S.A.R. of world's smallest carbohydrate-binding protein Odoranolectin using Liquid Glycan Arrays (LiGA)

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Introduction

Glycans are essential carbohydrates that play a role in cell recognition, immune responses, and disease progression [1]. Changes in glycosylation, the process of adding glycans to proteins or lipids, are linked to diseases like cancer. In cancer, altered glycosylation helps tumour cells evade the immune system [2]. Fucosylation is one such modification that supports cancer growth and immune escape, making it a promising target for therapies. Lectins are proteins that bind specific glycans, useful in identifying and separating glycans, especially in cancer biomarkers. However, most lectins have limitations in drug development because of their size, immunogenicity, and potential toxicity. Odorranalectin (Odo), a small, fucose-binding lectin-mimicking peptide (17 amino acid residues) derived from Odorrana grahami, exhibits high affinity toward tumour-associated glycans while maintaining low immunogenicity and potential for nasal drug delivery [3, 4].

Strategy of synthesis of modified Odorranalectins

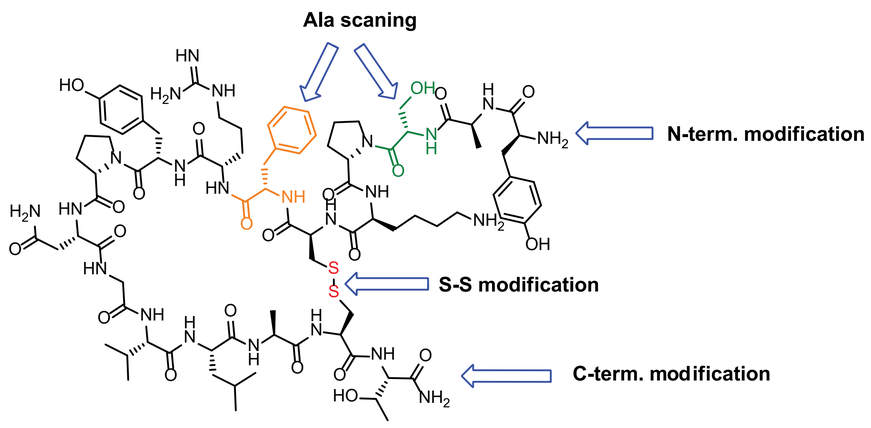


Fig. 1. The native form of Odo and points for modification

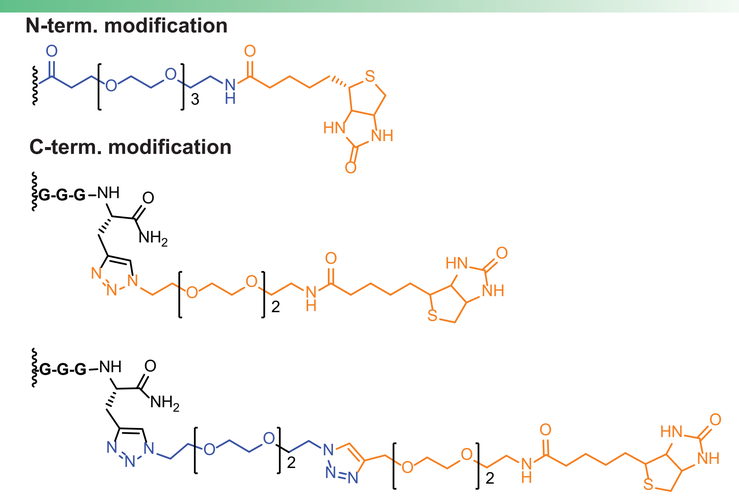


Fig. 2. Linkers for N-term. and C-term. modification

Library and Binding Assay

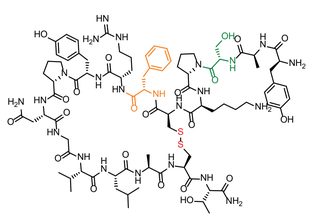
N	Sequence	a	b	c	d	e	f	g	h	i	j	k	l	m
