Glycopeptides

Saccharide-Induced Peptide Conformation in Glycopeptides of the Recognition Region of LI-Cadherin**

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Dedicated to Professor Dieter Hoppe on the occasion of his 65th birthday

Glycoproteins of the outer cell membranes are components of the glycocalix involved in fundamental recognition processes.^[1] Among them, the cadherins are crucial to cell adhesion and to the formation of tissues. The epithelial E-cadherin, having five extracellular consensus repeat domains,^[2] also plays an important role in cell differentiation.^[3] Its interaction is homotypic and homophilic. The homophilic recognition site identified by X-ray analysis and multidimensional NMR spectroscopy is located in the N-terminal domain and consists of three antiparallel β sheets— β C, β F, and β G—arranged around the central recognition motif HAV (His-Ala-Val, Figure 1 a).^[4,5]

Glycopeptide partial sequences of the homophilic recognition region of E-cadherin were shown to induce differentiation in transformed but E-cadherin-expressing keratinocytes.^[6] The effect proved dependent upon the conformation of the glycopeptide and was only observed if the saccharide forced the peptide backbone to adopt a turnlike conformation (Figure 1a). Except for the amino acid sequence not much is known about the structure of LI-cadherin,^[7] which was discovered in 1994 in rat liver and intestine. LI-cadherin mediates the Ca2+-dependent cell adhesion^[8] and is involved in intestine development as well as in differentiation processes.^[9] In the N-terminal domain a recognition motif Ala-Ala-Leu was identified.^[7] It can be concluded from sequence comparison that LI-cadherin has a homophilic recognition region similar to that in E-cadherin, in which a turn sequence (SOG) C-terminally follows the recognition motif AAL (Figure 1b). To prove this hypothesis, glycopeptide sequences I (L⁹⁵AALDSQGAIV¹⁰⁵) of the LI-cadherin recognition region^[7] were synthesized and subjected to conformational analysis by NMR spectroscopy.

The saccharide parts of these glycopeptides were systematically varied in the form of tumor-associated mucin antigens in order to determine the effect of type and size of the saccharide upon the peptide conformation. This appeared attractive, as no conclusions regarding a saccharide-depend-

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Figure 1. Homophilic recognition region of a) E-cadherin (based on X-ray crystal structure) and b) L1-cadherin (postulated, based on sequence homologies).

ant effect could be drawn from previous conformational studies on single mucin glycopeptides.^[10–15]

The 9-fluorenylmethoxycarbonyl (Fmoc)-protected Oglycosyl serine building blocks were synthesized from Fmocserine tert-butyl ester^[16] via the T_N antigen serine derivative $\mathbf{1}^{[17]}$ (Schemes 1 and 2). Its selective deprotection led to compound 3,^[18] which served as the substrate for the construction of all extended saccharide side chains. All glycosylation reactions and manipulations of protecting groups must be carried out preserving both the base-labile Fmoc group and the acid-labile tert-butyl ester. The 4,6benzylidene acetal 4 was formed under controlled acid catalysis. Subsequent Helferich glycosylation using the galactosyl bromides 5 and 6 afforded the T antigen serine derivatives 7 and 8, respectively. Selective hydrolysis of the benzylidene acetal 7 to give 7a was achieved by treatment with hot aqueous acetic acid. Acetylation and treatment with trifluoroacetic acid yielded the protected T antigen serine building block 7b (Scheme 1).

Analogous acidolysis (step a in Scheme 1) converted **1** into the T_N antigen serine derivative **2**. Xanthate **9** of sialic acid benzyl ester was coupled regio- and stereoselectively to

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Scheme 1. a) TFA/CH₂Cl₂ 1:1, anisole, 6 h, RT, 73% (HPLC); b) NaOCH₃, CH₃OH, pH 8.5, RT, 72%; c) benzaldehyde dimethyl acetal, *p*-toluenesulfonic acid, pH 4, CH₃CN, RT, 63–77%; d) **5/6**, Hg(CN)₂, CH₂Cl₂/ CH₃NO₂, 14 h, RT, 66% (**7**), 91% (**8**); e) AcOH/H₂O 4:1, 85 °C, 1 h, 64–90%; f) 1. Ac₂O/pyridine, 0 °C/4 h \rightarrow 14 h, RT, 97%; 2. TFA, anisole, 1.5 h, RT, 53% (HPLC); g) **9**, AgOTf, CH₃SBr, CH₃CN/CH₂Cl₂, –65 °C, 38– 44%; h) Ac₂O/pyridine, 0 °C/4 h \rightarrow 14 h, RT, 99%; i) TFA/CH₂Cl₂ 1:1, anisole, 6 h, RT, 99%; j) **9**, AgOTf, CH₃SBr, CH₃CN/CH₂Cl₂, –65 °C, 20% (R=Ac), 71% (R=Bn); k) **5**, Hg(CN)₂, CH₂Cl₂/CH₃NO₂, 14 h, RT, 44%; l) R=Ac: 1. Ac₂O/pyridine, 14 h, RT, 99%; 2. TFA, anisole, 1.5 h, RT, 70% (HPLC); R=Bn: TFA, anisole, 1.75 h, RT, 61% (HPLC). Bn = benzyl, OTf=trifluoromethanesulfonate, TFA=trifluoroacetic acid.

