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Ultrasonic and Densimetric Characterization of the Association of Cyclic AMP with the cAMP-Binding Domain of the Exchange Protein EPAC1

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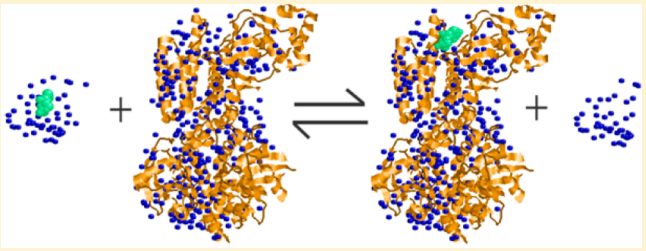
ABSTRACT: We employed a combination of densimetric and ultrasonic velocimetric techniques to characterize the volumetric properties of the association of the cAMP-binding domain (CBD) of EPAC1 with cAMP at 25 °C in a pH 7.6 buffer. The binding of cAMP to the CBD of EPAC1 is accompanied by changes in volume, ΔV, and adiabatic compressibility, ΔK_S, of -59 ± 4 cm^3 mol^{-1} and $(34 \pm 9) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively. We use these volumetric results in conjunction with the structural data to estimate a change in hydration, Δn_h, accompanying the binding. We calculate that approximately 103 water molecules are released to the bulk from the associating surfaces of the protein and the ligand. This number is ∼30% larger than the number of water molecules in direct contact with the associating surfaces while also being within the error of our Δn_h determination. Therefore, we conclude that cAMP binding to EPAC1 may involve, in addition to the waters from within the first coordination sphere, also some waters from the second coordination sphere of the protein and cAMP. Our analysis of the compressibility data reveals that the protein becomes more rigid and less dynamic upon the cAMP binding as reflected in a 4 ± 0.5% decrease in its intrinsic coefficient of adiabatic compressibility. Finally, we estimate the hydration, ΔS_{hyd}, and configurational, ΔS_{conf}, contributions to the binding entropy, ΔS_b. We find that the binding entropy is determined by the fine balance between the ΔS_{hyd} and ΔS_{conf} terms. In general, we discuss insights that are derived from a combination of volumetric and structural properties, in particular, emphasizing how measured changes in volume and compressibility can be interpreted in terms of hydration and dynamic properties of EPAC1 in its apo- and holo-forms.



INTRODUCTION

EPAC1 is a guanine nucleotide exchange factor for the small GTPases Rap1 and 2, which is directly activated by cAMP.[1,2] Rap proteins are GTP-binding proteins that serve as molecular switches between a GDP-bound inactive and a GTP-bound active state. EPAC1 acts by accelerating the slow intrinsic GDP dissociation from Rap, which subsequently associates with GTP. Two highly homologous isoforms of EPAC, namely, EPAC1 and EPAC2, which are found in mammalian cells, consist of a single polypeptide chain with an N-terminal regulatory and a C-terminal catalytic region.[3,4] The regulatory domain of EPAC1 contains an N-terminal dishevelled/Egl-10/pleckstrin (DEP) domain, followed by the cAMP-binding domain (CBD).[2] CBD is responsible for the cAMP-mediated EPAC activation, whereas the DEP domain is responsible for its membrane binding and subsequent localization.

The binding of cAMP to the CBD of EPAC1 or EPAC2 causes a hinge-like rotation of a C-terminal helix (α6) toward the β-barrel core characteristic of CBDs.[5,6] The binding and the associated structural reorganization of the protein are accompanied by a complex interplay of the enhanced and quenched dynamics of amino acid residues that are proximally as well as remotely located from the binding site in both the α and the β subdomains of the protein.[6] Importantly, our NMR studies have suggested that the cAMP-mediated alteration in the dynamic profile of the protein is involved in the entropically controlled allostery of the cAMP-dependent EPAC activation.[6−12]

While enabling one to map out the cAMP-dependent enhancement/quenching of the protein dynamics at residue resolution, NMR measurements alone do not readily provide the assessment of the global change in protein dynamics as reflected in the mean-square fluctuations of the protein volume, $\langle \delta V_M^2 \rangle$. The latter assessment is important, since $\langle \delta V_M^2 \rangle$ correlates with the configurational entropy of the protein in its apo- and holo-forms. The mean-square fluctuations of the protein volume, $\langle \delta V_M^2 \rangle$, is directly proportional to the intrinsic coefficient of isothermal compressibility, β_M, of the protein molecule[13,14]

$$\langle \delta V_M^2 \rangle = k_B T V_M \beta_M \quad (1)$$

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where k_B is Boltzmann's constant and T is the absolute temperature.

