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Structural Basis for Molecular Recognition in an Affibody:Affibody Complex

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Affibody molecules constitute a class of engineered binding proteins based on the 58-residue three-helix bundle Z domain derived from staphylococcal protein A (SPA). Affibody proteins are selected as binders to target proteins by phage display of combinatorial libraries in which typically 13 side-chains on the surface of helices 1 and 2 in the Z domain have been randomized. The Z_{Taq}:anti-Z_{Taq} affibody–affibody complex, consisting of Z_{Taq}, originally selected as a binder to *Taq* DNA polymerase, and anti-Z_{Taq}, selected as binder to Z_{Taq}, is formed with a dissociation constant $K_d \sim 100$ nM. We have determined high-precision solution structures of free Z_{Taq} and anti-Z_{Taq}, and the Z_{Taq}:anti-Z_{Taq} complex under identical experimental conditions (25 °C in 50 mM NaCl with 20 mM potassium phosphate buffer at pH 6.4). The complex is formed with helices 1 and 2 of anti-Z_{Taq} in perpendicular contact with helices 1 and 2 of Z_{Taq}. The interaction surface is large (~1670 Å^2) and unusually non-polar (70 %) compared to other protein–protein complexes. It involves all varied residues on anti-Z_{Taq}, most corresponding (*Taq* DNA polymerase binding) side-chains on Z_{Taq}, and several additional side-chain and backbone contacts. Other notable features include a substantial rearrangement (induced fit) of aromatic side-chains in Z_{Taq} upon binding, a close contact between glycine residues in the two subunits that might involve aliphatic glycine Hα to backbone carbonyl hydrogen bonds, and four hydrogen bonds made by the two guanidinium $N^{\eta}H_2$ groups of an arginine side-chain. Comparisons of the present structure with other data for affibody proteins and the Z domain suggest that intrinsic binding properties of the originating SPA surface might be inherited by the affibody binders. A thermodynamic characterization of Z_{Taq} and anti-Z_{Taq} is presented in an accompanying paper.

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Introduction

The high levels of binding affinity and specificity of antibodies have been exploited extensively in medicine and biotechnology, and the large range of applications has motivated development of novel artificial binding proteins to be used as cost-effective alternatives or which allow for more flexibility with regard to conditions such as pH, temperature, redox potential etc.[1,2] Beside their practical applicability, engineered proteins provide attractive model systems for studying fundamental aspects of structure, stability and function. We are using a class of engineered binding proteins named affibody proteins as models in studies of the mechanisms of molecular recognition and protein–protein binding interactions.

Affibody binders are based on the three-helix bundle scaffold of the Z domain, which was originally engineered from the B domain of staphylococcal protein A (SPA).[3] The Z domain shows, like the other five homologous SPA domains, antibody Fc binding activity, but it lacks the Fab affinity found in the parental domain.[4] The Fc binding surface of the Z domain is used as template for creation of affibody binding proteins. Thirteen residues in the binding interface are randomized to

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Abbreviations used: NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy.

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create a combinatorial library from which specific binders can be selected using phage display.[5] Binders to a large number of protein targets, with dissociation constants in the range micromolar to approximately nanomolar have been reported.[6–9]

In previous work, we examined structural and thermodynamic properties of the complex between the Z_{SPA-1} affibody and its target, the Z domain.[10–13] Overall, these studies illustrate how seemingly simple structural topologies may conceal a great deal of complexity that arises when coupled folding/binding and induced fit operate simultaneously in molecular recognition. On the other hand, it was possible to deduce the structural basis for the selection of Z_{SPA-1} as a Z-domain binder.[11] We dissected the coupled folding and binding thermodynamics, and suggested that the origin of moderate affinity (dissociation constant $K_d \sim 1$ μM) of Z_{SPA-1} is the entropic penalty associated with conformational stabilization upon binding, rather than defects in the binding interface or the poor folding stability of Z_{SPA-1}.[13]

