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Revealing the peptide presenting process of human leukocyte antigen through the analysis of fluctuation

Saeko Yanaka[1,7], Takamasa Ueno[2,3], Kouhei Tsumoto[1,4,5] and Kenji Sugase[6]

[1]*Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo 108-8639, Japan*
[2]*Center for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan*
[3]*International Research Center for Medical Sciences (IRCMS), Kumamoto University, Kumamoto 860-0811, Japan*
[4]*Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Tokyo 108-8639, Japan*
[5]*Medical Proteomics Laboratory, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan*
[6]*Bioorganic Research Institute, Suntory Foundation for Life Sciences, Osaka, 618-8503, Japan*
[7]*Present address: Bioorganic Research Institute, Suntory Foundation for Life Sciences, Osaka, 618-8503, Japan*

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Structural fluctuation on microsecond to millisecond time scales has been reported to play an important role in proteins that undergo significant structural change during their expression of function. In these proteins, the structural change was obvious in the crystal structures. However, protein motions in solution could contribute to the function of proteins, even if no significant structural difference is observed in crystal structure of different states while they function. In this review, we introduce our recent report on the stabilization mechanism of human leukocyte antigen, and the possibility of fluctuation contributing to several biophysical properties of proteins.

Key words: Major Histocompatibility Complex (MHC), NMR, thermodynamics, protein dynamics, protein stability

Fluctuation in proteins

Proteins move in their environment to express their function. Motions exist in different time scales, where they contribute to different functions. Especially important biochemical reactions exist on microsecond to millisecond time scales, and the motions at these time scales are called "fluctuation" in the field of structural biology. The analysis of protein motion in these timescales would be important for revealing the mechanism of chemical reactions occurring in living things. NMR is a powerful tool for studying motions at different time scales (Fig. 1). Among them, relaxation dispersion is an effective method for a quantitative measurement of fluctuation. Relaxation dispersion enables the detection of structures, which exists only in a few percentages. Since around 2000, several reports emerged on the precise reaction steps of proteins using relaxation dispersion. For example, it was revealed that an intrinsically disordered protein forms a transient intermediate structure during binding to its partner. The structure was different from the final bound structure, and was defined as encounter complex [1]. Another example is about an enzyme's reaction. Wright and his colleagues reported that the time scale of the catalytic reaction and the fluctuation of the active loop was correlated in dihydrofolate reductase, and that the reaction was inhibited when the active loop was mutated to a non-fluctuating form [2]. These examples show the importance of fluctuation on functions for proteins that are known to undergo large structural change when they function. However, the role of fluctuation has not been investigated for proteins that showed no significant structural change in crystal structures during the steps of their function. In this review, we introduce the impact of fluctuation on an important immunological process, antigen presentation by human leucocyte antigen (HLA).

Corresponding authors: Kouhei Tsumoto, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. e-mail: tsumoto@k.u-tokyo.ac.jp; Sugase Kenji, Suntory Foundation for Life Sciences, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka, 618-8503, Japan. e-mail: sugase@sunbor.or.jp

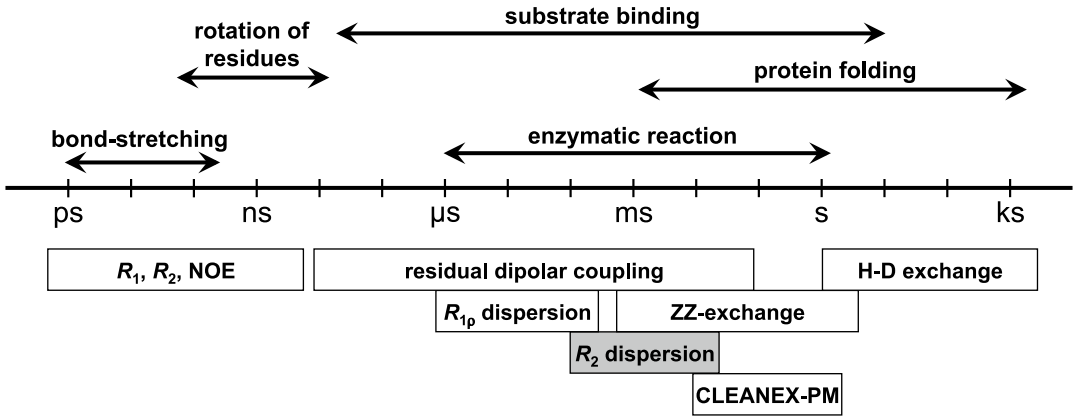

Figure 1 NMR measurements for protein motions. The graph was reprinted from seibutubuturi.

