enthalpy-entropy Compensation Phenomenon. Limitations for the Use of Some Basic Thermodynamic Equations

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Abstract

The thermodynamic analyses of proteins, protein-ligands and protein-nucleic acid complexes involves the entropy–enthalpy (S-H) compensation phenomenon. We have examined the question whether the observed compensation is artificial or reflects anything more than the well-known laws of statistical thermodynamics (so-called extra-thermodynamic compensation). We have shown that enthalpy–entropy compensation (EEC) is mainly the trivial consequence of the basic thermodynamic laws and there are no experimental evidences for existence of the extra-thermodynamic compensation. In most cases EEC obtained in the experiments through the plot enthalpies (H) and entropies ($T S$) versus one another is meaningless due to the large correlated errors in H and $T S$ unless special measures are taken to minimize, quantify and propagate these errors. Van't Hoff equation can be used for entropy calculation in limited cases when enthalpy is measured in independent experiments. Eyring equation cannot be used for calculation of entropy in any case and should be excluded from scientific use. Both equation, Van't Hoff and Eyring cannot be used for simultaneous calculation of the enthalpy and entropy values using one set of data. All the data obtained in this way should be recognized as erroneous.

Keywords

Entropy - enthalpy compensation; protein thermodynamics

1. INTRODUCTION

Enthalpy–entropy compensation (EEC) is a well-known phenomenon manifested in many chemical and biochemical systems [1-16]. Linear plots of enthalpies vs. entropies are often treated as authentic representations of an extrathermodynamic relationship, which is sometimes called the isokinetic effect or enthalpy-entropy compensation effect. EEC is the

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CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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main obstacle on the way to optimizing many of the physical properties of prospective compounds necessary for drug development. For instance, EEC is clearly observed in the analysis of the experimental binding thermodynamics for approximately 100 protein-ligand complexes [17]. It appears that the molecular interactions reflected in a better binding enthalpy are critical for the development of improved drugs. However, drug candidates are difficult to make enthalpically-optimized. If enthalpy/entropy compensation were an inevitable phenomenon, it would be impossible to improve the binding affinity of a compound [18]. Although the physical bases of EEC are still not completely understood but the enthalpy–entropy compensation paradox (EECP) comes out as a contradiction between understanding of EEC as a real phenomenon including some extrathermodynamic properties of the system and an artefact that arises due to statistical reasons. What is the nature EECP from the thermodynamic point of view?

2. RESULTS AND DISCUSSION

Two basic equations of chemical thermodynamics and chemical kinetics are the Gibbs equation (1) and Arrhenius equation (2).

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

$$k = Ae^{-E_a/RT} \quad (2)$$

where

G is a free energy, H – enthalpy, S – entropy, A – preexponential factor, k - rate constant, E_a – activation energy (enthalpy), R – gas constant and T – absolute temperature in Kelvin.

In the particular case of the ligand binding to the protein, the enthalpic component (ΔH) of the Gibbs equation quantifies the change in heat associated with binding, while the entropic component ($T\Delta S$) quantifies a change in disorder of the overall system (including the protein, the ligand, cofactor, receptor, and surrounding solvent).

The most popular forms of equations (1) and (2) are their derivatives, Van' t Hoff (3) and Eyring or Eyring–Polanyi (4) equations in their linear form:

$$\ln K_a = -\Delta H/R^*(1/T) + \Delta S/R \quad (3)$$

$$\ln (k/T) = -\Delta H^\ddagger/R^*(1/T) + \ln(k_B/h) + \Delta S^\ddagger/R \quad (4)$$

where $H^\ddagger$ is activation enthalpy, $S^\ddagger$ - activation entropy, k_B – Boltzmann's constant, h – Planck's constant

It is obvious that $\ln K_a$ and $\ln (k/T)$ are the linear functions of the reverse absolute temperature mainly because of the narrow temperature range. This significantly reduces the change in heat capacity, C_p and, as a consequence, H does not depend on temperature, and the graph of $\ln K_a = f(1/T)$ will be linear. The Van 't Hoff ($\ln K_a = f(1/T)$) and Eyring ($\ln (k/T) = f(1/T)$) plots derived from equations (3) and (4) led to many examples of the correlation observed between the estimates of the enthalpy and entropy of a reaction obtained from temperature-dependence data described in the numerous (hundreds) literature sources. That correlation (EEC) is practically linear with the correlation coefficient exceeding 0.95. The reasons for the H/S linear correlation such as extra-thermodynamic, empirical, error-related, solvation, and so on are discussed in the literature [1-4, 6, 10, 11, 17, 19]. In general, there are two explanations for EEC: EEC is an artefact and EEC is a real phenomenon, origin of which should be ascertained for each particular reaction. Following the above discourses, the enthalpy-entropy compensation paradox (EECP) can be expressed as "a statement that appears to be self-contradictory but may include a latent truth". Thus, the main question on the way to resolve EECP for the particular reaction is: "Is EEC real or artificial?"