In this work, we study the volumetric properties of the association of the cAMP-binding domain (CBD) of EPAC1 (residues 149−318) with cAMP. To this end, we employ densimetric and ultrasonic velocimetric measurements to determine changes in volume, ΔV, and adiabatic compressibility, ΔK_S, accompanying the binding of cAMP to EPAC1. We use these results in conjunction with the available structural data on the highly homologous EPAC2−cAMP complex to estimate changes in protein dynamics and hydration linked to the binding event.

The dynamic and hydration changes mapped here for the CBD of EPAC1 will also serve as a foundation for understanding cAMP recognition in other cAMP-dependent systems controlled by structurally homologous CBDs, such as protein kinase A (PKA) and the hyperpolarization activated and cyclic-nucleotide gated (HCN) ion channels.[15,16] It should be noted in this regard that all macromolecular events taking place in living organisms are accompanied by alterations in solute−solvent interactions (hydration). The hydration-related energetics provides a major contribution to the driving force in macromolecular events, including protein and nucleic acid folding and binding reactions.[17] Despite the significance of their contribution to the energetics of macromolecular folding and binding events, hydration changes are difficult to quantify, while evaluation of the hydration-related thermodynamic profile is even a more formidable task. This situation is unfortunate, since it lowers our ability to predict the conformational preferences of biopolymers as well as the affinity and specificity of their recognition reactions. One approach to tackling this problem is to establish hydration libraries on macromolecular folding and binding and to globally correlate these libraries with the energetics of individual events. Changes in hydration for specific macromolecular reactions can be quantified, for example, by the judicious use of volumetric measurements in conjunction with structural data on the system under question.[18−25] The work presented here on EPAC1 is a step in this direction and provides insights into the relative configurational and hydration determinants of ligand-binding entropies.

MATERIALS AND METHODS

Materials. The cAMP-binding domain of human EPAC1 including residues 149−318 was expressed and purified according to protocols that have been published.[26] cAMP was purchased from Sigma Aldrich Canada (Oakville, Ontario, Canada). All measurements were performed in a pH 7.6 buffer consisting of 20 mM Tris and 50 mM NaCl.

The concentration of EPAC1 was determined from the absorbance measured at 25 °C with a Cary 300 Bio spectrophotometer (Varian Canada, Inc., Mississauga, ON, Canada) using a molar extinction coefficient ε_{280} = 12 490 M^{-1} cm^{-1}. The latter has been calculated according to the additive scheme proposed by Pace et al.[27] In densimetric and ultrasonic velocimetric experiments reported in this work, the protein concentration was on the order of ∼1 mg/mL (∼60 μM).

Ultrasonic and Densimetric Measurements. All densimetric and ultrasonic velocimetric measurements reported here were carried out at 25 °C. Densities were measured using a vibrating tube densimeter (DMA-5000, Anton Paar, Graz, Austria). The precision of density measurements was ±1.5 × 10^{-6} g cm^{-3}. The partial molar volume, $V°$, of the protein was calculated from the relationship

$$V° = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C) \quad (2)$$

where ρ and ρ_0 are the densities of the protein solution and the solvent, respectively, and M and C are the protein's molecular weight (19.2 kDa) and molar concentration, respectively.

The partial molar adiabatic compressibility, $K_S°$, was calculated from the densimetric and ultrasonic data (see below) using the expression[28−30]

$$K_S° = \beta_{S0}(2V° - 2[U] - M/\rho_0) \quad (3)$$

where $[U]$ is the relative molar sound velocity increment, $[U] = (U - U_0)/(U_0 C)$, U and U_0 are the sound velocities in the protein solution and the neat solvent, respectively, and β_{S0} is the coefficient of adiabatic compressibility of the solvent.

