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Sculpted Immunogens; B-Cell Epitope Optimization Using Constrained Secondary Structure Libraries

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Abstract—A library of conformationally constrained reverse turn mimetics of the HIV V3 loop was screened against the mAb 50.1. Addition of a T_h-cell epitope to the reverse turn template provided an immunogen which, when immunized (either in alum or IFA), generated a high titer, highly specific response to HIV gp120. Copyright © 1996 Elsevier Science Ltd

Introduction

Peptide vaccines offer several advantages over more traditional (whole killed, live attenuated) vaccines, including simplified preparation, increased safety and decreased liability, and the ability to focus the immune response to a selected antigenic determinant of the pathogen. In practice, a major drawback of peptide vaccines is their general inability to elicit a high titer, high specificity antibody response to the native protein from which the peptide sequence was chosen. This is due to the fact that the three-dimensional conformation of the antigenic determinant is critical for specific recognition by antibodies. Due to their structural flexibility, short peptides are intrinsically not suitable for the preservation of the conformational and topological features required to induce specific high titer, high affinity antibodies.

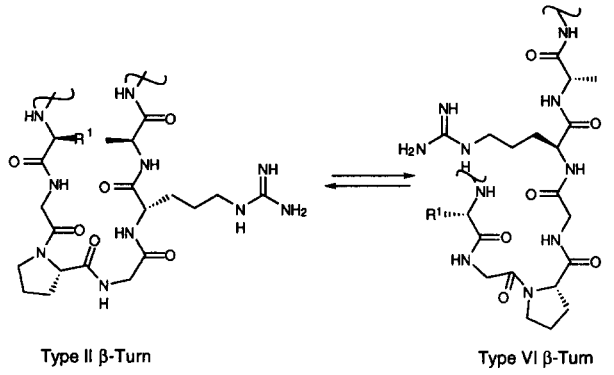
HIV V3 Loop

The human immunodeficiency virus (HIV) infects cells following the binding of the viral envelope glycoprotein gp120 to the cell surface receptor CD4.[1,2] However, the binding and infective processes can be separated.[3,4] Antibodies to the principal neutralizing determinant in the V3 loop prevent infection, but do not inhibit binding to CD4.[5–7] It has also been suggested that proteolytic cleavage of this loop, probably by a trypsin-like serine protease(s) at the cell surface, is a prerequisite to viral infection,[8] and that cleavage is enhanced by sCD4 (soluble CD4) binding and inhibited by neutralizing antibodies to the V3 loop.[9]

Gp120 is cleaved specifically at a single site in the V3 loop between R315 and A316 by thrombin.[10] Thrombin cleavage of gp120 is enhanced by sCD4 binding and is abrogated by transient exposure to nonionic detergent,[9,11] underlining the importance of substrate conformation. Hattori and coworkers reported that trypstatin, a Kunitz-type proteinase inhibitor of coagulation factor Xa, prevented infection.[8] A strong homology exists between the active site inhibitory region of trypstatin and the highly conserved GPGR region of the gp120 V3 loop.[8] The GPGR sequence from residues 312–315 is found in 85–90% of HIV isolates and is believed to contain critical structural features which are conserved, despite variability in the remaining flanking regions of the loop.[12] Additionally, Gotoh *et al.* have shown that factor Xa from chick embryo is a critical determinant in paramyxovirus activation in chick embryos, thus highlighting the importance of proteolytic cleavage to viral infection.[13,14] Mutations at G312, G314, or R315 produce non-infectious virions, although the P313 to A313 mutation did not prevent infection.[15] Secondary structure analysis,[12] solution NMR,[16] and X-ray crystallographic studies[17] suggest that this sequence, immediately preceeding the thrombin cleavage site, forms a type II β-turn, with Pro313 at the $i+1$ position, and with the adjacent segments forming antiparallel β-strands.[12,16]

Thrombin exhibits substantial secondary structure specificity for a β-sheet-turn-sheet-motif, with the reverse turn appositely orienting substrates for binding to thrombin.[18–20] We compared the predicted secondary structure for the consensus sequence ... RKSIHIGPGRAFY[12] and other high probability sequences for the principal neutralizing determinant (PND) region of the gp120 V3 loop with the thrombin-bound conformation of fibrinopeptide.[21,22]

We proposed that the enhancement of the cleavage rate observed upon CD4 binding to gp120 results from CD4-induced *trans/cis* isomerization and stabilization of the *cis* Pro313 configuration.[23] In the $i+2$ position of a β-turn, the *cis* form of Pro313 would be compatible with a type VI reverse turn at the tip of the V3 loop.