In general, an idea of EEC follows from the Gibbs equation (1) itself due to the minus sign before the entropy term. If H and S for the particular reaction are changing in one direction (either increase or decrease) then their changes being transformed into G are mutually compensated and there is a little change in the value of G . In turn, if the range of ΔG 's is much smaller than the range of ΔH 's, then - with respect to H , $G \sim$ constant and linear H - S compensation follows immediately from equation (1). This synchronous one-directional change in H and S across a wide range of the physiological temperatures is true for the most biochemical reactions such as ligand binding, protein folding etc. Aside from H / S compensation followed from equation (1) the changes in solvation can also contribute to EEC. If the water is tightly bound to the system, its contribution to the enthalpy and entropy of binding will also be largely compensatory [1]. The processes include solvation of ions and nonelectrolytes, hydrolysis, oxidation-reduction, ionization of weak electrolytes. It is proposed that the linear enthalpy-entropy relationship be used as a diagnostic test for the participation of water in protein processes [11]. It was concluded that the large fluctuations in enthalpy and entropy observed are too great to be a result of only conformational changes and must result from variations in the amounts of water immobilized or released on forming complexes [20]. EEC is also influenced by the molecular conformational changes inside ligands upon their solvation and due to conformational changes inside a protein upon ligand association [21]. The enthalpy-entropy compensation is not only a 'ubiquitous property of water', but a property of all weak intermolecular interactions in aqueous solution including hydrogen bonding [6]. This can explain why such compensation is so prevalent in biological systems. Thus, qualitatively EEC follows from the thermodynamic fundamentals and confirmed by numerous experimental facts. We have the EEC in its pure view.

In contrast, extra-thermodynamic compensation, which reflects anything more than the well-known laws of statistical thermodynamics, shows a linear relationship between H and S but cannot be deduced from the basic laws of thermodynamics. The slope of the dH/dS line, the compensating temperature (T_c) is the basic parameter for characterization of extra-

thermodynamic compensations. T_c can reveal some mechanistic or extra-thermodynamic information about the system; for instance, some additional information about the interaction between different components of the system. In fact, the acquisition of extra-thermodynamic information is the main objective for experiments to study EEC. There are numerous works dedicated to obtaining such additional extra-thermodynamic information and a question arises whether any such information was obtained? Actually, it was not. The detailed statistical analysis of the enthalpy-entropy compensation effect have shown that without careful statistical considerations any extra-thermodynamic factors are indistinguishable from the statistical compensation effect [22-25]. A general statistical mechanical model of a complex system suggests that extra-thermodynamic compensation, if it exists, is unlikely to be observed in real experimental systems and difficult to interpret if it does [16].

At the same time, for many (perhaps most) cases, it has been suggested that the EEC is an artifact for several reasons.

First, the determination of two parameters from one single equation is impossible since two quantities are not *a priori* mutual independent [26]. This suggests that the excellent correlation often observed between H ($H^\ddagger$) and S ($S^\ddagger$) [27] mainly reflects the fact that both thermodynamic parameters are in reality two measures of the same thing [4, 26]. In fact, the beauty of the equations (3) and (4) led the many to the wrong idea that both parameters, H ($H^\ddagger$) and S ($S^\ddagger$) can be evaluated from one set of the experimental data. Such recommendation is traced through a great deal of the biochemical text-books and manuals and is misleading. Let's consider a linear equation $y=mx+n$ containing two monomials, "mx" and "n" which are in fact the derivatives of the same entity. Indeed, $n=mx_1$ and $y=mx-mx_1$ where $x_1=\text{const}$ is the intercept of x-axis at $y=0$. In attempt to establish correlation between two quantities, "mx" and "n" which are H/R and S/R respectively, equation 3 establishes correlation between "mx" and "mx1". Thus, the H/S plot usually shows the perfect correlation which in fact is essentially correlation between two derivatives of the same entity measured in different scale. This implies the basic (albeit trivial) conclusion. If two quantities are the terms of the same linear equation, this is a necessary and sufficient condition for these two quantities to be linear correlated and use of equations 3 and 4 is misleading since actually we measure correlation between two derivatives of the same entity under different names.

Second, the phenomenon of EEC descends from the statistical artefacts when the data obtained over very narrow range of temperature are extrapolated to infinite temperature [4, 16, 24, 25]. Errors of incorrect statistics applied to the chemical data are the source of incorrect interpretation and EEC arises due to statistical reasons, that is, of mathematical origin, and no chemical reason needs be associated with it [24, 28]. The linear plots of the enthalpy-entropy data for which the correlation coefficient exceeds 0.95 is more often due to the propagation of measurement errors than to chemical variations. Most of the enthalpy-entropy compensation effects presented in the reviews on this subject appears to be indistinguishable from the statistical compensation effect [11, 22, 29]. The correlation would not be significant even at the 95% confidence level if $T_c - 2\sigma < T < T_c + 2\sigma$ where σ is the estimated standard error in T_c from the fit [16].