The sound velocities in protein solutions were determined at ∼7.2 MHz by analyzing the amplitude-frequency characteristics of an ultrasonic resonator as described previously.[31−33] The analysis of the frequency characteristics of the resonator was performed by a Hewlett-Packard model E5100A network/spectrum analyzer (Mississauga, Ontario, Canada).

Densimetric and ultrasonic titrations were performed at 25 °C by adding aliquots of cAMP to EPAC1 solution following previously described experimental protocols.[34,35] All experiments have been performed at least three times with the average values of $[U]$ and $V°$ being used for $K_S°$ determination.

Determination of Intrinsic Volumes and Solvent-Accessible Surface Areas. The complex of the CBD of EPAC1 (residues 149−318) with cAMP has not been structurally characterized. However, the structure of the highly homologous isoform EPAC2 associated with cAMP has been solved by X-ray crystallography.[5] The atomic coordinates of the cAMP-EPAC2 complex are available from the RSCB Protein Data Bank (http://www.rcsb.org) (PDB entry 3CF6). We use this structure to calculate the solvent-accessible surface areas, S_A, and intrinsic volumes, V_M, for the complex, apoprotein (the complex minus cAMP), and free cAMP (the complex minus the protein). We calculated the solvent-accessible surface area, S_A, for each structure as the sum of the accessible surface areas of all atoms in the structure. The intrinsic volumes, V_M, of apoEPAC2, free ligand, and the ligand−protein complex were calculated as molecular volumes as described by Richards.[36,37]

The PDB file was stripped of water molecules and cleaned using VMD (version 1.9.1) on a Linux platform. The program MSP (Molecular Surface Package) Version 3.9.3 was obtained from Dr. Michael Connolly at www.biohedron.com and used to calculate the solvent-accessible surface area and molecular volume for each structure, using a 1.4 Å probe radius on a Linux platform.

RESULTS AND DISCUSSION

Binding Affinity. Figures 1 and 2 present, respectively, the changes in volume, ΔV, and relative molar sound velocity increment, $\Delta[U]$, of EPAC1 as a function of the ligand-to-protein binding ratio, r. The binding profiles in Figures 1 and 2 have been approximated by an analytical function representing a one-to-one stoichiometric binding reaction[25]

$$X = X_0 + \alpha\Delta X \quad (4)$$

where X is a binding-dependent observable (in this case, volume or relative molar sound velocity increment), X_0 is the

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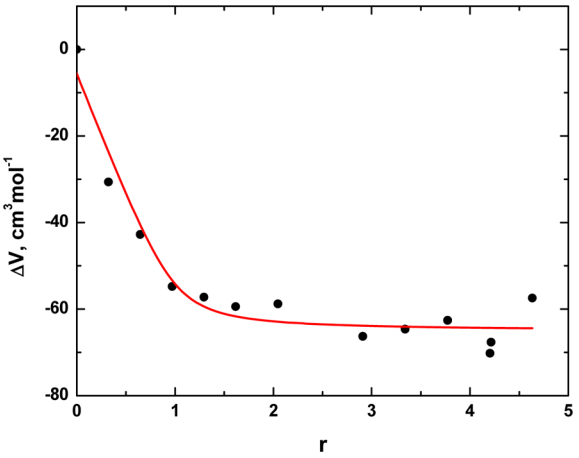

Figure 1. Change in the partial molar volume of the CBD of EPAC1 plotted against the cAMP-to-protein molar ratio, r. The initial concentration of CBD is 60 μM. The experimental points are fitted using eq 4 (solid line).