the partially unprotected T_N antigen and T antigen serine derivatives 3 and 7a at -65 °C to give the sialyl- T_N antigen (10) and 2,6sialyl-T antigen (12) structures, respectively. After Oacetylation and acidolysis of the tert-butyl esters the sialyl-T_N (11) and 2,6-sialyl-T antigen (13) serine building blocks were obtained (Scheme 1). Alternative formation of the 2,6-sialyl-T structure by Helferich glycosylation of 10 (step k in Scheme 1) afforded 12 with lower yield than that obtained for the sialvlation of 7a. The 2,3-sialyl-T derivative required for the complete set of tumor-associated mucin saccharide antigens was synthesized from precursor 8 (Scheme 2). Its O-deacetylation with catalytic sodium methoxide in methanol proceeded very slowly even at pH 9.5,^[19] showing that more rapid transesterification reactions (e.g. of 1) proceed first by O-acetyl migration to the 6-position.



Scheme 2. a) NaOCH₃, CH₃OH, pH 9.5, 24 h, RT, 39%; b) 9, AgOTf, CH₃SBr, CH₃CN/CH₂Cl₂, 3 h/−50°C→14 h/−30°C, 49%; c) 1. Ac₂O/pyridine, 14 h, RT, 95%; 2. AcOH/H₂O 4:1, 85°C, 1 h, 76%; d) Ac₂O/pyridine, 14 h, RT, 99%; e) TFA, anisole, 1 h, RT, 85% (HPLC); f) 9, AgOTf, CH₃SBr, CH₃CN/CH₂Cl₂, 3 h/−50°C→14 h/−30°C, 36%; g) TFA, anisole, 1.5 h, RT, 93%.

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The subsequent regio- and stereoselective sialylation with the xanthate 9 at -50 °C stereoselectively produced the 2,3sialyl-T serine derivative 14. After acetylation of the free hydroxy groups of the galactose portion and solvolysis of the benzylidene acetal, the partially unprotected galactosamine derivative 15 was furnished. Its O-acetylation and acidolysis gave the 2,3-sialyl-T serine building block 16. By selective sialylation of 15, again using xanthate 9 at -50 °C, the most complex structure, the glycophorin antigen 17, was formed. Subsequent acidolysis of the *tert*-butyl ester resulted in the formation of building block 18. The solid-phase syntheses of LI-cadherin glycopeptides I were carried out using a Wang anchor^[20] on Tentagel resin^[21] preloaded with Fmoc valine according to published procedures^[22] (Scheme 3).



Scheme 3. Solid-phase syntheses of glycopeptides **20–25** containing the amino sequence LAALDSQ-GAIV.

For comparison, the non-glycosylated LI-cadherin peptide **19** was also synthesized using the same procedures. The yields of products isolated after acidolytic cleavage from the solid support as well as the yields of peptide **19** and glycopeptides **20–25**^[23] after cleavage of the remaining benzyl groups by hydrogenolysis, removal of the O-acetyl groups by transesterification, and purification by HPLC are listed in Table 1.

To analyze their preferred conformation, peptide **19** and glycopeptides **20–25** were investigated by NOESY and ROESY NMR spectroscopy in $[D_6]DMSO$ solution. For discussion of the most important NOE contacts, the amino acids of the LI-cadherin sequence **I** are designated from the N terminus L¹ to the C terminus V¹¹. Based on the observed

 $\textit{Table 1:} Yields of solid-phase synthesis of peptide 19 and glycopeptides 20–25. <math display="inline">^{[a]}$

Building block	-	2	7 b	11	13	16	18	
(Glyco-)Peptide	19	20 78	21	23	24	22	25	
Yield (overall) [%]	99	57	23	24	57	17	21	

[a] The glycopeptides were prepared by solid-phase synthesis using the glycosyl serine building blocks **2**, **7b**, **11**, **13**, **16**, and **18**. [b] After cleavage from solid phase.

NOE contacts,^[24] a three-dimensional structure of each (glyco)peptide (Figure 2) was determined by molecular dynamics calculations and energy minimization by an MM2

force field method.^[25,26] According to published procedures, the found dihedral angles within the turn region (Table 2) were compared to the ideal dihedral angles reported for the known turn types.^[27] The deviations from these turn types are given in Table 2 as the sums of the differences in these angles.

