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Ring-Toss: Capping highly exposed tyrosyl or tryptophyl residues in proteins with β-cyclodextrin

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Abstract

We have used UV difference spectroscopy and fluorescence spectroscopy to study the perturbation by β-cyclodextrin of tyrosyl or tryptophyl residues located at each of the 10 variable consensus contact positions in the third domain of turkey ovomucoid. The goal was to monitor the accessibility of the side chain rings of these residues when located at these positions. The results indicated that the tyrosyl or tryptophyl rings are most highly exposed when located in the P_1 position followed by the P_4 position. It was possible to determine the association constants for β-cyclodextrin binding at these positions. When located at the P_2, P_5, P_6 and P_3' positions, the rings of the tyrosyl or tryptophyl residues were exposed but less so than at the P_1 or P_4 positions. By contrast, when located at the P_1', P_2', P_{14}' and P_{18}' positions, the tyrosyl or tryptophyl residues were insufficiently exposed to be perturbed by β-cyclodextrin, although they reacted positively to dimethyl sulfoxide solvent perturbation. These findings indicate that β-cyclodextrin perturbation provides a convenient way to detect highly exposed tyrosyls or tryptophyls in proteins. Furthermore, we evaluated the ability of β-cyclodextrin to inhibit the interaction of turkey ovomucoid third domain variants with different P_1 residues. The results showed that the presence of β-cyclodextrin had little effect on the association constant when the P_1 residue was a glycyl residue, but greatly decreased the association constant when the P_1 residue was a tyrosyl or tryptophyl residue. Thus, β-cyclodextrin may be used to selectively modulate the interaction between proteinase inhibitors and their cognate enzymes.

Keywords: β-cyclodextrin; Exposed tyrosyl/tryptophyl residues; Proteinase inhibitor; Protein–protein interaction; UV difference spectroscopy; Fluorescence spectroscopy

1. Introduction

Cyclodextrins (CDs) are truncated cone-shaped molecules with hydrophilic rims and hydrophobic cavities [1–9]. The most commonly observed mode of association of cyclodextrins with different compounds involves inclusion of the less polar portion of the guest within the cavity, while the hydrophilic part of the guest remains solvent exposed [6,10–12]. Cyclodextrins have attracted much interest not only in theoretical research but also in industrial applications [4]. The most common CDs are α-, β-, and γ -CD each composed of six, seven, and eight α-1, 4 linked D-glucopyranose units arranged in a circular fashion, respectively. Cyclodextrins have been reported to interact with hydrophobic residues at the surface of proteins, leading to an increase in solubility and suppression of protein aggregation [13–15]. They also serve as substrates for some α-amylases [16,17]. Modified β-CD has been shown to protect NIH3T3 cells against oxidative stress [18]. Interaction of CDs with DNA and RNA has also been studied [19] and this interaction has been utilized for targeted in vitro gene delivery [20].

One of the first studies of cyclodextrins interaction with amino acids was performed by Warrington [21], at a time when solvent perturbation of exposed aromatic residues in proteins was becoming an increasingly popular technique in protein chemistry [22,23]. In these studies, the interaction of β-CD with acetyl-tyrosine ethylester was analyzed using difference spectral

Abbreviations: β-CD, β-cyclodextrin; OMTKY3, turkey ovomucoid third domain; DMSO, dimethylsulphoxide; SGPA, *Streptomyces griseus* proteinases A; SGPB, *Streptomyces griseus* proteinase B; PSA, protein surface water-accessible areas; OMSVP3, silver pheasant ovomucoid third domain; we use K_f to designate the association of β-cyclodextrin with the protein and K_a as the usual association constant for OMTKY3 variants with cognate proteinases

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technique and the results indicated that addition of either 1% (w/v) (10 mM) β-CD or 20% (w/v) sucrose solution produced a large red shift in the ultraviolet spectrum of the model compound [21]. A much lower concentration of β-CD than sucrose was needed to produce the same difference spectral change of the model compound. This was apparently due to formation of a non-covalent complex between β-CD and the model compound. The existence of a non-covalent complex between β-CD and aromatic amino acids has been confirmed by crystallographic studies [24].

Warrington [21] also attempted to study the exposed aromatic amino acid residues in proteins by using β-CD. He chose bovine ribonuclease A which contains 6 tyrosines, 3 of which ionize normally and 3 do not ionize, suggesting that only half of these tyrosyls are exposed [25,26]. Difference spectrum studies confirmed this finding [23]. However, the addition of 1% (w/v) β-CD to ribonuclease produced no observable red shift [21]. Evidently, the exposed tyrosyls in ribonuclease A are not sufficiently exposed to effectively interact with β-CD. Warrington [21] also tried bovine plasma albumin, α-chymotrypsin, and lysozyme although no difference spectrum signal was obtained.

