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Fragment molecular orbital (FMO) study on stabilization mechanism of neuro-oncological ventral antigen (NOVA)–RNA complex system

Ikuo Kurisaki [a,*], Kaori Fukuzawa [b], Tatsuya Nakano [c], Yuji Mochizuki [d], Hirofumi Watanabe [a,e], Shigenori Tanaka [a,e,**]

[a] *Graduate School of Engineering, Kobe University, 1-1, Rokkodai, Nada, Kobe 657-8501, Japan*
[b] *Mizuho Information and Research Institute, Inc., 2-3, Kanda Nishiki-cho, Chiyoda-ku, Tokyo 101-8443, Japan*
[c] *Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[d] *Department of Chemistry and Research Center for Smart Molecules, Faculty of Science, Rikkyo University, 3-34-1, Nishi-ikebukuro, Toshima-ku, Tokyo 171-8501, Japan*
[e] *Japan Science and Technology Agency, CREST, Japan*

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ABSTRACT

We report the molecular mechanism of protein–RNA complex stabilization based on the electronic state calculation. Fragment molecular orbital (FMO) method based quantum mechanical calculations were performed for neuro-oncological ventral antigen (NOVA)–RNA complex system. The inter-molecular interactions and their effects on the electronic state of NOVA were examined in the framework of *ab initio* quantum calculation. The strength of inter-molecular interactions was evaluated using inter-fragment interaction energies (IFIEs) associated with residue–RNA base and residue–RNA backbone interactions. Under the influence of inter-molecular interactions, the change of electronic state of NOVA upon the complex formation was examined based on IFIE values associated with intra-NOVA residue–residue interactions and the change of atomic charges by each residue. The results indicated that non-specifically recognized bases contributed to the stability of the complex as well as specifically recognized bases and that the secondary structure of NOVA was remarkably associated with the change of electronic state upon the complex formation.

1. Introduction

The RNA-binding proteins (RBPs) express their function and play essential roles in the RNA metabolism when they bind to the target RNA molecules [1]. The mechanism of stable complex formation with RNA can be an important issue to understand how an RBP acts on only its targets. Since the 1990s, more than 600 RBP–RNA complex structures have been resolved, and the observations of atomic details of RBP–RNA interactions and of global structural change upon complex formation have provided qualitative insights into the mechanism of complex stabilization [2–6].

In the light of achievement of the structural biology, atomic simulations of biomacromolecules have been employed to obtain quantitative and qualitative understanding of the molecular mechanism based on observation of atomic structure of RBP–RNA complexes. Classical molecular dynamics (MD) studies succeeded in revealing the mechanisms for the difference of binding free energies by mutating RNA bases or amino acid residues [7–10], the inter-molecular interaction based on hydrogen bond formation [11], the analysis of network of intra-molecular interaction [12], the molecular dynamics of the huge molecular machine complex [13,14], the conformational rearrangement in the catalytic reaction [15] and the effect of RNA binding on the structure of RBP [16,17].

Ab initio quantum mechanics (QM) calculations are employed to investigate those problems which classical MD simulations cannot work out or treat, such as reliable estimations of energies associated with the van der Waals interactions and the explicit treatment of quantum effect (e.g. inter- and intra-molecular charge transfer and charge redistribution). However, high-level *ab initio* QM calculations require unrealistically enormous computation time even for a system consisting of less than 1000 atoms. Thus, the application of QM calculations have been limited to the local structure cut out from the whole structure around the binding interface of RBP–RNA complex [18,19]. The effects from the rest of the structure have often been ignored there, although they are often indispensable to understand the molecular mechanism of stabilization of complex formation.

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[*] Corresponding author. Present address: Research Organization of Science and Engineering, Ritsumeikan University, 1-1-1, Noji-Higashi, Kusatsu 525-8577, Japan. Tel.: +81 78 803 7991; fax: +81 77 561 5203/5077/78 803 7761.

[**] Corresponding author at: Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada, Kobe 657-8501, Japan. Tel.: +81 78 803 6620; fax: +81 78 803 7761.

E-mail addresses: kurisaki@insilico.h.kobe-u.ac.jp, kurisaki@fc.ritsumei.ac.jp (I. Kurisaki), kaori.fukuzawa@mizuho-ir.co.jp (K. Fukuzawa), nakano@nihs.go.jp (T. Nakano), fullmoon@rikkyo.ac.jp (Y. Mochizuki), watanabe@radix.h.kobe-u.ac.jp (H. Watanabe), tanaka2@kobe-u.ac.jp (S. Tanaka).

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Thus, in this study, we performed QM calculations for whole RBP–RNA complex to elucidate the mechanism of complex stabilization from the aspect of electronic state. To circumvent the difficulty in computational cost of conventional QM calculations, we employed fragment molecular orbital (FMO) method developed by Kitaura et al. [20–23].

The FMO method has realized the QM calculation of biomacromolecule consisting of over 1000 atoms within realistic computation time with keeping high accuracy as well as conventional *ab initio* QM calculations. This method has been improved successively [24] and applied to many biomacromolecules [25–30].

To consider the problem of protein–RNA complex stabilization, we selected neuro-oncological ventral antigen (NOVA) as a model system. NOVA contains three K-homology (KH) domains [31,32] which are known as one of canonical RNA-binding domains. One of NOVA KH domains (NOVA-2 KH3) bound to an RNA has been resolved by X-ray crystallographic analysis [33] (Fig. 1(A)). The system consists of ca. 2000 atoms, which is a reasonable size concerning computational cost. Upon the complex formation with RNA, NOVA-2 KH3 specifically recognizes the "UCAY" (Y: pyrimidine base) quartet [36] (Fig. 1(B)). An observation of the X-ray structure indicates that specific and non-specific amino acid residue–RNA base interactions and pinioning an RNA by the GxxG motif and the variable loop, named as "molecular vise", are also important for stabilization of complex formation (Fig. 1(C)). Note that NOVA-2 KH3 is denoted by NOVA below, for simplicity.