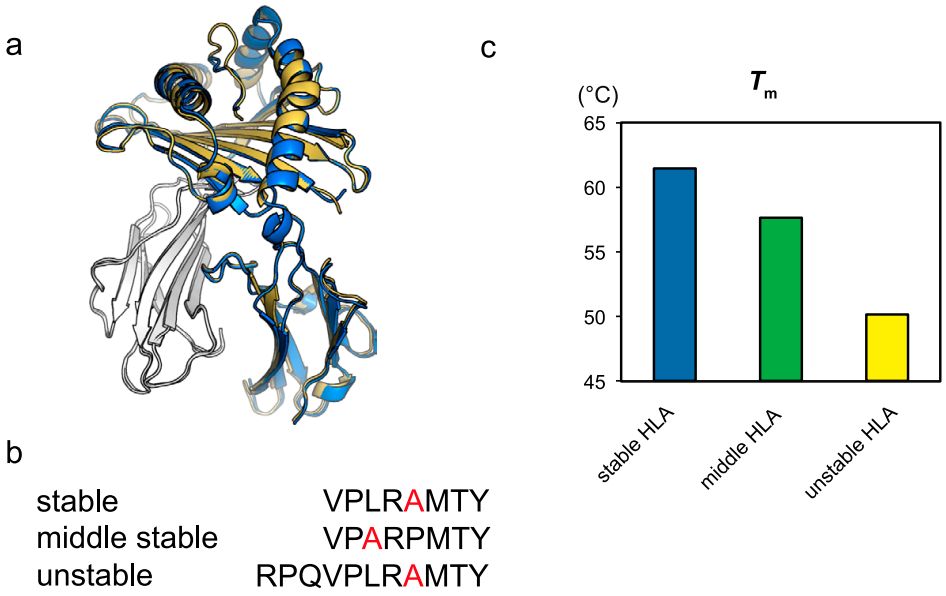

Figure 2 (a) Similarity of the crystal structures of stable HLA (blue) and unstable HLA (yellow). (b) The peptide sequences presented by the three HLAs (c) The graph shows the melting temperatures of the three pHLAs.

Introduction of HLA

HLA plays an important role in infection defense. HLA presents many kinds of antigenic peptides to cognate T cell receptors (TCR), leading to activation of the immune system. Each peptide presented by HLA is recognized by different set of TCRs. For effective activation of the immune system, the proper and stable presentation of peptides by HLA is essential. In our previous researches [3], we have investigated the stability of HLA and reported that the stability of HLA drastically changed by the presented peptide. However, the crystal structures of HLAs are very similar, and thus the stability difference is difficult to explain [3] (Fig. 2). Thus, we postulated that the difference in stability is caused by the difference of motion in solution, and conducted relaxation dispersion analysis using NMR. In our experiment, we used three HLAs presenting different peptides, which has different thermo-stabilities (Fig. 2). Although the three peptides are highly similar in its structure, the melting temperature of the HLA binding them vary by more than 5°C.