		SH, N-term-PEG4-Biotin	S-S, N-term-PEG4-Biotin	CH_2, N-term-PEG4-Biotin	PFS, N-term-PEG4-Biotin	DFS, N-term-PEG4-Biotin	DCA, N-term-PEG4-Biotin	MBX, N-term-PEG4-Biotin	TMBB, N-term-PEG4-Biotin	TMBB, N-term-PEG4-Biotin	pIPy, N-term-PEG4-Biotin	DFBP, N-term-PEG4-Biotin	S-S, C-term-PEG4-Biotin	S-S, C-term-PEG4-Biotin
1	YASPKCFRYPNGVLACT	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k		
2	YAAPKCFRYPNGVLACT	2a	2b											
3	YASPKCERYPNGVLACT	3a	3b											
4	YASPKCARYPNGVLACT	4a	4b											
5	$_D$Y$_D$A$_D$S$_D$P$_D$K$_D$C$_D$F$_D$R$_D$Y$_D$P$_D$N$_D$G$_D$V$_D$L$_D$A$_D$C$_D$T	5a	5b											
6	YASPKCFRYPNGVLACTGGGZ												6l	6m
7	SPGCFRYPNGVKVCD	7a	7b											
8	RLCYMVLPCP	8a	8b											
9	24AA-YASPKCFRYPNGVLACT	9a	9b											
10	YASPKC$_D$FRYPNGVLACT	10a	10b											

Table 1. Library Odos

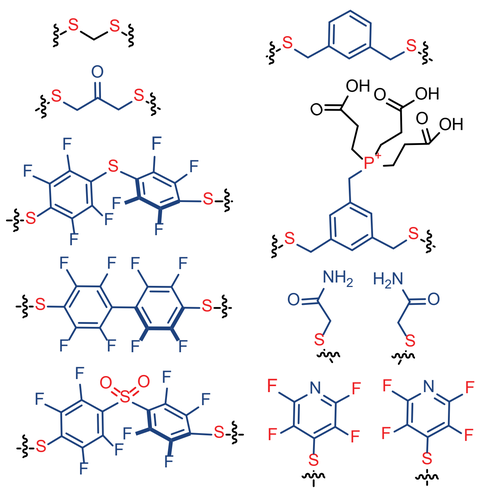


Fig. 3. Linkers for Cys

The Liquid Glycan Array (LiGA) is a sophisticated platform used to map glycan-binding profiles of lectins with high accuracy. Developed by our group [5], it utilizes glycan-functionalized, DNA-barcoded M13 bacteriophages and Streptavidin-coated plates to enhance binding efficiency and optimize signal-to-noise ratios, providing superior sensitivity (Fig. 4). Using this advanced technology, we investigated the glycan-binding affinities of biotin-labeled Odorranalectin (Odo) variants against our largest glycan libraries. These experiments offer critical insights into Odo's selectivity and binding behaviour, supporting its potential applications in glycobiology and targeted drug delivery.

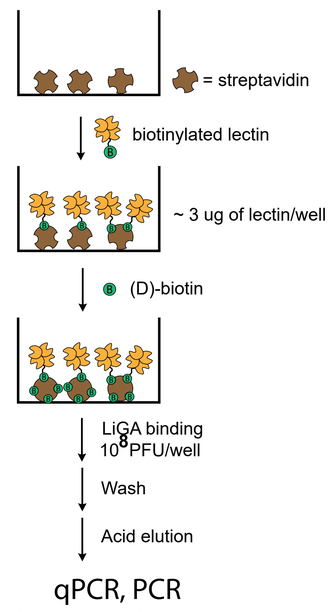


Fig. 4. Scheme of Binding Assay

Results

What is a better C-term. or N-term. modification?

