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The 'order-to-disorder' conformational transition in CD44 protein: An umbrella sampling analysis

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ABSTRACT

The molecule of CD44, a membrane protein being the major cell surface receptor for hyaluronan, is postulated to undergo the conformational rearrangement called the 'order-to-disorder' transition. The experimental studies suggest that the Tyr161 residue is crucial for maintaining the equilibrium between the 'ordered' (O) and 'partially disordered' (PD) forms of CD44. The molecular modeling study based on the umbrella sampling protocol was carried out separately for the wild-type CD44 and Tyr161Ala mutant in order to gain more insight into the molecular mechanism of the O-PD transition and to clarify the role of the Tyr161 amino acid residue. The calculated free energy profiles associated with the initial stages of the O-PD conformational transition allow to identify the crucial steps of this process and their molecular details. The differences between the wild-type CD44 and the Tyr161Ala mutant are very insignificant which allows for speculating that, surprisingly, the role of Tyr161 in the O-PD transition is not connected with disrupting the attractive Glu48-Tyr161 and Leu52-Tyr161 interactions but with other types of (unknown yet) interactions located outside the β7-β8 loop or with the existence of the PD-like structure in which the terminal lobe remains located under the β7-β8 loop.

1. Introduction

CD44 is a principal cell surface receptor for hyaluronan (HA) and plays an important role in a wide variety of biological and pathological events. The experimental studies let to postulate that the CD44 molecule can undergo the conformational changes which switch the receptor 'on' or 'off' under appropriate circumstances [1]. According to Ref. [2], such conformational type switching may be induced by the binding of HA but the subsequent studies [1] showed that large rearrangements in the CD44 molecule can occur either in the presence or the absence of ligand. Our previous study reported in Ref. [3] was based on the structures of CD44 in the complex with the HA heptamer and resolved by Banerji et al. [4]. Two conformational forms (called 'A' and 'B') of the CD44/HA complex were identified, differing in orientation of a crucial HA-binding residue (Arg45). Thus, it was suggested that there is an interconversion between these two conformers upon ligand (i.e. HA) binding. One can also expect that the 'B' form represents a higher-affinity ligand-bound state as it corresponds to a more intimate contact between the HA chain and CD44. One of the results of our previous investigations [3] was the conclusion that the structures corresponding to the two crystal forms ('A' and 'B', according to Ref. [4]) can be distinguished only in some cases when considering the values of dihedral angle φ at the Tyr46 residue (this free energy barrier is, however, not high and can be overcome several times during the unbiased molecular dynamics simulation lasting ~100 ns). On the other hand, the free energy barrier associated with the Arg56-HA distance (i.e. the main parameter varying between 'A' and 'B') is much lower and, as a result, both 'A' and 'B' states reduce to an average, dynamic structure. Such behavior was observed for both the liganded and unliganded forms of CD44.

Apart from summarizing our previous results, we would like to comment on the very closely related studies reported by Jamison et al. The paper [5] was unknown to us during publishing our results collected in [3] (thus, it was not mentioned there) but the obtained results deserve more profound comparison with our data. The authors concluded [5] that there exists significant (~6.5 kcal/mol) free energy barrier associated with the changing Arg56-HA distance; furthermore, this barrier is correlated with the varying value of the dihedral angle φ at the Tyr46 residue which was identified as molecular switch governing the HA-CD44 interaction strength. In the case of our study, the φ-related free energy barrier was not correlated with the Arg45-HA distance (see Fig. 2 in Ref. [3]). Due to the similarities in both studies (e.g. using the same types of structures, computational methods, etc.), such contradictory results are most likely caused by using different potentials (force fields) for the interactions in the system. Currently, it is hard to judge which

