

Surface-induced dissociation of multimeric protein complexes

The vast majority of proteins carry out their biological functions by fashioning intricate assembly lines or working in unison as complex macromolecular machines. Structural studies of these protein complexes are vital for understanding their functional role in cellular processes such as enzyme catalysis, signal transduction and DNA replication, especially as many proteins are only operable within the context of the complex. One such example is the pentameric C-reactive protein (CRP), a pentraxin protein upregulated by the human body in response to inflammation [1]. CRP, shown at the right, consists of five identical subunits, and its serum levels have been used as an indicator of coronary disease.

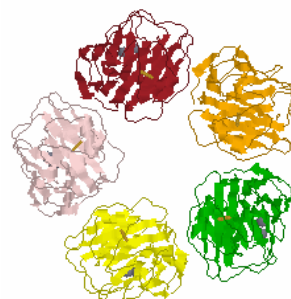


Figure 1. X-ray crystal structure of pentameric C-reactive protein (PDB 1gnh)

Recent advances in mass spectrometry have made it possible to preserve intact protein assemblies such as CRP in the gas-phase such that aspects of their quaternary structure may be revealed. Accurate molecular weight and stoichiometric information can be obtained through MS experiments, while the dissociation of protein complexes through tandem MS holds the potential to probe their sub-oligomeric architecture. Slow-heating activation methods such as collision-induced dissociation (CID) or blackbody infrared radiative dissociation (BIRD)

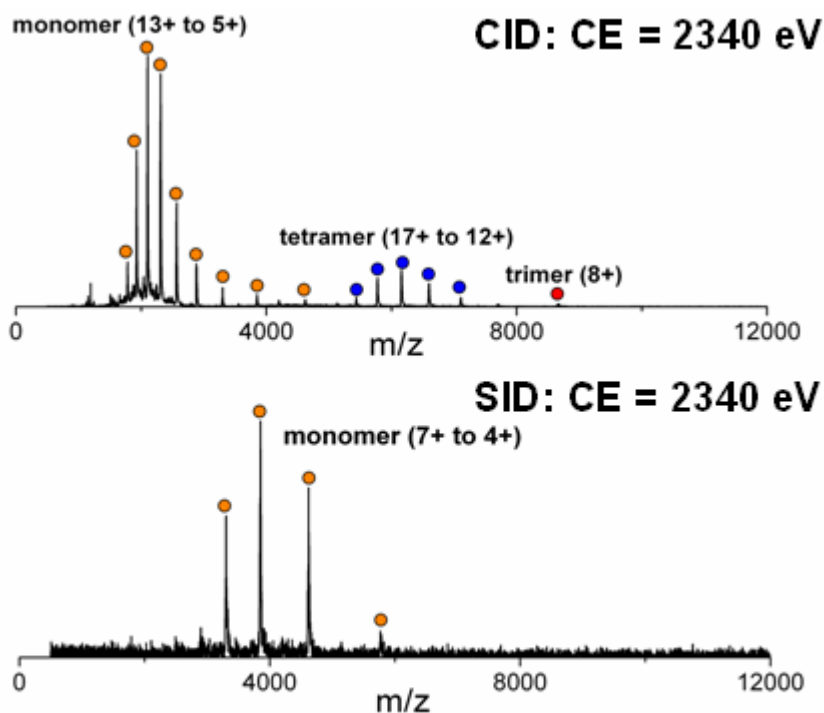


Figure 2. MS/MS spectra of the 26+ charge state of pentameric C-reactive protein. (A) Collision-induced dissociation at collision energy 2340 eV (90V x 26+). (B) Surface-induced dissociation at the same collision energy.

invariably results in the asymmetric ejection of an unfolded, highly charged monomer from the complex and a complementary (n-1)-mer, limiting the amount of structural insight that can be gained [2-4]. Our research group has recently demonstrated that surface-induced dissociation (SID) however, is a promising alternative for activating protein complexes, and has the potential to reveal the biologically relevant details absent in CID experiments [5]. CID and SID of C-reactive protein are compared in Figure 2 with SID yielding symmetrically charged monomers from the intact pentamer, while CID leads to a very asymmetrical pathway resulting in highly charged monomer and tetramer product ions. Analysis of CRP over a wide range of SID collision energies suggests that this complex may follow a dissociation pathway that forms monomeric product ions directly from the pentamer, rather than by sequentially stripping each subunit from the oligomer. We are currently investigating the dissociation pathways of protein complexes by both CID and SID in effort to extend the ability of MS/MS to probe the quaternary organization of subunits within the intact complex.

