Binding of Hapten to a Single-Chain Catalytic Antibody Demonstrated by Electrospray Mass Spectrometry

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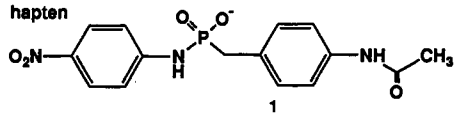
Catalytic antibodies (abzymes) have been elicited for a variety of chemical reactions. Abzymes have been developed to catalyze high-energy[1] and highly disfavored[2] reactions through induction of an immunological response using a hapten that structurally resembles the transition-state or high-energy species encountered in a given reaction.[1] In general, catalytic antibodies exhibit high affinities (<1 nM) for hapten binding; this interaction energy acts to stabilize intermediates along the course of the chemical reaction and consequently drives the reaction. The mechanism of action of catalytic antibodies has been the focus of several recent studies which used various enzymological protocols.

The enzymological techniques used to study abzymes include presteady-state kinetics,[3] substrate structure–activity relationships,[4] and mutagenesis.[5,6] Electrospray mass spectrometry is also emerging as an important new enzymological tool[7,8] having the ability to detect relatively small chemical modifications in proteins. Although intact antibodies (M_r = 150 000) are too large to be routinely studied with this technique, recent innovations in antibody engineering[9] have allowed us to generate a single-chain Fv fragment ($C_{1162}H_{1814}N_{316}O_{368}S_{10}$, M_r = 26 419.9). In this communication we employ pneumatically-assisted electrospray (ion spray) mass spectrometry to antibodies in order to examine the complex found between a catalytic antibody Fv fragment and its hapten.

The catalytic antibody, NPN43C9, as a cloned single chain F_v fragment,[5] was purified to homogeneity by SDS–PAGE before electrospray analysis. The catalytic antibody was introduced into a Perkin-Elmer SCIEX API III Mass analyzer (error ~±0.01%) either alone or complexed to its inducing hapten (phosphonamidate (**1**)) or inhibitor (*p*-nitroaniline (**2**)) at a concentration of 20 μM in distilled H_2O at pH 5.

hapten



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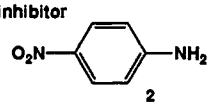
inhibitor



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The results obtained for the antibody–hapten complex under two ionization conditions are shown in Figure 1. The electrospray ionization source allows for an ion's kinetic energy to be adjusted through the declustering potential. Declustering potentials on the order of 70 V or greater usually promote the dissociation of noncovalent complexes as well as covalent fragmentation; lower potentials (<70 V) are conducive to the observation of noncovalent complexes (protein complexes have been analyzed[8] at declustering potentials of 40 V). The antibody–hapten complex was observed even at declustering potentials greater than 130 V. The noncovalent complex (M_r 26 769 ± 2.7) predominates in the reconstructed mass spectrum, consistent with the tight association (K_d < 1 nM)[5] between hapten and single-chain catalytic antibody (M_r 26 419.8 ± 2.6). Complete dissociation was observed at a declustering potential of 175 V, where the antibody peak is still present but the complex is no longer observed, consistent with its noncovalent nature.[8] The considerably weaker binding of the inhibitor *p*-nitroaniline (K_d = 10 μM)[3] was indicated by the complete lack of any observable complex formation in the mass spectrum (data not shown).

An apparent change in the charge state distribution occurred upon addition of the hapten. The mass spectrum of the antibody prior to hapten addition showed the presence of the charge states between 18+ and 12+. However, only two charge states, 12+ and 13+, were observed for the hapten–antibody complex at the same declustering potential (125 V), as shown in Figure 2. These results are interesting in light of recent modeling studies of the SCA[10] which predict that the hapten-binding region contains at least three positively-charged residues (His L91, Arg L96, His H35). The observed decrease in the net positive charge of the complex suggests that the bound hapten prevents the ionization of these side chains near the abzyme active site. Since the SCA is believed to be a nucleophilic catalyst,[3-5] the binding-induced deprotonation might be important for abzyme reactivity. Alternatively, the altered charge state distribution may also reflect global conformational change as caused by temperature[11] and pH[12] changes, organic solvents,[13] and metal binding.[14] The dissimilar charge envelopes could represent native or denatured forms of the protein or, as observed in pH- and metal-dependent spectral changes, an alternative folded conformer. Since the SCA antibody tends to irreversibly aggregate when unfolded (unpublished observations), the latter possibility seems unlikely. Therefore, the change noted for the single-chain catalytic antibody may reflect both proton displacement in the binding site and global changes in the conformation of the antibody.