The experiments described above were performed at a time when proteins were poorly characterized and site-specific mutagenesis was not practical. Our laboratory has long been working on turkey ovomucoid third domain (OMTKY3, Fig. 1), which is a standard mechanism [27], canonical [28] Kazal [29] superfamily inhibitor of serine proteinases and strongly inhibits many serine proteinases [30]. OMTKY3 has 10 variable contact positions, which are shown in blue in Fig. 1 and labeled as P_1, P_2, P_4, P_5, P_6, P'_1, P'_2, P'_3, P'_{14} and P'_{18} according to the Schecter and Berger notation [31]. In order to generate a sequence to reactivity algorithm for the entire Kazal family of inhibitors, all 19 possible singly coded variants at each of the 10 variable contact positions were expressed in our laboratory [30]. Therefore, a set of single Tyr or Trp substitution variants at all 10 variable consensus contact positions in OMTKY3 (P_nY and P_nW, see Fig. 1) was available. OMTKY3 has 3 tyrosyls, one in the contact region, P'_2, and the others are outside: P_8 and P'_{13}. Thus, P'_2Y is the same as OMTKY3. All other P_nY variants have an additional Tyr in the P_n contact position.

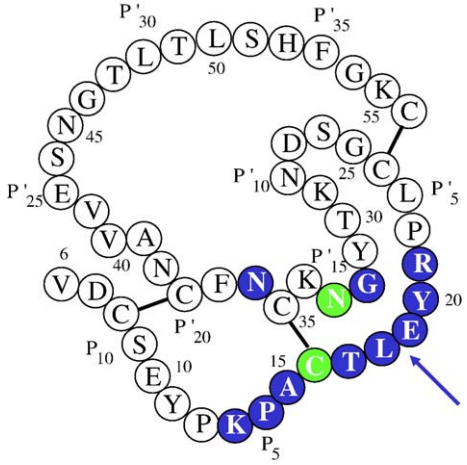

Fig. 1. Covalent structure of turkey ovomucoid third domain, OMTKY3, a Kazal superfamily proteinase inhibitor. The three disulfide bridges are indicated by bars. Residues are numbered sequentially from Val^6 to Cys^{56}. The arrow points to the reactive site peptide bond between the P_1 and P'_1 residues. Residues preceding the reactive site are labeled P_1, P_2 … P_n; those following: P'_1, P'_2 … P'_n. The 12 colored residues comprise the consensus contact residue set. Of these 12, the two in green are structural and have accepted very few mutations in evolution. The remainder, named the variable consensus contact residue set, is marked in blue and has accepted many mutations in evolution.

In the present work, perturbation by β-cyclodextrin of tyrosine and tryptophan residues substituted at various contact positions of OMTKY3 was studied by UV difference spectroscopy and fluorescence spectroscopy. The contact residues of standard mechanism serine proteinase inhibitors lie on the surface of the protein and are most likely to be freely accessible to the surrounding solvent. Thus, solvent perturbation by dimethylsulphoxide (DMSO) was carried out to infer the number of tyrosyls in OMTKY3 that are exposed to the solvent in order to understand whether the additional tyrosyl or tryptophyl residue in the variants P_nY and P_nW is solvent exposed. Then, the difference and fluorescence spectra of P_nY and P_nW in the absence and presence of β-CD were measured and the formation constants for the association (K_f) were obtained, if possible. Finally, we measured the association constants (K_a) of two proteinases, *Streptomyces griseus* proteinase A (SGPA) and *Streptomyces griseus* proteinase B (SGPB), with the inhibitor variant that has a glycyl, tyrosyl or tryptophyl residue at the P_1 position in the absence and presence of β-cyclodextrin. It is known that the binding of protein proteinase inhibitors to their cognate enzymes usually involves the burial of the P_1 residue side chain into a well-defined cavity of the enzyme [32]. By including the tyrosyl or tryptophyl side chain into its cavity, β-CD may decrease the binding of the inhibitor with its cognate enzymes.

2. Materials and methods

β-cyclodextrin was purchased from ICN. Single glycyl, tyrosyl or tryptophyl substitution variants at all 10 variable consensus contact positions in turkey ovomucoid third domain (OMTKY3) were expressed in our lab as described previously [30,33]. α-Chymotrypsin was purchased from Worthington. *Streptomyces griseus* proteinase A (SGPA) and *Streptomyces griseus* proteinase B (SGPB) were purified from pronase bought from Sigma. All substrates for the K_a measurements were bought from Bachem.

The concentration of *N*-acetyl-tyrosine ethylester and acetyl-tryptophan ethylester was obtained by measuring the absorbance at 276 nm ($\varepsilon = 1.4 \times 10^3$ M^{-1} cm^{-1}) and 280 nm ($\varepsilon = 5.5 \times 10^3$ M^{-1} cm^{-1}), respectively. OMTKY3 concentration was routinely measured by amino acid analysis. α-chymotrypsin concentration was determined by titration with OMTKY3. The concentration for P_nY and P_nW variants was obtained by titration with α-chymotrypsin. The molar extinction coefficients for P_nY and P_nW variants were then obtained by measuring the absorbance.

Difference spectra were measured on a Cary-300 spectrophotometer in pH 6.5 0.05 M phosphate buffer. The concentrations of acetyl-tyrosine ethylester, acetyl-tryptophan ethylester, P_nY and P_nW variants of OMTKY3 are about 8×10^{-4} M, 2×10^{-4} M, 2×10^{-4} M and 1.2×10^{-4} M, respectively. The formation constants (K_f) and molar extinction coefficient changes at infinite β-CD