From the said above, Van't Hoff (3) and Eyring (4) equations cannot be used for simultaneous calculation of the values of H ($H^\ddagger$) and S ($S^\ddagger$) using one set of data. All the data obtained through this way should be dismissed as erroneous. Moreover, if Van't Hoff (3) equation can be used for entropy calculation in limited cases when G and H are measured through independent experiments (mostly using isothermal titration calorimetry (ITC)), the Eyring equation (4) cannot be used in any case due to impossibility to measure the value of activation energy (enthalpy) in independent experiments. Thus, the Eyring equation (4) should be excluded from the scientific use and the values of activation energy (enthalpy) should be calculated using the Arrhenius equation (2).

Third, since theoretically only calorimetric studies can provide data indicative of additional thermodynamic compensation, it is worth considering whether the calorimetric studies show any obvious evidence of such compensation.

2.2 Window Effect

The ITC experiment can give estimates like K_a (G) and H from one experiment with the subsequent calculation of S from the Van't Hoff plot. Analysis of the data confirming the existence of enthalpy-entropy compensation effect for more than 400 receptor-ligand complexes is provided using strictly ITC data [30]. This data was proclaimed as the first validation of the existence of EEC from an extensive set of ITC measurements of a diverse set of ligands binding to biological receptors. However, apparent correlation between H and TS can arise because the measured values of G tend to occupy a restricted range while H and S can vary over a much wider range - a phenomenon sometimes termed the "window effect" [3, 31]. For instance, the range of K_a values of 10^2 to 10^8 M^{-1} and corresponding G values of 10- 45 kJ mol^{-1} can be obtained from ITC measurements. Thus, the range of observable G values is experimentally limited and may give rise to apparently linear plots; and plots of H/S appear linear, with slope of T_c although there is no any compensation whatsoever. All experimental data collected so far, from a range of calorimetric studies including [30], fall within such windows.

2.3 Correlated Errors in H and $T S$ are the Reason for Appearance of EEC

Association of Biomolecular Resource Facilities (ABRF) Molecular Interactions Research Group (MIRG) developed a standardized model system and distributed it to a panel of ITC operators to assess inter-laboratory variability in analyzing a model system with readily measurable K_a and H values from ITC studies [32]. It was shown the precision in determining H is 24% and K_a is 22% which are much higher than those ($\pm 3\%$ estimates) reported for ITC instruments [33]. Error in K_a translated into error in G was only 0.13 kcal/mol or relative error of $\sim 1.6\%$ which compared with a 2.5 kcal/mol error in H ($\sim 24\%$) is negligible. Thus, the uncertainty in H dominates, resulting in an equal and opposite error in $T S$ followed by the appearance of artificial entropy-enthalpy compensation. The conclusion is made that it is meaningless to plot H and $T S$ versus one another due to their large correlated errors, unless caution is expressed in minimizing, performing quantitative calculations and propagating these errors [2].

2.4 Heat Capacity (Cp) and Temperature Effects

Usually observed large temperature dependencies of H and S upon protein-ligand interactions or protein folding are simply thermodynamic consequences of the heat capacity change (C_p effect) [3, 16] since

$$C_p = \frac{\delta H}{\delta T} = T \frac{\delta S}{\delta T}$$

The mere presence of a finite heat capacity can lead to entropy-enthalpy compensation effects with change in temperature [34] and thus, depending on the range of temperatures examined and the C_p change, a plot of H versus S for a series of experiments at different temperatures may be linear.

In the end, I cannot disagree with the conclusion made many years ago [35] that the correlation between H and S *often is of trivial character* and the result of improper application of some equations of equilibrium thermodynamics (page 83).

CONCLUSIONS

Enthalpy–entropy compensation (EEC) is mainly the trivial consequence of the basic thermodynamic laws.

Theoretically, there may be some extra thermodynamic compensation effect, which reflects something more than the known laws of statistical thermodynamics.

Up to date there is no any experimental evidences of existence of the extra-thermodynamic compensation.

Van' t Hoff and Eyring equations cannot be used as a single source for calculation of the enthalpy and entropy values. All the data obtained in this way should be recognized as erroneous.

Van' t Hoff equation can be used for entropy calculation in limited cases when G and H are measured through independent experiments using isothermal titration calorimetry (ITC). However even in this case the extra-thermodynamic compensation cannot be revealed due to “window effect” and the large correlated errors in enthalpy and entropy measurements.

Eyring equation cannot be used for calculation of the activation thermodynamic parameters in any case and should be excluded from scientific use. The value of activation energy (enthalpy) should be calculated using the Arrhenius equation

ACKNOWLEDGEMENTS

I thank Dr. A.I. Dragan for the stimulating and useful discussion. This work was supported by National Institute of General Medical Sciences Grant 5P01GM068036.

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