For the NOVA–RNA system, we performed FMO calculations. The inter-molecular interactions and their effects on the electronic state of NOVA were quantitatively analyzed to elucidate the molecular mechanism of complex stabilization. The strength of intermolecular interactions was estimated based on inter-fragment interaction energy (IFIE), FMO-derived effective interaction energy between fragments. The contributions of specific- and non-specific-interactions were quantitatively discussed. Furthermore, the effects of these interactions on the electronic state of NOVA were examined in terms of the change of atomic charges and the IFIEs between amino acid fragments. The relationship between structure and the change of electronic state upon the complex formation was thus discussed.

2. Materials and methods

2.1. Preparing structure for FMO calculation

We downloaded NOVA–RNA complex structure [33] (PDBID: 1EC6) from Protein Data Bank (PDB) [37] and used it for FMO calculations. In this PDB file, two NOVA–RNA complex structures are registered. We selected the complex consisting of chain A and D because it has no missing residues. The chain A is NOVA-2 KH3 and the chain D is RNA bound to NOVA.

Generally, position of hydrogen atom cannot be identified by X-ray crystallography. Thus, the structure was complemented and energetically optimized by the following procedure. At first, hydrogen atoms were added to NOVA–RNA complex structure using LEaP module of Amber8 [38] and the structure was optimized by molecular mechanics (MM) calculation with AMBER force field 99 parameters [39]. In the optimization procedure, (1) only hydrogen atoms were first optimized in vacuum and then (2) all atoms were optimized in the generalized Born solvation model developed by Hawkins et al. [40,41]. The heavy atoms (i.e. not hydrogen atom) were constrained to be pulled back by the harmonic potential to the initial position, where the force constant was set to 25 kcal/mol Å^2 to avoid artificial hydrogen bond formation between fragments. In each optimization process, 45,000 steps of conjugate gradient (CG) method followed 5000 steps of steepest descent (SD) method and all abnormal contacts between atoms were removed. These calculations were performed under the free boundary condition.

We performed molecular dynamics (MD) simulations for the NOVA–RNA complex. Periodic boundary condition was set for rectangle box. The box was set so that the minimum distance from the box face to molecule surface is 12 Å. The system was solvated by adding TIP3P model water [42] in the box. The system was electrically neutralized by adding 14 sodium ions. Amber force field 99 SB parameter [43] was used to compute forces acting between atoms. The Particle Mesh Ewald method was used to calculate long-range interactions employing 12 Å as real-space cutoff. The time step for integration was set to 1 fs. The initial velocities were assigned from a Maxwellian distribution at the temperature of thermostat. The vibrational motions associated with a hydrogen atom were frozen

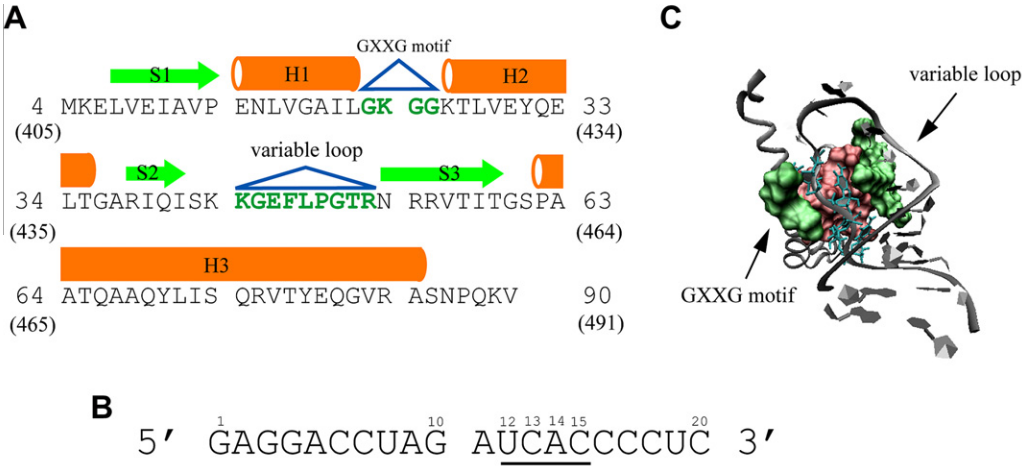

Fig. 1. Primary and secondary structures of NOVA-2 third KH domain, tertiary structure of NOVA-2 third KH domain–RNA complex and the bound RNA sequence. (A) Primary and secondary structures of NOVA-2 containing third KH domain (residues #10–72) and C-terminal region (residues #73–90). The numbers in the parenthesis are the original residue numbers of full sequence. The locations of GXXG motif (residues #22–25) and variable loop (residues #43–52) are indicated by green bold letter. Orange cylinders and green arrows represent α-helix and β-sheet, respectively. (B) The RNA sequence in the crystal structure of NOVA–RNA complex (PDBID: 1EC6). The digits on the symbols of RNA base represent the position in the RNA. The specifically recognized sequence is indicated by underscore. (C) Tertiary structure of NOVA-2 third KH domain and the bound RNA (PDBID: 1EC6) [33]. Gly-X-X-Gly motif and variable loop, RNA binding interface of the KH domain and specifically recognized bases are indicated by green surface, red surface and blue stick, respectively. The secondary structure is defined by using "STRIDE" program [34]. The molecular graphics here and below are drawn by "Visual Molecular Dynamics (VMD)" [35] software. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)