Scheme 1. The frame-shift moving Pro[313] from a *trans* configuration in the $i+1$ position of a type II β-turn to a *cis* configuration in the $i+2$ position of a type VI β-turn.

Based upon molecular modeling, we believed that this conformational shift would be required to permit cleavage by thrombin, factor Xa or a related thrombin-like cellular protease (Scheme 1).

B-Cell Epitope Optimization Using Constrained Libraries

To determine the optimum construction for our B-cell epitope, we screened a SMART Library™ (Small Molecular Arrays of Restricted Templates) against the V3 directed mAb 50.1.[17] Based upon the previous analysis, we incorporated into our constrained B-cell epitope library both the B (*before* binding) and A (*after* binding) type constructions (Fig. 1). The synthesis was performed essentially according to the previously described protocol (Scheme 2).[24] The synthesis involves the coupling of the first modular component piece **9**, to the amino terminus of a growing peptide chain **10**. Coupling of the second component **11**, removal of the

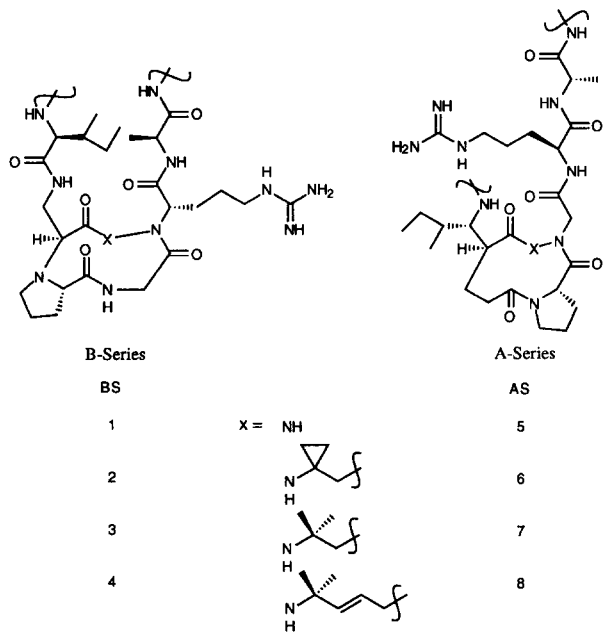
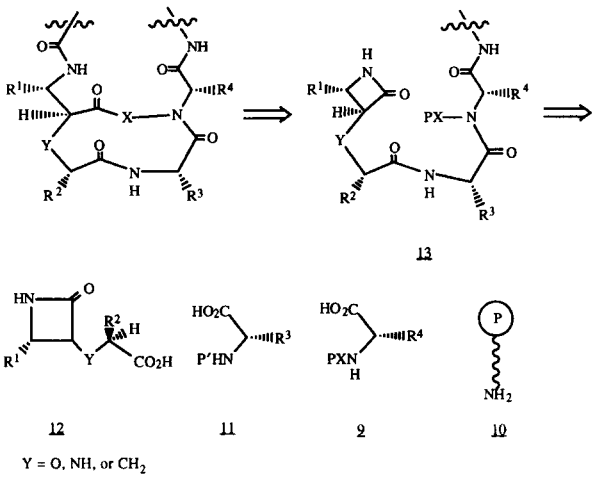


Figure 1.



