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Redesigning Protein Function: Towards Synthetic Vaccines and MHC Mimetics

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Abstract. Template Assembled Synthetic Proteins (TASP) approach has been applied to mimic 3D-conformations of the hen egg white lysozyme antigen (HEL) and the major histocompatibility complex (MHC) and some of their biological properties.

1. Introduction

The design of peptide based antigens has attracted considerable interest in view of the potential for development of synthetic vaccines [1][2]. In order to obtain a specific antibody response, conformational constraints such as cyclized or rigid building blocks have been proposed for stabilizing the native conformation of a protein-derived peptide antigen [3][4]. We have shown earlier (see preceding paper and references therein) that topological templates may serve as device for inducing specific 3D-conformations in protein design, thus being ideal candidates to mimic, *e.g.*, discontinuous epitopes or contact regions of peptide binding proteins involved in cell-cell interactions. This application of the Template Assembled Synthetic Proteins (TASP) approach [5–11] is exemplified for the design and synthesis of TASP antigens and MHC models for molecular recognition studies.

2. TASP Molecules as Antigens

2.1. *Models for HEL*

Hen egg white lysozyme (HEL) has been one of the most widely used protein antigens in the study of the various aspects of immune responses at the molecular level. The helix 87–97 of HEL represents an attractive model for investigating the impact of peptide conformation and its relevance for antigenicity. Most notably, the stabilization of the native structure in synthetic models, seems to represent a prerequisite for obtaining highly specific antibodies able to recognize the native molecule [3][4]. In order to test this hypothesis, we have designed and synthesized TASP molecules with a 4-α-helix bundle topology, in which the antigenic helical segment of HEL is assembled on a suitable template. Such prototype TASP molecules have been synthesized applying solid-phase peptide synthesis strategies and showed indeed increased helical content compared to the linear individual peptide block [7].

In a second generation of antigenic TASP molecules, the lysozyme derived helical blocks were arranged in an all antiparallel fashion on a topological template [12]. To this end, HEL derived peptide segments containing aldehyde functions at the C- or N-terminus were condensed to a selectively addressable topological template *via* oxime-bond formation. As followed by HPLC, the oxime-bond formation proceeded under mild conditions to completion; interestingly, the ligation of the last two helical blocks did

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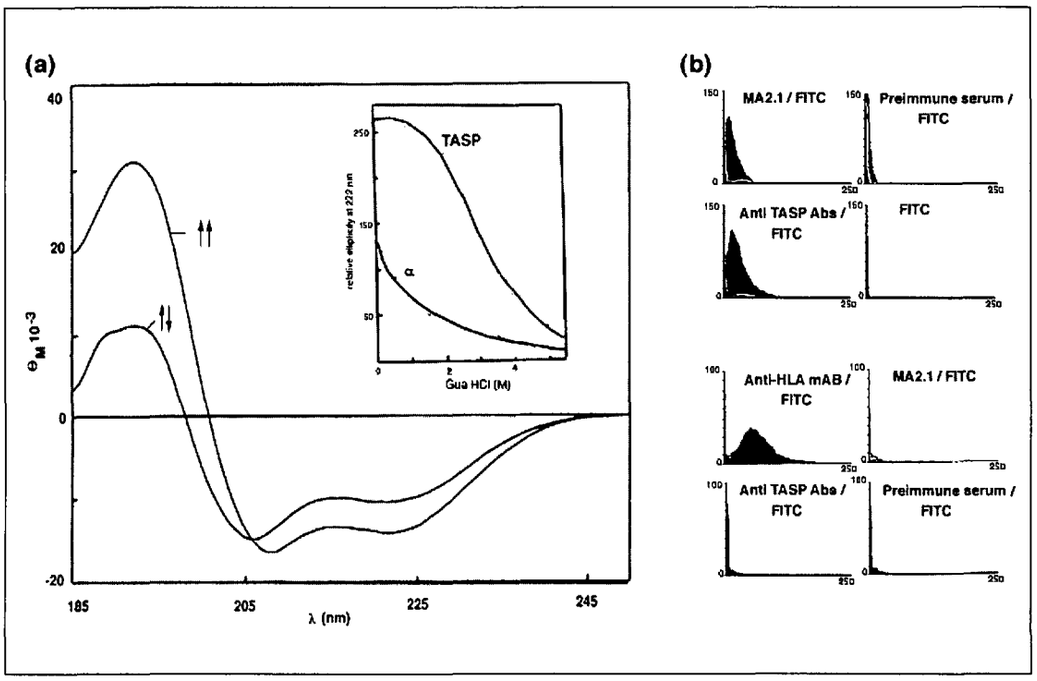

Fig. 1. a) *CD Spectra of the parallel* (↑↑) *and antiparallel* (↑↓) T_4-$(4\alpha_{15})$ *in aqueous phosphate buffer pH 7 at a concentration of* 10^{-4} M; *inset: denaturation of* T_4-$(4\alpha_{15})$ *and* α_{15} *by increasing concentrations of guanidine hydrochloride;* b) *flow cytometry analysis of anti MHC TASP antibodies on HLA-A2 positive* (upper curve) *and negative cells* (lower curve)