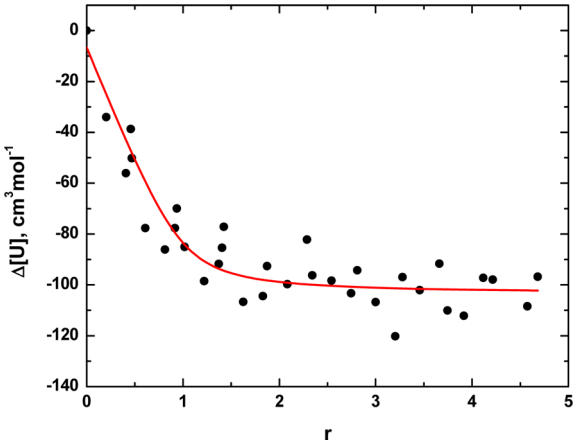

Figure 2. Change in the relative molar sound velocity increment of the CBD of EPAC1 plotted against the cAMP-to-protein molar ratio, r. The initial concentration of CBD is 60 μM. The experimental points are fitted using eq 4 (solid line).

initial value of X in the absence of the ligand, ΔX is the maximum change in X upon protein saturation with the ligand, and $\alpha = [PL]/[P_T]$ is the fraction of the ligated protein. The latter is computed as

$$\alpha = 0.5(r + 1) + Y^{-1} - [0.25(r - 1)^2 + (r + 1)/Y + Y^{-2}]^{1/2} \quad (4a)$$

where $Y = 2K_b[P_T]$, $K_b = [PL]/([P][L])$ is the binding constant, $[PL]$ is the concentration of the cAMP–EPAC1 complex, $[P]$ is the concentration of the apoprotein, $[P_T] = [PL] + [P]$ is the total concentration of the protein, and $[L]$ is the concentration of the free ligand.

Table 1 lists our evaluated changes in volume, ΔV, relative molar sound velocity increment, $\Delta[U]$, and adiabatic compressibility, $\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U])$ (see eq 3), accompanying the complexation of cAMP with EPAC1. Table 1 also presents the association constants, K_b, evaluated from fitting the volume and sound velocity binding profiles. The two binding constants, K_b, are in excellent agreement with each other, while also being in close agreement with the published binding constants determined using isothermal titration calorimetry and a competitive [^{3}H]cAMP radiolabel assay.[38,39] The agreement lends credence to our experimental protocols and obtained results.

Table 1. Thermodynamic Characteristics of the Association of cAMP with the CBD of EPAC1

ΔV, cm^3 mol^{-1}	−59 ± 4
$\Delta[U]$, cm^3 mol^{-1}	−97 ± 5
ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}	34 ± 9
K_b (volume), 10^5 M^{-1}	3.9 ± 3.0
K_b (sound velocity), 10^5 M^{-1}	3.7 ± 2.1

A Change in Hydration. In this section, we use the change in volume, ΔV, in conjunction with structural information to quantify a change in hydration accompanying the cAMP–EPAC1 binding. To this end, we use the empirical protocols reported previously.[25] The structural data on the cAMP–EPAC2 binding are presented in Table 2. A change in volume associated with a ligand–protein binding event can be parsed into the intrinsic, ΔV_M, thermal, ΔV_T, and interaction, ΔV_I, contributions:[22,25]

$$\Delta V = \Delta V_M + \Delta V_T + \Delta V_I \quad (5)$$

Table 2. Molecular Volumes, V_M, and Solvent Accessible Surface Areas, S_A, of the cAMP–EPAC1 Complex, apoEPAC1, and Free cAMP

protein	S_A, Å^2	V_M, cm^3 mol^{-1}
complex	7957	11 600
apoEPAC1	8155	11 350
cAMP	480	168

The intrinsic volume, V_M, of a solute is the volume inaccessible to any part of a spherical probe with a radius of 1.4 Å that is rolled over the surface of a solute.[36,37] The thermal volume, V_T, is defined as the volume of the effective void created around the solute due to thermally induced mutual vibrational motions of solute and solvent molecules as well as steric and structural effects.[40−42] The interaction volume, V_I, is the change in volume due to solute–solvent interactions.[41]