Additional experiments were performed to gain information on the binding energetics in the antibody–hapten complex. The declustering potential was varied while the intensity of the complex (charge state 12+) with respect to unbound SCA (charge state 12+) was monitored. The plot of declustering potential versus bound SCA/unbound SCA (charge state 12+) was nonlinear.

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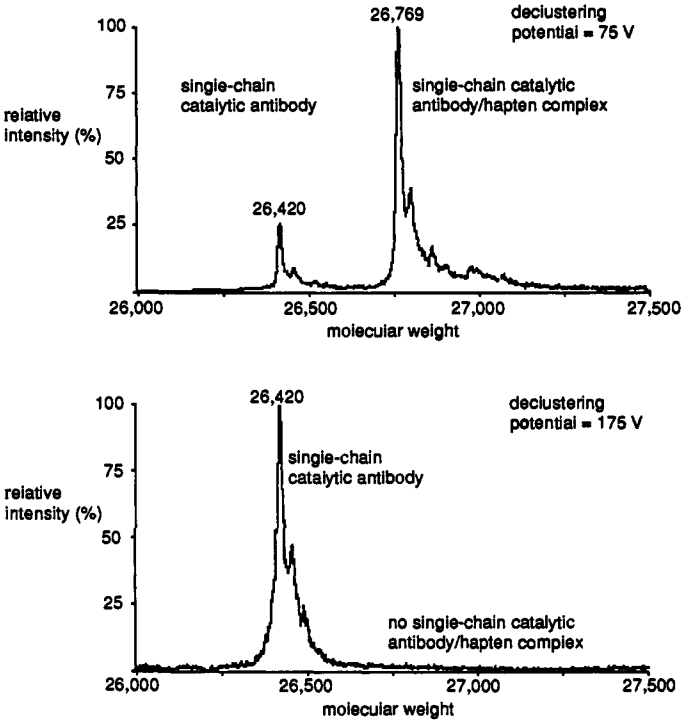

Figure 1. Noncovalent antibody–hapten complex as observed with pneumatically-assisted electrospray (ion spray) mass analysis at a declustering potential of 75 V (above) and 175 V (below).

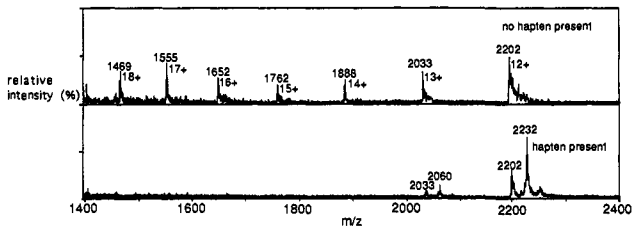

Figure 2. Charge state distribution of the SCA observed under identical conditions before and after addition of the hapten. The mass spectrum of the antibody prior to hapten addition showed the presence of all charge states between 18+ and 12+, while only two charge states, 12+ and 13+, could be observed for the hapten–antibody complex.

This nonlinearity was attributed to simultaneous variations in the dissociation of the complex and the charge state; variation of charge state distribution as a function of declustering potential is well-known. The possibility of gaining binding information would require monitoring the full distribution of bound charge states for the antibody and antibody–hapten complex, a requirement beyond the mass range of this instrumentation.

The electrospray mass spectrometry technique shows promise in the study of tight noncovalent interactions of macromolecules. The significant differences between the hapten–antibody and inhibitor–antibody complexes in these experiments, especially at the high declustering potentials necessary for complete hapten–antibody dissociation, are indicative of site-specific hapten binding. This system represents an excellent model for our future deuterium exchange experiments and studies of the physical interactions of the catalytic antibody fragment with various inhibitors and substrates.

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