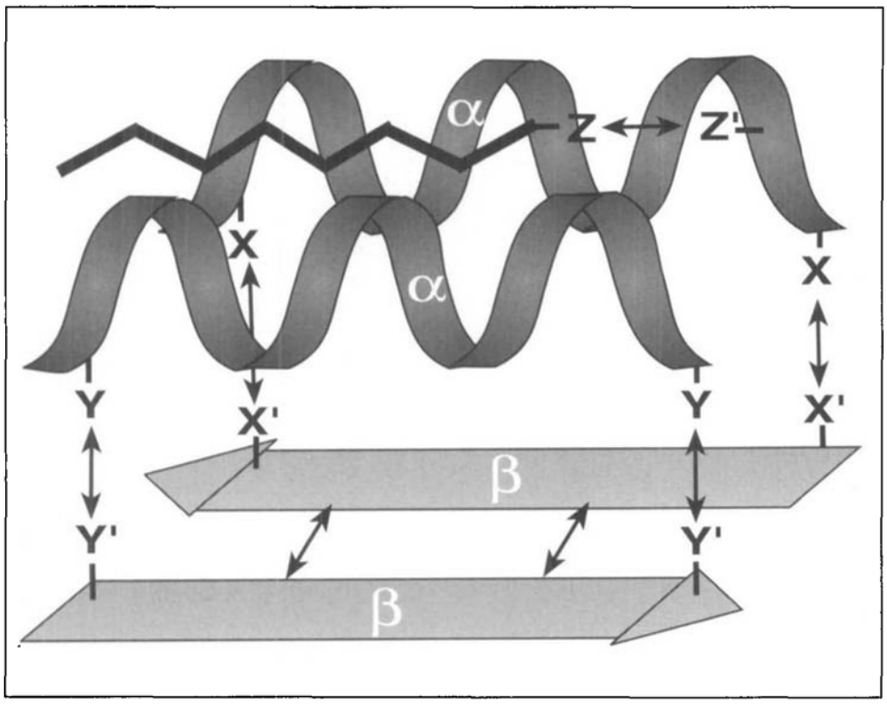

Fig. 2. *MHC II as 'locked-in' tertiary fold: the antiparallel helices α_1 and α_2 are covalently fixed to a template molecule which mimicks the underlying β-sheet structure of the native molecule.* ↔: chemoselective ligation (oxime, thioether bond); X,X'; Y,Y': functional groups (–CHO, $-ONH_2$; –SH, –CHBr) for covalently ligating the individual building blocks; Z, Z': the antigenic peptide is attached to α_1 *via* thioether formation.

not significantly interfere with the prior attached two helices. CD Spectroscopic investigation revealed high helical content and a significant increase in secondary-structure formation due to template-induced long-range interactions of the attached helices. Surprisingly, the parallel arrangement of the helices resulted in higher thermodynamic stability of the TASP compared to the antiparallel arrangement (*Fig. 1, a*). This finding should have a fundamental impact in future protein design strategies.

2.2. *Models for MHC*

Cell-surface glycoproteins of the major histocompatibility complex (MHC) are involved in antigen presentation for the stimulation of T cells and activation of an immune response. The three-dimensional structure of human MHC class I reveals a characteristic folding topology which can serve as an interesting example of considerable immunological relevance for the *de novo* design according to the TASP concept. For this purpose, a segment of the α_1 helix of MHC I was attached to a cyclic decamer peptide resulting in a TASP molecule exhibiting a pronounced tendency for α-helix formation in different solvents (*Fig. 1, a*) with a denaturation behavior similar to natural proteins (*Fig. 1, a, inset*) [4].

The presence of anti MHC I antibodies was detected by ELISA and immunofluorescence experiments confirmed the binding specificity of anti MHC TASP antibodies on HLA-A2 expressing cells (*Fig. 1, b*). Furthermore, we succeeded in raising specific anti MHC-TASP antibodies without prior conjugation to a carrier protein, which is normally required for the use of peptides as antigens.

In conclusion, the present approach allows for the stabilization of native conformations in antigenic peptide fragments giving access to the presentation of conformational epitopes and resulting in antibodies of high specificity.

3. MHC as 'Locked-in Tertiary Folds'

So far, limitations in the synthesis strategies and the low probability of designed polypeptides to fold into a unique, stable tertiary structure impeded more complex mimicry of functional domains of proteins. An interesting extension of the TASP approach represents the design of structural motifs based on the principles of a 'molecular kit' as depicted in *Fig. 2*.

Here, the folded structure is enforced and stabilized by covalent bonds resulting in 'locked-in tertiary folds' as demonstrated by the example of the antigen presenting domain of MHC II. The MHC structural motif is assembled starting from three structural building blocks, *i.e.*, a cyclic peptide mimicking the underlying β-sheet part and two helical blocks forming the binding groove for antigenic peptides. As a further design element, a selective binding site within one of the helices offers the possibility to either attach selectively an antigenic peptide or to use MHC models immobilized in an affinity chromatography column as synthetic receptors for peptide antigen screening.

The design and total synthesis of these folding motifs are based on recent progress in the synthetic methodology of peptides, most notably in chemoselective ligation procedures and orthogonal protection chemistry. The conformational analysis reveals a pronounced increase in the thermodynamic stability of the 'locked-in folds' compared to natural chain topologies. In addition, preliminary binding tests indicate that the newly engineered motifs conserve their functional properties.

In summary, the elaboration of the original concepts of template-based protein design allows for the efficient chemical synthesis of TASP molecules exhibiting immunogenic or binding properties of native proteins.

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