The relation of HLA fluctuation and stability

We compared the fluctuation profile of the stable, middle, and unstable HLA using relaxation dispersion. The results showed that the fluctuating residues are concentrated in the peptide-binding domain. We observed a greater extent of fluctuation to the minor state in the stable HLA compared to

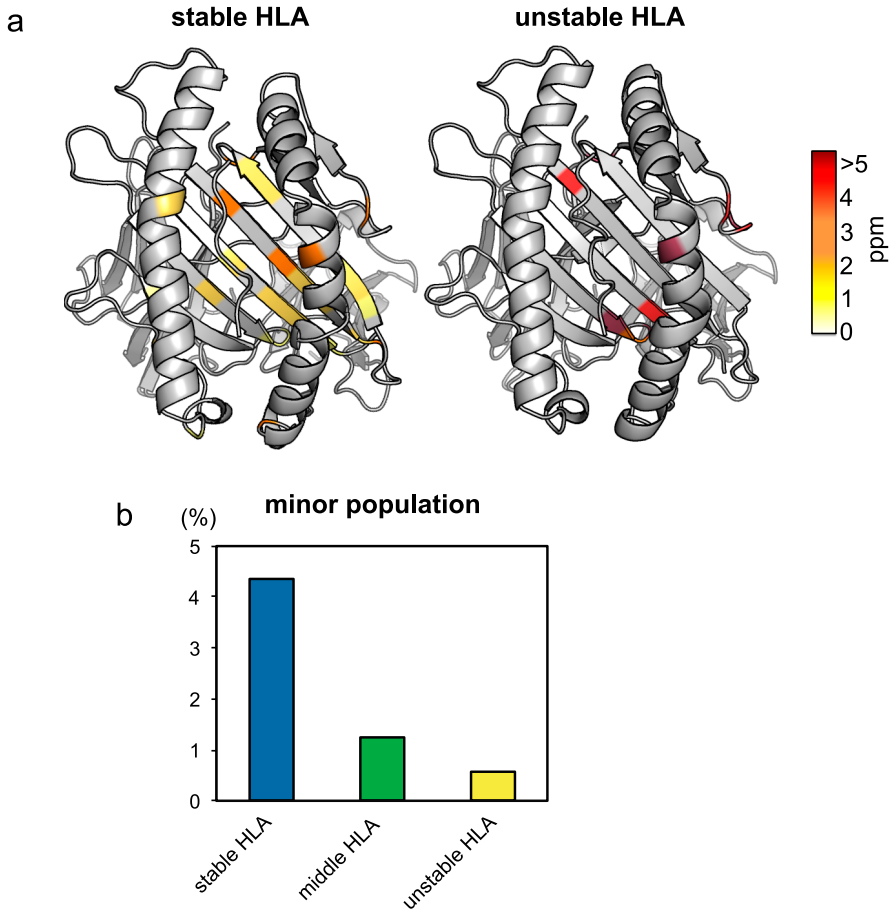

Figure 3 (a) Conformational fluctuations of the stable HLA, and the unstable HLA. The amplitude of fluctuation is shown as chemical shift differences (Δω) on the crystal structures of HLA (PDBID:1A1N) as a continuous color scheme from gray to red. The peptide-binding domain of the structure is shown. (b) The graph shows the minor populations of the three pHLAs.

the middle stable and unstable HLA (Fig. 3a), and the formation of minor state correlated well with the stability of HLAs (Figs. 2, 3). In order to elucidate the reason why the fluctuating HLA is more stable, we conducted the analysis of ΔC_p using the kinetic parameters obtained from the fluctuation experiments conducted at different temperatures. The observed ΔC_p was –0.88 kcal/mol [4]. It is generally appreciated that a negative ΔC_p value for a protein complex provides strong evidence that dehydration has occurred due to interaction and/or induced fit [5]. The peptide-binding domain is composed of many fluctuating hydrophobic residues (Fig. 4), and dehydration in a hydrophobic area generally contributes to the formation of a strong interaction. Therefore, the minor state most likely forms a more packed dehydrated conformation. It is likely that the interaction between the peptide-binding domain and the peptide becomes stronger in the dehydrated minor state. From these results we concluded that the minor state is important for the stabilization of the HLA to prevent disintegration of the complex. It is previously reported that HLAs disintegrate with rate constants of 1×10^{-6}–4×10^{-6} s^{-1} [3,6]. In contrast, the rate constants of the major-to-minor transition observed in this study (9–34 s^{-1}) were much faster than the disintegration rate [4]. This suggests that a HLA rapidly forms the more packed minor state before it enters the disintegration pathway.