A change in the intrinsic contribution, ΔV_M, of 82 cm^3 mol^{-1} (11 600 − 11 350 − 168) is the difference between the molecular volumes of the holoprotein (11 600 cm^3 mol^{-1}) and the sum of the molecular volumes of the apoprotein (11 350 cm^3 mol^{-1}) and the free ligand (168 cm^3 mol^{-1}) (presented in Table 2). As a first approximation, a change in thermal volume, ΔV_T, is proportional to the change in solvent accessible surface area, ΔS_A:[25]

$$\Delta V_T = \delta_L \Delta S_{AL} + \delta_P \Delta S_{AP} \quad (6)$$

where δ_L and δ_P are the thicknesses of thermal volume of the ligand and the protein, respectively, and ΔS_{AL} and ΔS_{AP} are the changes in the solvent accessible surface area of the ligand and the protein, respectively (note that $\Delta S_A = \Delta S_{AL} + \Delta S_{AP}$).

Using a δ_L of 0.6 Å (the estimate for small molecules[41−43]) and a δ_P of 1.0 Å (the estimate for proteins[42,44,45]) and assuming $\Delta S_{AL} = \Delta S_{AP} = 0.5\Delta S_A$, a change in thermal volume, ΔV_T, is given by $\Delta V_T = 0.5(\delta_L + \delta_P)\Delta S_A = 0.8\Delta S_A$.[25] The change in solvent-accessible surface area, ΔS_A, equals −678 Å^2 (7957 − 8155 − 480) as can be calculated from the solvent-accessible surface areas of the holoprotein, apoprotein, and cAMP presented in Table 2. The change in thermal volume,

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ΔV_T, accompanying the binding of cAMP to EPAC1 is, thus, equal to −542 Å^3 (−0.8 × 678) or −326 cm^3 mol^{-1}. With the estimates of ΔV_M and ΔV_T, a change in interaction volume, ΔV_I, can be determined from eq 6 to be 185 ± 4 cm^3 mol^{-1} (−59 − 82 + 326).

A change in interaction volume, ΔV_I, reflects redistribution of water molecules between the bulk and hydration phases. The value of ΔV_I is given by the sum

$$\Delta V_I = \sum_i \Delta n_{hi}(V_{hi} - V_0) \quad (7)$$

where Δn_{hi} is the number of water molecules taken up by the ith solvent-exposed domain of the ligand or the protein upon their association, and V_{hi} and V_0 are the partial molar volumes of water of hydration of the ith solute domain and bulk water, respectively. Under the assumption of the uniformity of the hydration shells of the ligand and the protein, eq 7 simplifies to the form $\Delta V_I = \Delta n_h(V_h - V_0)$, from which $\Delta n_h = \Delta V_I/(V_h - V_0)$. The partial molar volume of water solvating proteins and nucleic acids, V_h, is roughly 10% smaller than that of bulk water.[46−48] Assuming $(V_h - V_0) = -1.8$ cm^3 mol^{-1}, the value of Δn_h is −103 ± 2 (−185/1.8). Thus, we conclude that, although the crystal structure of activated EPAC suggests that at least three water molecules remain trapped at the cAMP/EPAC interface,[5] approximately 103 water molecules become released to the bulk from the hydration shells of cAMP and EPAC1 following their association.

A note of caution is in order regarding the uncertainty of the determined number of released water molecules (±2). It solely reflects the experimental error of our measured changes in volume, ΔV, and does not include any error related to the structurally derived changes in intrinsic volume, V_M, and solvent accessible area, S_A. Presently, it is difficult to estimate the error of V_M and S_A determination as only a single structure of the cAMP−EPAC complex is available in the RSCB Protein Data Bank. On the basis of our previous works on protein−ligand association,[18,20,25] the real error of Δn_h determination is within the 25−50% range (i.e., ±25−50).

Our determined number of water molecules released to the bulk, 103, is ∼30% larger than 75, the number of water molecules in direct contact with the associating surfaces. The latter can be evaluated by dividing the net change in solvent-accessible surface area, ΔS_A, of 678 Å^2 by 9 Å^2, the effective cross-section of a water molecule. The difference of 30% is within the error of our Δn_h determination of 25−50%. Thus, we conclude that cAMP binding may involve, in addition to the waters from within the first coordination sphere, also some waters from the second coordination sphere of the protein and cAMP.

