*Peptides: Chemistry, Structure and Biology*
*Pravin T.P. Kaumaya and Robert S. Hodges (Eds.)*
*Mayflower Scientific Ltd., 1996*

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# Identification and Applications of Idiotype-specific Peptides for Two Murine B-Cell Lymphoma Cell Lines

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## Introduction

Over 90% of human non-Hodgkin's lymphomas are B-cell type with specific cell surface immunoglobulins (or idiotypes). These surface idiotypes are potential therapeutic targets [1, 2]. Combinatorial peptide library methods based on the "one-bead one-chemical" concept (Selectide Process) were used to identify idiotype-specific peptide ligands of two murine lymphoma cell lines (WEHI-279 and WEHI-231) with surface IgMκ. The peptides were synthesized on solid-phase beads using a "split synthesis" method [3, 4] resulting in a huge library of peptide beads such that each solid-phase bead expressed only one peptide entity [4]. The peptide-bead library ($10^6$-$10^7$) was then screened with the purified surface idiotypes derived from two murine lymphoma cell lines. With an enzyme-linked assay system, the positive beads turned color. The colored beads were then physically isolated and the amino acid sequence of the peptide determined by an automatic protein microsequencer.

## Results and Discussion

Table 1 shows the peptide motifs identified for the two murine lymphoma cell lines (WEHI-279 and WEHI-231). Both L- and D-amino acid peptide libraries were screened.

**Table 1.** *Idiotype-specific peptide motif.*

| | Cell Lines | |
|---|---|---|
| Peptide Library | WEHI-231 | WEHI-279 |
| L-amino acid library (7-mer, 9-mer, and 11-mer) | WYTP<br>WYDD<br>WY(V/I)P | RWID<br>RWFD |
| D-amino acid library (8-mer) | wGey(i/v)_v_<br>lw_ _pew(i/v)<br>kw_Gp_w | _t_Gm_k_<br>_Gr_ _w_ _ |

The D-amino acid peptide ligands are particularly interesting as they are likely to be more resistant to proteolysis *in vivo* and therapeutically more useful.

Some of the ligands shown in Table 1 were resynthesized on beads and their ability to be stained by either the purified idiotypes or whole cell extract (extracted with 0.5% NP-40) coupled with a secondary antibody-enzyme conjugate system was confirmed. In addition, some of these peptide-beads (120 μm diameter) were able to bind strongly to intact cells (~8 μm diameter) resulting in a rosette. In addition to binding specifically to their corresponding idiotypes, these peptides (in a tetrameric form) upon binding to the intact lymphoma cells were able to induce signal transduction resulting in an elevated level of protein tyrosine phosphorylation.

We are currently working on the design and synthesis of oligomeric idiotype-specific peptides with the appropriate hydrophilic linkers. One of the bifunctional hydrophilic linkers that we have designed and synthesized is $FmocNHCH_2CH_2CH_2(OCH_2CH_2)_2$-$OCH_2CH_2CH_2NH$-$COCH_2CH_2COOH$. This was synthesized by mixing a readily available and inexpensive hydrophilic molecule 4,7,10-trioxo-1,13-tridecane diamine with equal molar amount of succinic anhydride followed by derivatizing the primary amino group with Fmoc-OSu. We have succeeded in using this linker in conjunction with lysine to synthesize tetrameric idiotype-specific peptides (analogous to the multiple antigen peptide system [5]). These oligomeric peptides are then conjugated with radio-nuclide or toxin for targeted-therapy of these lymphoma cells both *in vivo* and *in vitro*. In addition, we also plan to use these peptides as a model system to develop idiotype-specific peptide reconstituted liposomes for drug delivery studies.

## References

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