Why does HLA fluctuate to a minor state? A number of structurally different antigenic peptides are selected and loaded onto HLA. Our results suggest that the major state of HLA seems to accommodate an antigenic peptide more loosely, compared with the well-packed minor state, as if it allows various structurally different peptides to temporally bind. We proposed such a two-step "transient induced-fit model" for the binding of a peptide to HLA. In the first step, the major state loads a peptide loosely, without the need for conformational adjustment, and in the second step, conformational tuning to the well-packed minor state enables the HLA to avoid rapid disintegration (Fig. 4).

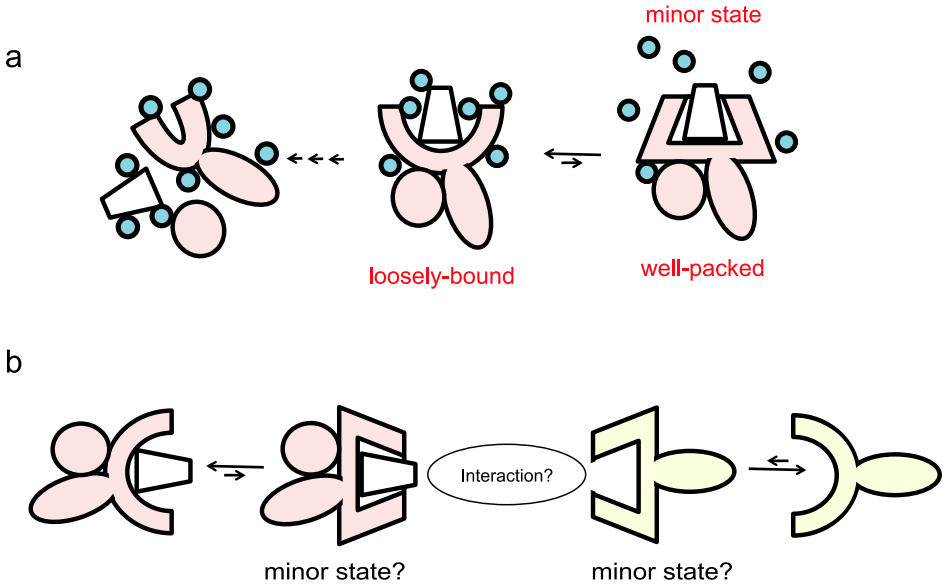

Figure 4 (a) The pHLA transient induced-fit model. A schematic illustration of pHLA fluctuation is shown. The HLA is shown in pink, and the peptide is represented as a trapezoid. Water molecules are shown as light blue circles. (b) A schematic illustration of fluctuating TCR binding with fluctuating HLA is shown. The HLA is shown as in (a), and TCRs are shown as yellow.

Future perspective

Through our research, it was revealed that proteins, which do not obviously change their structure, are fluctuating in solution, and that its stability can be regulated by fluctuation. Although we first focused on the stability of HLA in our analysis, there is high chance that other several biophysical properties are correlated with fluctuation. For example, fluctuation could contribute to the specificity regulation of HLA and TCR binding. Previous studies reported that the ΔC_p of HLA and TCR binding suggested folding event occurring in TCR or HLA. Although the structural change of TCR loop could be the source, the amount of ΔC_p reported is approximately 1 kcal/mol, which is too large for the structural change in TCR alone [7]. The rest of the ΔC_p can be attributed to the structural change in HLA. However, when we observe the crystal structure of HLA, little change is observed. Thus, the folding event that we observed in our NMR experiments could also occur in HLA-TCR interaction. HLA-TCR recognition is known to be specific. However, that the affinity of HLA-TCR interaction is set to a limited range. More over, the structural motifs, which is used for interaction is overlapped. To explain the high specificity with such limits, criteria besides static structures are needed. We suppose that the fluctuation during interaction could play a role in the specific recognition of HLA and TCR, and we are now investigating this issue. In the near future, we hope to reveal the role of fluctuation in other proteins as well.

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