Water molecules solvating proteins are highly heterogeneous with respect to their structural, dynamic, and thermodynamic properties.[49−52] Consequently, the thermodynamic impact of the 103 released water molecules is difficult to quantify in terms of the accompanying changes in free energy, enthalpy, and entropy. However, as we have previously estimated based on Makhatadze's and Privalov's results,[53] water molecules solvating all functional protein groups exhibit, at room temperature, a similar partial molar entropy that is 1.3 ± 0.4 cal mol^{-1} K^{-1} smaller than that of bulk water.[18] By multiplying this number by Δn_h, of 103 we calculate a highly favorable hydration contribution to the binding entropy, ΔS_{hyd}, of 134 ± 41 cal mol^{-1} K^{-1} (1.3 × 103). As shown below, the favorable entropic contribution of hydration is offset by an unfavorable change in configurational entropy of the protein and the ligand.

A Change in Protein Dynamics. Protein dynamics is reflected in the mean-square fluctuations of the intrinsic volume, $\langle \delta V_M^2 \rangle$, which is proportional to the intrinsic coefficient of isothermal compressibility, β_M, of the protein (see eq 1). To estimate a change in β_M brought about by EPAC1 association with cAMP, we use our measured change in compressibility, ΔK_S, in conjunction with the estimated number of water molecules released to the bulk, Δn_h. Note that ΔK_S can be presented as the sum of the intrinsic, ΔK_M, and hydration, ΔK_h, contributions:

$$\Delta K_S = \Delta K_M + \Delta K_h \quad (8)$$

Under the assumption of the uniformity of the hydration shells of the ligand and the protein, we obtain $\Delta K_h = \Delta n_h(K_h - K_0)$. The partial molar adiabatic compressibility of water solvating proteins and nucleic acids, K_h, is roughly 15−20% smaller than that of bulk water (8.1 × 10^{-4} cm^3 mol^{-1} bar^{-1}).[48,54] With Δn_h of −103 ± 2 and $(K_h - K_0) = -1.3 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, the value of ΔK_h is (134 ± 3) × 10^{-4} cm^3 mol^{-1} bar^{-1} (103 × 1.3 × 10^{-4}). From eq 8, we estimate a change in the intrinsic compressibility of EPAC1, ΔK_M, of −(100 ± 10) × 10^{-4} cm^3 mol^{-1} bar^{-1} (34 × 10^{-4} − 134 × 10^{-4}). Given $\Delta K_M = \beta_M \Delta V_M + V_M \Delta \beta_M$ and assuming $\Delta V_M \approx 0$, we obtain $\Delta \beta_M = \Delta K_M / V_M$. With V_M of 11 350 cm^3 mol^{-1}, we calculate $\Delta \beta_M$ of −(0.9 ± 0.1) × 10^{-6} bar^{-1} (100 × 10^{-4}/11 350). Thus, the binding of EPAC1 to cAMP renders the holoprotein 4 ± 0.5% (0.9 × 10^{-6}/25 × 10^{-6}) less compressible compared to the apoprotein. Similar decreases in the intrinsic compressibilities of 1, 4, and 5% have been observed for hexokinase upon its association with glucose,[20] lysozyme upon its binding to $(GlcNAc)_3$,[25] and ribonuclease A caused by its complexation with 2′- or 3′-CMP,[18] respectively.

According to eq 1, the observed 4% decrease in β_M reflects a similar decrease in the mean-square fluctuations of the intrinsic volume, $\langle \delta V_M^2 \rangle$ (or 2% decrease in δV_M). The ligand-bound state of EPAC1 is, thus, more rigid and less dynamic compared to its unbound state. As shown below, the change in the protein dynamics correlates with a decrease in configurational entropy.