However, an ideal model system for protein–protein interactions must allow for detailed studies of structural and biophysical properties of all components in the binding equilibrium, i.e. also those of the free components. And, even though the solution structure of the Z domain has been determined,[14,15] the Z_{SPA-1} affibody shows many of the characteristics of a protein molten globule,[11] and these properties prohibit structure determination of the free Z_{SPA-1}[12]. The Z_{Taq}:anti-Z_{Taq} complex studied here is formed between two different affibody proteins. The Z_{Taq} affibody was selected as a strong binder to *Thermus aquaticus* (*Taq*) DNA polymerase.[16] Z_{Taq} was subsequently used as a target to obtain an affibody-binding affibody (anti-Z_{Taq}) in analogy with anti-idiotypic antibodies.[7] The dissociation constant for the Z_{Taq}:anti-Z_{Taq} complex is K_d ~100 nM (see the accompanying paper[17]), which is ten times stronger than that for the Z:Z_{SPA-1} complex, and both affibody proteins are properly folded, so their solution structures can be determined. Here, we present high-precision structures of Z_{Taq}, anti-Z_{Taq}, and the Z_{Taq}:anti-Z_{Taq} complex determined under identical experimental conditions. The structures reveal a number of intriguing properties, and suggest a common feature of complexes involving SPA domains and affibody binders. In addition, this work provides for further structure-based analyses of protein–protein binding thermodynamics and investigation of the roles of flexibility (manifested as dynamics) and induced fit in protein–protein recognition.

Results

Structure determination

NMR resonance assignments of Z_{Taq}, anti-Z_{Taq} and the Z_{Taq}:anti-Z_{Taq} complex are reported elsewhere.[18] The structures were determined at 25 °C and pH 6.4 using 20.2 restraints per residue for Z_{Taq}, 22.7 for anti-Z_{Taq} and 23.5 for the complex, including 206 intermolecular nuclear Overhauser effects (NOEs). Structural statistics of the final ensembles are shown in Table 1. The 40 structures in each ensemble do not contain any

Table 1. Structural statistics

	Complex (Z_{Taq} /anti-Z_{Taq})	Z_{Taq}	anti-Z_{Taq}
Non-redundant NOE restraints			
Intra residue	358/375	332	381
Sequential ($\|i-j\|=1$)	267/317	263	311
Medium-range ($2 \le \|i-j\| \le 4$)	309/301	294	339
Long-range ($\|i-j\| \ge 5$)	162/157	171	179
Total	1096/1145	1060	1210
Intermolecular	206		
Dihedral angle restraints			
ϕ	46/44	45	46
ψ	45/42	40	43
χ_1	7/15	8	16
Hydrogen bonds	19/20	9	0
XPLOR van der Waals energy	−1858±23	−885±17	−761±13
Ramachandan statistics			
Most favored regions (%)	93.8	95.0	90.7
Allowed regions (%)	6.2	5.0	9.3
r.m.s. deviation from restraints			
Distances (Å)	0.063±0.001	0.066±0.001	0.066±0.002
Dihedral angles (deg.)	0.65±0.05	0.86±0.09	1.00±0.05
r.m.s. deviation from ideal geometry			
Bond lengths (Å)	0.0069±0.0001	0.0065±0.0002	0.0077±0.0002
Bond angles (deg.)	0.88±0.01	0.88±0.02	0.97±0.02
Impropers (deg.)	0.72±0.01	0.70±0.03	0.77±0.03
Coordinate precision[a]			
Backbone heavy-atoms (Å)	0.19±0.05	0.23±0.07	0.19±0.05
All heavy atoms (Å)	0.61±0.06	0.74±0.09	0.66±0.05

[a] Compared to the mean structure. The r.m.s.d. was calculated on fragments beginning with the first residue of helix 1 and ending with the last residue of helix 3, i.e. residues 4–55 (Z_{Taq}, complex), 6–55 (anti-Z_{Taq}, complex), 6–55 (Z_{Taq}, free) and 5–55 (anti-Z_{Taq}, free).

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