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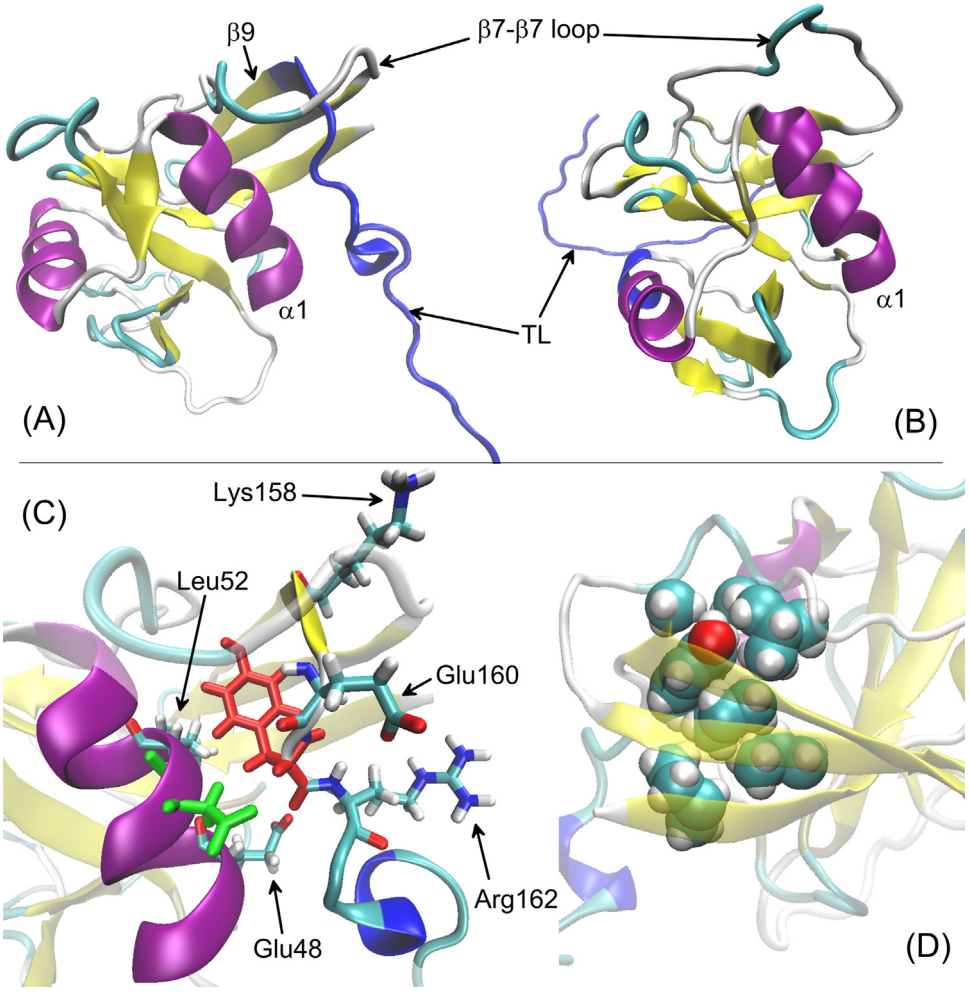

Fig. 1. (A) The 'ordered' (O) and (B) 'partially disordered' (PD) structures of the CD44 molecule, based on the PDB data (entries 1POZ and 2I83, respectively). The terminal lobe (TL) residues are marked in blue. (C) The vicinity of the Tyr161 residues showing some crucial amino acids (e.g. Glu48, Leu52, Lys158, Glu160 and Arg162). The residues defining the reaction coordinate (e.g. Tyr161 and Asp51) are shown in red and green, respectively. (D) Hydrophobic cluster found in the vicinity of Tyr161. Sidechains of Tyr161, Ile145, Ala138, Leu135, Ala55, Leu24 and Ile22 are shown in the ball representation. The pictures were prepared by using the VMD software [17].

force field is 'better' for reproduction of the HA-CD44 interactions, without further data. Moreover, it is worth noting that even the presence of a large free energy barrier separating the 'A' and 'B'-like states does not automatically mean that these two conformational forms exhibit different affinities for HA. The additional study is then required to be performed according to the procedures applied by Jamison et al. for obtaining the free energy changes accompanying the HA binding to CD44 in both 'A' and 'B' states (The convention of amino acid residues numbering in the above part of the paper corresponds to the murine CD44 and the X-ray structures by Banerji et al. [4]; in the further parts the numbering corresponding to the NMR structures (i.e. PDB: 1POZ and 2I83) will be used).

According to the NMR studies by Takeda et al. [2] and Ogino et al. [1], there exist two fundamental conformational forms of CD44; the first is very close to the structures obtained by Banerji et al., whereas in the other rearrangement of the β-strands in the extended lobe and disorder of the structure in the following C-terminal region can be observed. This observation and the subsequent studies led to the hypothesis that the order-to-disorder transition of the C-terminal region is induced by HA binding. However, it should be emphasized that both the 'ordered' (O) and 'partially disordered' (PD) forms of CD44 contribute to HA binding. Moreover both of them can exist in parallel either in the presence or absence of HA; the ligand appearance shifts the dynamic equilibrium state toward the PD form.

Fig. 1 presents schematically the structures representing the PD and O states of CD44 molecule. The range of the 'order-to-disorder' (O-PD) transition include mainly the lobular extension of the CD44 HA binding domain (HABD). The formation of the β-strand in the region corresponding to β9 of the O state is not observed in the PD one. In addition, β8 became rearranged relative to β0. Further, there is not enough data to state if the terminal residues (158–178) go under the loop between β7 and β8 as it is observed in the unbound (O) state. The residues 158–178 will be referred to as TL (terminal lobe). The detailed similarities and differences between PD and O forms are not reported here; the reader is referred to the original papers or to the related PDB entries. It is worth noting that the relation between the 'A'/'B' and O/PD pairs of conformational states has not been studied so far; the main problem is the lack information about the location of the HA ligand bound to the PD form of CD44 (the definitions of 'A' and 'B' are based on the Arg45-ligand distance).

The Tyr161Ala mutant exhibits both: (i) the PD conformation exclusively; (ii) much higher affinity for HA binding, compared to the native CD44. In the light of this correlation, it has been concluded that the PD form binds HA more strongly than the O one. However, understanding the reasons for such correlation is not trivial, as the Tyr161 residue lies nearly on the opposite side of the CD44 molecule with respect to the identified ligand anchoring site and the NMR structures of Tyr161Ala mutant do not exhibit any