From these values the following conclusions were drawn (Figure 2). Peptide 19 adopts a looplike conformation (NH contacts in the sequence DSO) in the vicinity of the turn sequence DSQG. It does not resemble any ideal turn conformation (contacts from NH-A⁹ to α - and γ -CH-L⁴). The structure is reminiscent of a beginning helix that is not continued towards the C terminus. The C terminus folds back to the turn sequence (α -CH-Q⁷ to α -CH-V¹¹), resulting in a kind of double loop. The situation for the T_N-antigen glycopeptide 20 is similar. Nevertheless, comparison of the dihedral angles (Table 2) suggests a β III' turn formed within the sequence

LDSQ, which, however, cannot assume a stable β -sheet structure (contacts between α -CH-L⁴ and α -CH-V¹¹, and between γ -CH-L⁴ and α -CH-I¹⁰).

The conformation of the sequence DSQG of T-antigen glycopeptide **21** is most like a β -II turn (Table 2, Figure 2). The saccharide apparently stabilizes this conformation (contacts between CH-NAc and α -CH-D⁵, α -CH-S⁶, and NH-D⁵). The N-terminal sequence and the C-terminal sequence adopt a conformation similar to a β sheet (γ -CH-L⁴ and γ -CH-I¹⁰, β -CH-L⁴ and NH-V¹¹, and NH-D⁵ and β -CH-I¹⁰).

In sialyl-T antigen LI-cadherin glycopeptides **22**, **24**, and **25** the larger saccharides exhibit a marked influence on the conformation of the peptide backbone, which according to NOE contacts apparently forms an increasingly closer loop

Table 2: Dihedral angles of (glyco)peptides within the amino acid sequence LAALDSQGAIV.^[a]

	Antigen	Turn sequence	Ca	alculated dihedral angles			Deviation from the ideal turn (type β)						(type γ)	
			${I\!\!\!\!/}_{i+1}$	Ψ_{i+1}	$arPsi_{i+2}$	Ψ_{i+2}	I	ľ	П	Π'	III	ÎII′	γ	γ′
20	T _N	LDSQ	43.8	39.1	69.7	28.7	361	74	224	354	362	41	115	125
23	ST _N	DSQ	77.1	-63.0									20	260
21	Т	DSQG	-19.3	121.9	66.2	-5.3	354	200	62	473	345	218	119	277
24	(2→6)ST	DSQG	160.4	-2.9	59.0	-0.7	397	165	365	357	397	168	158	283
22	(2→3)ST	LDSQ	81.9	-36.8	-98.0	-168.8	326	446	646	292	321	449	45	239
25	S ₂ T	DSQG	45.1	-79.5	-159.5	0.9	225	375	545	136	272	376	34	245

[a] The smallest sums of deviation from the ideal dihedral angles are printed in bold.



Figure 2. Preferred conformations of peptide **19** and glycopeptides **20–25** of LI-cadherin determined by NOESY/ROESY experiments in $[D_6]DMSO$. The saccharide (more intensively colored) is positioned to the right of the corresponding peptide (C gray, O red, N blue).

with antiparallel alignment of the N- and C-terminal sequences. Contacts between NHAc and A⁹ (in **22** and **24**) and between H-2 (GalNAc) and NH-G⁸ (in **25**) suggest that the saccharide is oriented parallel to this loop (in Figure 2, right). From the deviations in the dihedral angles (Table 2) one can conclude that a γ -turn-like conformation that adopts an expanded and twisted form is most likely. This especially applies to 2,6-sialyl antigen glycopeptide **24**. A γ -turn conformation can also be assumed for the sequence DSQ of sialyl-T_N antigen glycopeptide **23**, with backfolding of the C terminus (NOE contacts between α -CH-Q⁷ and γ -CH-I¹⁰, and between NH-G⁸ and β -A⁹) similar to **20**. For the sialyl-T glycopeptides, the interactions between the peptide sequences in the N- and C-terminal regions are typical (**22**: between α -CH-A³ and NH-I¹⁰/NH-V¹¹; **24**: between NH-L⁴ and α -CH-

V¹¹, between β-CH-L⁴ and γ-CH-I¹⁰; **25**: between β-CH-L⁴ and γ- and δ-CH-I¹⁰). It is characteristic for the conformations of these compounds that only few direct interactions between the saccharide and peptide parts are observable in most cases and restricted to the GalNAc group (**22**: between AcNH-GalNac and α-CH-A⁹, between H-2-GalNAc and β-CH-S⁶; **24**: between CHAc-GalNAc and α-CH-D⁵/α-CH-A⁹; **25**: between H-2-GalNAc and NH-G⁸).

The described conformational analyses of LI-cadherin glycopeptides not only prove that the saccharide side chains exert a marked influence upon the conformation of the peptide chain (Figure 2) but also show that this effect is dependent on the type and size of the saccharide. Beyond this case,^[7] it is important to note that this conformational influence was investigated for the tumor-associated mucin saccharide antigens. With a glycopeptide vaccine from tumor-associated mucin MUC1 a specific immune response in mice could be induced. The induced antibody reacted neither with the non-glycosylated peptide of identical sequence nor with the tumor-associated saccharide antigen (sialyl-T_N), but only with the glycopeptide,^[28] probably indicating that the peptide chain adopts the recognized conformation only within the glycopeptide.

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