A Change in Configurational Entropy. Thermodynamic studies have characterized the association of EPAC1 with cAMP as an exothermic process with a binding affinity, K_b, of ∼3.3 × 10^5 M^{-1} (ΔG_b = −7.5 kcal mol^{-1}).[38,55] Our estimate of the binding enthalpy, ΔH_b, based on the isothermal titration calorimetric binding profiles presented by Rehmann et al.[38] is between −8 and 10 kcal mol^{-1}. Thus, the cAMP−EPAC1 binding is accompanied by an unfavorable change in entropy, $\Delta S_b = (\Delta H_b - \Delta G_b)/T$, of −2 to −8 cal mol^{-1} K^{-1} (−5 ± 3 cal mol^{-1} K^{-1}). A change in entropy, ΔS_b, for a ligand−protein association event can be presented as a sum of intrinsic (configurational), hydration, and translational terms[56]

$$\Delta S_b = \Delta S_{conf} + \Delta S_{hyd} + \Delta S_{rt} \quad (9)$$

where ΔS_{conf} is the change in the configurational entropy of the ligand and the protein, ΔS_{hyd} is the contribution due to a change in the hydration of the ligand and the protein, and ΔS_{rt} is the change in entropy due to the loss of rotational and translational degrees of freedom upon the binding. For a 1:1 stoichiometric binding, ΔS_{rt} equals −8 cal K^{-1} mol^{-1}.[56]

Given our estimated hydration contribution, ΔS_{hyd}, of 134 ± 41 cal mol^{-1} K^{-1}, the estimated change in configurational entropy, ΔS_{conf}, of the ligand and the protein is −131 ± 41 cal

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K^{-1} mol^{-1} (−5 − 134 + 8). Note that ΔS_{conf} is the sum of the changes in the configurational entropies of the ligands, $\Delta S_{conf}(L)$, and the protein, $\Delta S_{conf}(P)$. For a small nonpeptide ligand, $\Delta S_{conf}(L)$ is a linear function of the number of rotatable bonds (N_{rb}) and the total number of atoms (N_{atoms}):[56]

$$\Delta S_{conf}(L) = -1.76N_{rb} + 0.414N_{atoms} \quad (10)$$

With this relationship, we calculate the value of $\Delta S_{conf}(L)$ for cAMP to be −44 cal K^{-1} mol^{-1}. Thus, we estimate the binding-induced change in the configurational entropy of EPAC1, $\Delta S_{conf}(P)$, to be unfavorable and equal to −87 ± 41 cal K^{-1} mol^{-1} (−131 + 44). The configurational contribution of the binding free energy, $-T\Delta S_{conf}(P)$, is 26 ± 12 kcal mol^{-1}, which is 3.5 times as high in magnitude as the binding free energy, ΔG_b, of 7.5 kcal mol^{-1}.

Configurational entropy is a major determinant of protein association events.[56−63] In absolute value, ΔS_{conf} is, generally, larger than the entropy of a protein-binding reaction, ΔS_b, with the latter being governed by a fine balance between the large and opposing hydration and configurational contributions.[59] Consequently, determination of the ΔS_{conf} and ΔS_{hyd} contributions is required for developing empirical and/or theoretical predictive algorithms regarding the affinity and specificity of protein recognition events. Nevertheless, experimental determination of ΔS_{conf} is difficult. The empirical approach described in this study is a step in that direction that enables one to estimate the order of magnitudes of ΔS_{conf} and ΔS_{hyd} and the degree of their compensation.

CONCLUDING REMARKS

EPAC1 is a guanine nucleotide exchange factor for the small GTPases Rap1 and 2, which is directly activated by cAMP. In this work, we present a volumetric characterization of the association of the cAMP-binding domain (CBD) of EPAC1 with cAMP. We discuss the conceptual basis for resolving macroscopic properties (volume and compressibility) into microscopic events (protein hydration and dynamics). Our volumetric analysis performed in conjunction with the structural data on the cAMP/EPAC complex reveals that around 103 water molecules become released to the bulk from the interacting surfaces of the protein and the ligand. We find that the holoprotein is more rigid and less dynamic compared to its apo-form as reflected in a 4% decrease in its intrinsic coefficient of adiabatic compressibility. Our results enable us to estimate the favorable hydration contribution, ΔS_{hyd}, and the unfavorable configurational contribution, ΔS_{conf}, to the binding entropy, ΔS_b. The sign and magnitude of ΔS_b are determined by the fine balance between the ΔS_{hyd} and ΔS_{conf} terms.

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Notes

The authors declare no competing financial interest.

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