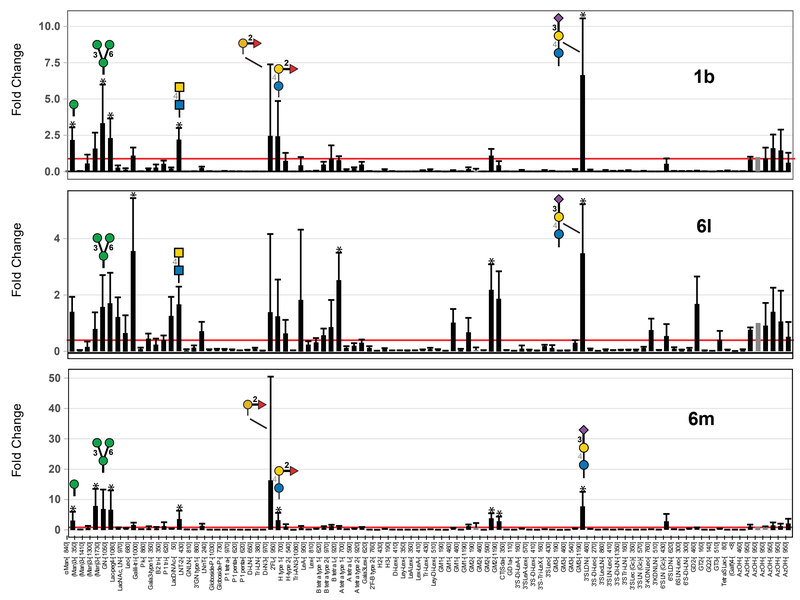


Figure 5. Binding of LiGA (EF) to biotin-labeled Odos immobilized on streptavidin coated well plates.

The results from Binding of LiGA (EF) to biotin-labeled Odos indicated that the choice of modification site, whether the N- or C-terminal, did not significantly impact the binding properties of the Odorranalectin variants (Fig.5). Based on the information, we decided to work with N-term. biotin-labeled Odo mutants.

Results from binding revealed nonspecific binding for compound 5b (D-Odo) (Fig.6). This unexpected observation raised further questions about the underlying cause. To ensure validity, control experiments were conducted using well-characterized lectins such as ConA, UEA, and CBM, which demonstrated expected specific binding profiles. The next step involved examining the influence of the linker between sugar and phage on recognition by mirror forms of Odorranalectin. Unexpectedly, neither 1b nor 5b demonstrated binding with sugars containing an Alk-COOH linker (Fig.7).