Scheme 2.

protecting group P′, and subsequent coupling of the third modular component **12** provides the nascent β-turn **13**. The critical step in this sequence involves the use of an azetidinone as an activated acylating species to effect the macrocyclization reaction. Upon nucleophilic opening of the azetidinone by the X moiety, a new amino terminus is generated for continuation of the synthesis. The library was screened by ELISA for the ability to inhibit the binding of gp120 to mAb 50.1. From this assay, two constructions—**7** (12AS) and **8** (14AS)—were selected as candidates for incorporation into full length tandem T–B immunogens (Fig. 2).

In particular, we synthesized analogs of the linear peptide immunogen CLTB 108 (Connaught Laboratories) (GPKEPFRDYVDRFYKNKRK-RIHIGPGR-AF) which incorporate either the 12-membered (12AS) or 14-membered (14AS) ring reverse turn templates at the crown of the V3 loop and elicited antisera in guinea pigs against these constructions (Fig. 3). Immunological evaluation confirmed our hypothesis that a conformationally constrained B-cell epitope has the ability to generate a high titer, high specificity antibody response. Table 1 shows the peptide specific antititer generated in guinea pigs after two boosts in either IFA or alum. The data show that the tandem constructions incorporating the constrained B-cell epitopes are capable of eliciting a high titer response.

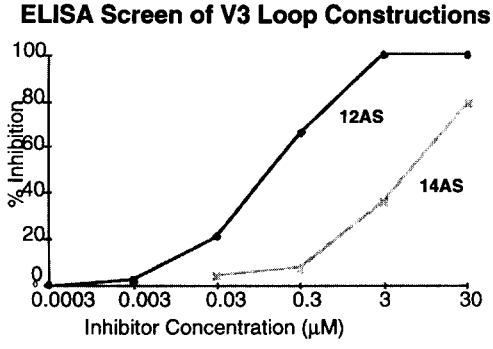


Figure 2. ELISA screen of V3 loop constructions.

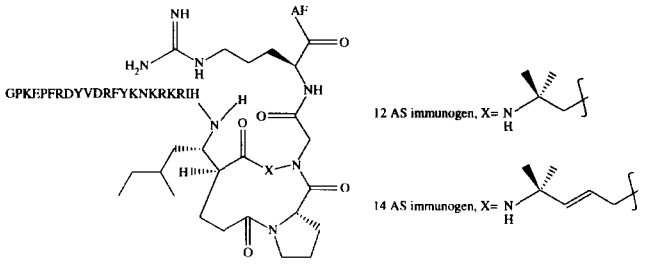


Figure 3.

Particularly noteworthy is the response of the 12AS construction in alum, which at present is the only adjuvant generally approved for humans.

Gratifyingly, the response is very specific for the native protein epitope in gp120 (Table 2). The 12AS tandem construction elicited exceptionally high antititer reactivity against gp160, in both IFA and alum. In alum, the response was four-fold higher than the response of the linear CLTB-36 (data not shown), which is presently being evaluated in the clinic.

The concept of determining an optimized B-cell epitope using a conformationally constrained library has broad applicability. This approach in principle can be utilized to find epitopes for either continuous or discontinuous determinants and should allow one to optimize the B-cell epitope component of a peptide vaccine in an antigen independent manner using only patient antisera.[25] In this regard, further investigations are in progress and will be reported in due course.

Table 1. Immunogenicity of constrained epitopes vs linear tandem epitopes in guinea pigs

Immunization	Adjuvant	Peptide-specific antititre		
		14AS	12AS	CLTB-108
14AS	ALUM	2700		900
	IFA	48,600		2700
12AS	ALUM		24,300	2700
	IFA		48,600	2700
CTLB-108	ALUM	300	300	900
	IFA	2700	2700	8100

Table 2. gp160 (gp120 MN/gp41 LAI) reactivity of guinea-pig antisera raised against peptidomimetics and linear tandem epitopes

Antisera against	Host	gp160-reactive titer
12AS/IFA	G.-pig	48,600
12AS/ALUM	G.-pig	48,600
14AS/IFA	G.-pig	16,200
14AS/ALUM	G.-pig	900
CLTB-108/IFA	G.-pig	12,150
CLTB-108/ALUM	G.-pig	900

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