Structure
Article

Two-State Conformations in the Hyaluronan-Binding Domain Regulate CD44 Adhesiveness under Flow Condition

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SUMMARY

The hyaluronan (HA) receptor CD44 mediates cell adhesion in leukocyte trafficking and tumor metastasis. Our previous nuclear magnetic resonance (NMR) studies revealed that the CD44 hyaluronan-binding domain (HABD) alters its conformation upon HA binding, from the ordered (O) to the partially disordered (PD) conformation. Here, we demonstrate that the HABD undergoes an equilibrium between the O and PD conformations, in either the presence or absence of HA, which explains the seemingly contradictory X-ray and NMR structures of the HA-bound HABD. An HABD mutant that exclusively adopts the PD conformation displayed a higher HA affinity than the wild-type. Rolling of the cells expressing the mutant CD44 was less efficient than those expressing the wild-type, due to the decreased tether frequency and the slow cellular off rate. Considering that the mutant CD44, devoid of the low-affinity state, exhibited impaired rolling, we conclude that the coexistence of the high- and low-affinity states of the HABD is essential for the CD44-mediated rolling.

INTRODUCTION

CD44 is a principal cell surface receptor for hyaluronan (HA), a major glycosaminoglycan component of the extracellular matrix (Aruffo et al., 1990). Cell adhesion and migration mediated by CD44 are implicated in a wide variety of biological and pathological events, including hematopoiesis, lymphocyte activation and homing, and tumor-cell migration and metastasis (Naor et al., 1997; Sugahara et al., 2006, 2003). CD44 expressed on lymphocytes mediates rolling adhesion on endothelial cells displaying HA under flow conditions (Clark et al., 1996; DeGrendele et al., 1996). Rolling adhesion is an important first step for the recruitment of leukocytes from the bloodstream to inflammatory sites and the secondary lymphoid organs and is only mediated by specialized adhesion molecules, such as selectins or α4 integrins (Alon et al., 1995b; Chen et al., 1997).

CD44 is a type I transmembrane glycoprotein receptor. The extracellular portion of CD44 comprises an N-terminal hyaluronan-binding domain (HABD), followed by a heavily glycosylated and alternatively spliced stem region. The short cytoplasmic tail of CD44 is connected to the actin cytoskeleton via ERM (ezrin, radixin, and moesin) proteins (Tsukita et al., 1994). The CD44 HABD contains a Link module, which is conserved among many other HA-binding proteins, including tumor necrosis factor stimulating gene-6 (TSG6) (Day and Prestwich, 2002). In addition, the N- and C-terminal flanking regions are necessary for the proper folding and the HA-binding activity of the HABD (Banerji et al., 1998; Peach et al., 1993). The three-dimensional structures of the HABD in the ligand-free state, revealed by the X-ray and nuclear magnetic resonance (NMR) studies, demonstrated that the Link module and the N- and C-terminal extensions form a single folded unit, which is composed of three α helices and eleven β strands, arranged in the order of β0-β0'-β1-α1-α2-β3-β4-β5-β6-β7-β8-β9-α3 (Takeda et al., 2003; Teriete et al., 2004).

We recently determined the solution structure of the HABD of human CD44 in complex with an HA hexamer (HA6) and demonstrated that HA binding induces an allosteric conformational rearrangement of the HABD, in which the C-terminal β9 strand and α3 helix become unstructured and the β8 strand is rearranged with respect to the β0 strand (Takeda et al., 2006). In addition, the heteronuclear NOE experiments showed that the C-terminal segment of the HABD has enhanced flexibility in the HA-bound state. Therefore, we refer to the conformation of the HABD in the unbound and HA-bound states as the "ordered (O)" and the "partially disordered (PD)" conformations, respectively, on the basis of the conformational differences in the C-terminal segment. However, the subsequently solved crystal structures of the HABD of mouse CD44 in complex with HA8 adopted the O conformation (Banerji et al., 2007). Therefore,