Binding of LiGA (EF) to biotin-labeled Odos

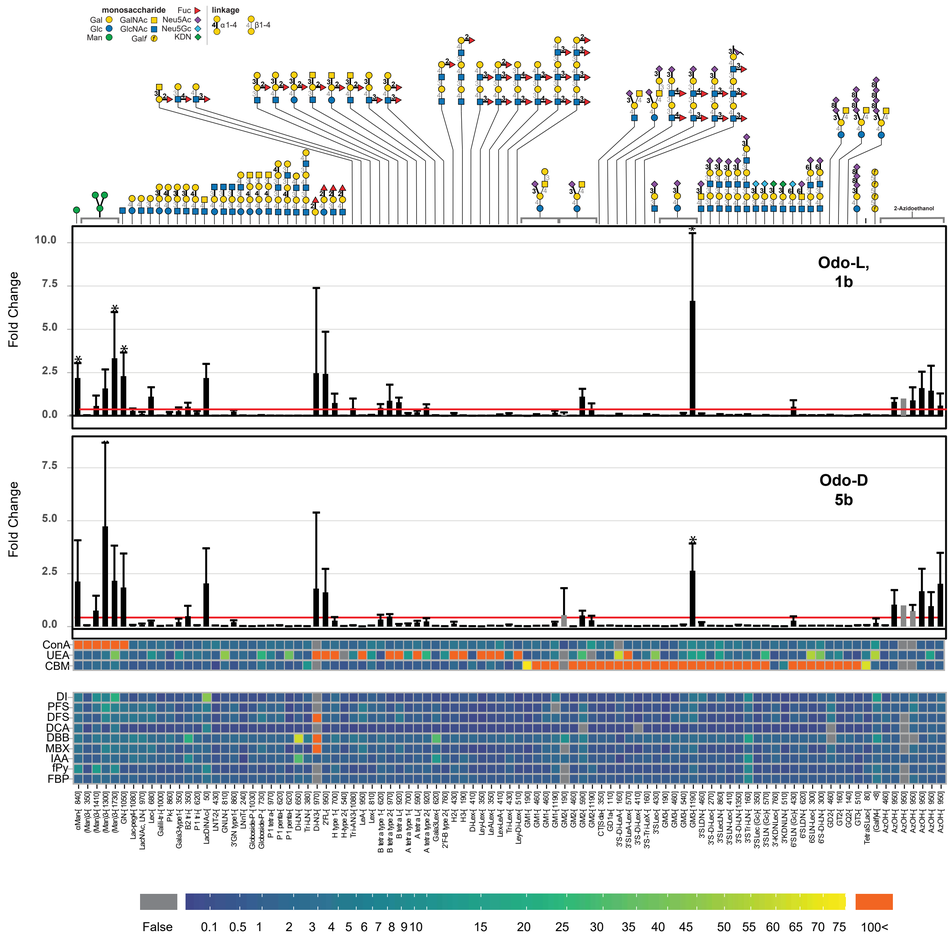


Figure 6. Heat map for binding of LiGA (EF) to biotin-labeled Odos immobilized on streptavidin coated well plates.

Nonspecific binding - Why?

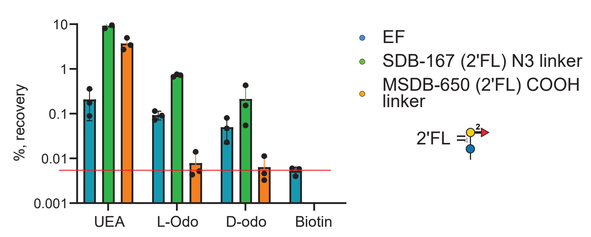


Figure 7. Recognizing UEA, L-and D-Odo fucosylated phages: LiGA (EF-library) (blue colour), 2'FL-azido linker (green colour) and 2'FL - carboxylic linker (orange colour).

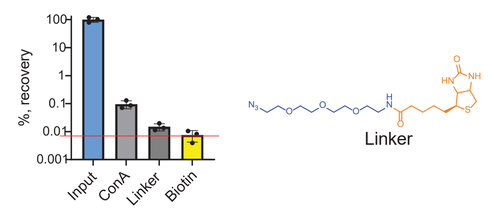


Figure 8. Binding of LiGA (EF) to linker.

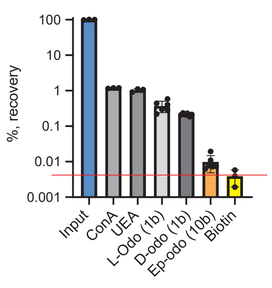


Figure 9. Binding of LiGA (Mega-Lib) to biotin-labeled Odos immobilized on scll plates.

After analyzing the binding of linkers with LiGA (Fig. 8), the structures of L-Odo, D-Odo, and epimeric Odo (Ep-Odo) (Fig. 9) were compared. The analytical data highlighted the critical role of the L- and D-Odo structures (in their β-forms) in determining binding specificity and efficiency.

Conclusion

Is Odorranalectin the smallest lectin in the world? The question remains open. Through our research, we established several key findings:

1. Odorranalectin is a macrocycle exhibiting lectin-modulated properties.
2. The linker between the sugar and phage plays a crucial role in recognition.
3. The β-forms of Odorranalectin are fundamental to its lectin-like behavior.
4. Both L- and D-Odo exhibit nonspecific binding tendencies.
5. Structure-activity relationship (SAR) studies identified a promising candidate from the library for in vivo studies, sparking discussions on the potential of D-Odo.

Acknowledgments

DERDA RESEARCH GROUP GlycoNet 48Hour Discovery

References

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