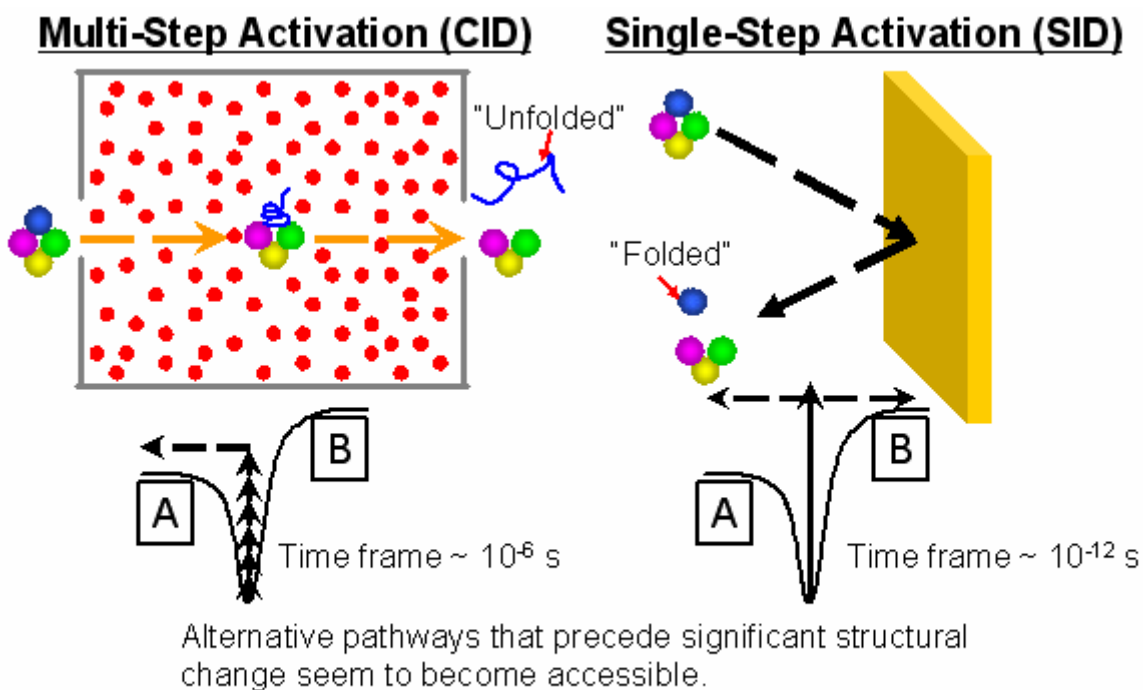


Figure 3. Comparison of CID and SID activation methods, illustrating a possible explanation for the different dissociation pathways observed from these techniques

Figure 3 displays how CID and SID might lead to the different dissociation pathways that have been observed for a wide range of complexes. By CID, the activation occurs gradually with internal energy being deposited through many low-energy collisions with inert gas atoms. Furthermore, the time frame of activation is in the microsecond regime. This is very different than SID, in which internal energy is deposited during a single collision event that occurs on a much shorter (i.e. picosecond) time-frame. Therefore, it may be possible for the complex to undergo significant structural rearrangement during CID activation, leading to unfolded monomers, with increased surface areas, that can accommodate the observed charge enrichment. On the other hand, a dissociation pathway involving a relatively folded transition state may be

accessible during an SID experiment since significant monomer unfolding is unlikely to occur on such a short time frame.

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