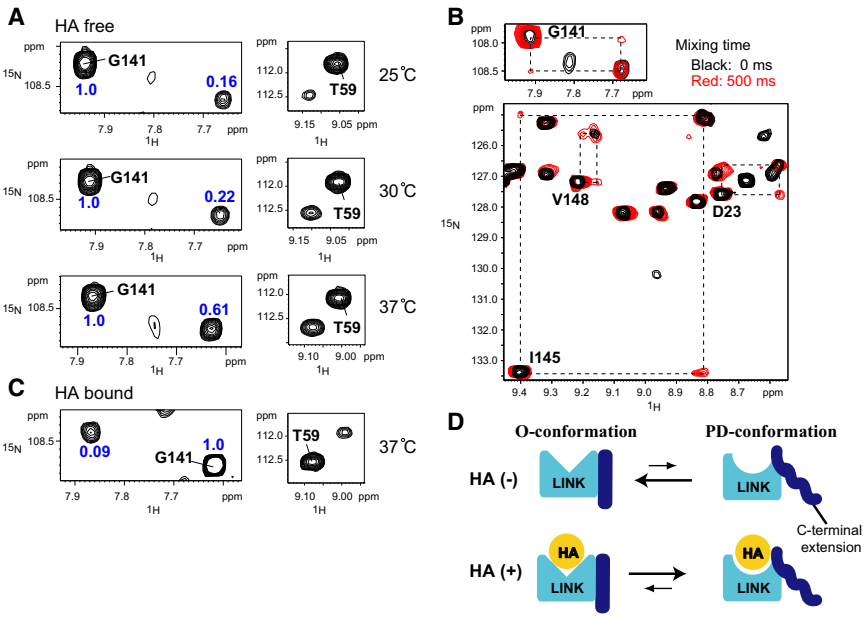

Figure 1. Two-State Conformational Equilibrium of CD44 HABD

(A) Selected regions of the ^{1}H-^{15}N HSQC spectra of the CD44 HABD in the absence of HA recorded at 25°C (top), 30°C (middle), and 37°C (bottom). Paired signals of the O conformation and the PD conformation are shown for T59 (right column) and G141 (left column), which are located at away from the HA-contact region. The relative intensities of the PD conformation signals with respect to the O conformation are shown in the spectra.

(B) N_{zz} exchange spectra of the ligand-free HABD, acquired without (black) or with a 500 ms mixing period (red). Cross peaks between the O and PD conformations were observed for residues such as G141 (upper spectrum), D23, I145, and V148 (lower spectrum), as indicated by dashed lines.

(C) Selected regions of the ^{1}H-^{15}N HSQC spectra of CD44 HABD in the HA-bound state, measured at 37°C. The relative intensities of the O conformation signals with respect to the PD conformation are shown in the spectra.

(D) Schematic drawings of the equilibrium of the CD44 HABD between the O conformation and the PD conformation. The size of the arrows drawn between the O state and the PD state are correlated with the changes in the conformational equilibrium in the presence and absence of HA.

the ligand-induced conformational change of the CD44 HABD is controversial and requires clarification.

In the present study, we demonstrated that CD44 HABD exchanges between the O and PD conformations in either the presence or absence of the HA ligand. The intrinsic two-state conformational transition of the HABD provides a clear explanation for the contradictory results between the X-ray and NMR structures, concerning the conformational rearrangement of the HABD upon HA binding. Furthermore, we prepared an engineered mutant that adopts only the PD conformation. This mutant exhibited higher affinity for HA, and when it was expressed on cell surfaces, the cells showed less efficient rolling behavior on the HA substrate under flow conditions. Based on these observations, we propose that the two-state equilibrium is essential for the CD44-mediated cell rolling.

RESULTS AND DISCUSSION

Conformational Equilibrium of CD44 HABD under the Physiological Conditions

In the previous study, we had completed the resonance assignment of all of the backbone signals of CD44 HABD in the ligand-free state that adopts the O conformation (Takeda et al., 2003). However, we noticed that a number of minor unassigned signals still remained on the HSQC spectrum (Figure 1A). The intensity ratios of those minor signals relative to the major signals exhibited a temperature-dependent increase, from 0.16 at 25°C to 0.64 at 37°C (Figure 1A). In addition, cross peaks were observed between the major and the minor signals in the N_{zz} exchange experiments at the mixing time of 500 ms (Figure 1B). Therefore, it was demonstrated that the HABD interconverts between two distinctive conformations with the exchange rate of hundreds of milliseconds. The chemical shifts of the minor signals in the ligand-free state mostly coincided with those of major signals in the HA-bound HABD state that adopts the PD conformation (Figures 1A and 1C). Likewise, the HSQC spectrum of the HA-bound HABD also provides minor signals at the position corresponding to the major signals of the HA-free state (Figure 1C). Based on these results, we conclude the CD44 HABD undergoes an equilibrium between the O and PD conformations, in either the presence or absence of HA, and the HA binding induces a shift of the equilibrium toward the PD conformation (Figure 1D).

Those results resolved the inconsistency regarding the conformation of the HABD in the HA-bound form, raised by previous X-ray and NMR studies (Banerji et al., 2007; Takeda et al., 2006). As demonstrated in this study, the HABD in the HA-bound form predominantly adopts the PD conformation, while the small fraction of the HA-bound HABD still adopts the O conformation. Therefore, under the physiological condition, both conformations, solved by the NMR and the X-ray crystallography, are present as a major and a minor population, respectively. Although less than 10% of the total HABD exists as the O conformation in the HA-bound state, it is likely that the sparsely populated O conformation was selectively crystallized because the PD conformation is unfavorable for crystallization, due to the flexibility of the C-terminal segment. In contrast, the major conformation in solution could be obtained in the structure determination by NMR of both the unbound (Teriete et al., 2004) and the HA-bound HABDs (Takeda et al., 2006), since the signal from the major conformation can be predominantly observed in NMR spectra.

The Y161A Mutant Constitutively Adopts the PD Conformation

In order to investigate the functional relevance of the two-state equilibrium of the CD44 HABD, we designed a mutant that would

