Carbohydrate Research 345 (2010) 1348–1359

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Kinetic and thermodynamic properties of MAG antagonists

Stefanie Mesch [a], Katrin Lemme [a], Hendrik Koliwer-Brandl [b], Daniel S. Strasser [a], Oliver Schwardt [a], Soerge Kelm [b], Beat Ernst [a,*]

[a] Institute of Molecular Pharmacy, Pharmacenter, University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland
[b] Center for Biomolecular Interactions Bremen, Glycobiochemistry, University of Bremen, POB 330440, D-28334 Bremen, Germany

ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 4 March 2010
Accepted 9 March 2010
Available online 16 March 2010

Keywords:
Carbohydrate mimetics
Siglecs
Myelin-associated glycoprotein (MAG)
Thermodynamics of carbohydrate–protein interactions
Kinetics of carbohydrate–lectin interactions

ABSTRACT

Paraplegia is caused by injuries of the central nervous system (CNS) and especially young people suffer from these severe consequences as, for example, the loss of motor functions. The lack of repair of the injured nerve strands originates from the inhibitory environment for axon regeneration in the CNS. Specific inhibitory proteins block the regrowth of nerve roots. One of these neurite outgrowth inhibitors is the myelin-associated glycoprotein (MAG), which is a member of the Siglec family (sialic acid-binding immunoglobulin-like lectin). In previous studies, we identified potent small molecule MAG antagonists. In this communication, we report new neuraminic acid derivatives modified in the 4- and 5-position, and the influence of various structural modifications on their kinetic and thermodynamic binding properties.

1. Introduction

Paraplegia is caused by injuries of the central nervous system (CNS). A therapy for full regeneration of injured nerve strands is not yet available. The lack of regeneration originates from the inhibitory environment in the CNS,[1,2] that is, specific inhibitors on residual myelin and on astrocytes, which are recruited to the site of injury.[3–5] In the last decade, several inhibitor proteins have been identified, one of them being the myelin-associated glycoprotein (MAG).[6] MAG is a transmembrane glycoprotein, belonging to the Siglec family (sialic acid-binding immunoglobulin-like lectin).[7,8] On the surface of neurons, MAG interacts with two classes of targets: proteins of the Nogo receptor family[9,10] and gangliosides, primarily the gangliosides GD1a and GT1b.[11–14] Although the relative role of Nogo receptors and gangliosides as MAG ligands has yet to be resolved, in some systems, neurite outgrowth can be initiated by sialidase treatment, suggesting that the sialic acid-mediated interactions of MAG predominantly contribute to the inhibitory process.[15] Therefore, blocking MAG with potent glycomimetic antagonists may be a valuable therapeutic approach to enhance axon regeneration. Based on the best known natural ligand of MAG identified to date, the ganglioside GQ1bα (Fig. 1), different series of antagonists have been developed.[16–21]

With neuraminic acid derivatives such as **1**,[16] Kelm et al. reported a remarkable simplification of the relevant tetrasaccharide binding epitope of GQ1bα. Further reported modifications are related to lipophilic interactions. Thus, antagonists with a lipophilic core, for example, the biphenyl derivatives **2**[18] or a lipophilic replacement of the α-(2→6)-linked Neu5Ac, for example, **3**[19] were synthesized (Fig. 1).

The concept of drug discovery is based upon selectively addressing particular biological targets preferably by low molecular weight compounds. In vitro determined drug–target interactions are classically rated in terms of binding parameters such as IC_{50}'s and K_D's. An alternative perspective on drug optimization is the residence time of the drug–target binary complex,[22] as quantified by the dissociation half-life ($t_{1/2}$). Potential advantages of a long residence time are extended duration of the pharmacological effect and target selectivity.[22,23] Especially in the field of carbohydrate–lectin interactions, this is a crucial point to address. As a result of the shallow and water accessible binding sites of lectins, carbohydrates bind with only low affinity and show very fast dissociation off-rates, leading to $t_{1/2}$ in the range of seconds. Examples

Abbreviations: AIBN, α,α′-azodiisobutyronitrile; aq, aqueous; BnBr, benzyl bromide; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; FAc, fluoro-acetyl; HBS-E, HEPES/NaCl/EDTA buffer; HBS-EP, HEPES–NaCl–EDTA–P20 buffer; ITC, isothermal titration calorimetry; K_D, dissociation constant; MS, mass spectrometry; Neu5Ac, *N*-acetylneuraminic acid; NgR, Nogo receptor; NMR, nuclear magnetic resonance; PDC, pyridinium dichromate; PPTS, pyridinium *p*-toluenesulfonate; RP, reversed phase; SPR, surface plasmon resonance; STD-NMR, saturation transfer difference nuclear magnetic resonance spectroscopy; THF, tetrahydrofuran.

* Corresponding author. Tel.: +41 267 15 51; fax: +41 267 15 52.
E-mail address: beat.ernst@unibas.ch (B. Ernst).

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.03.010

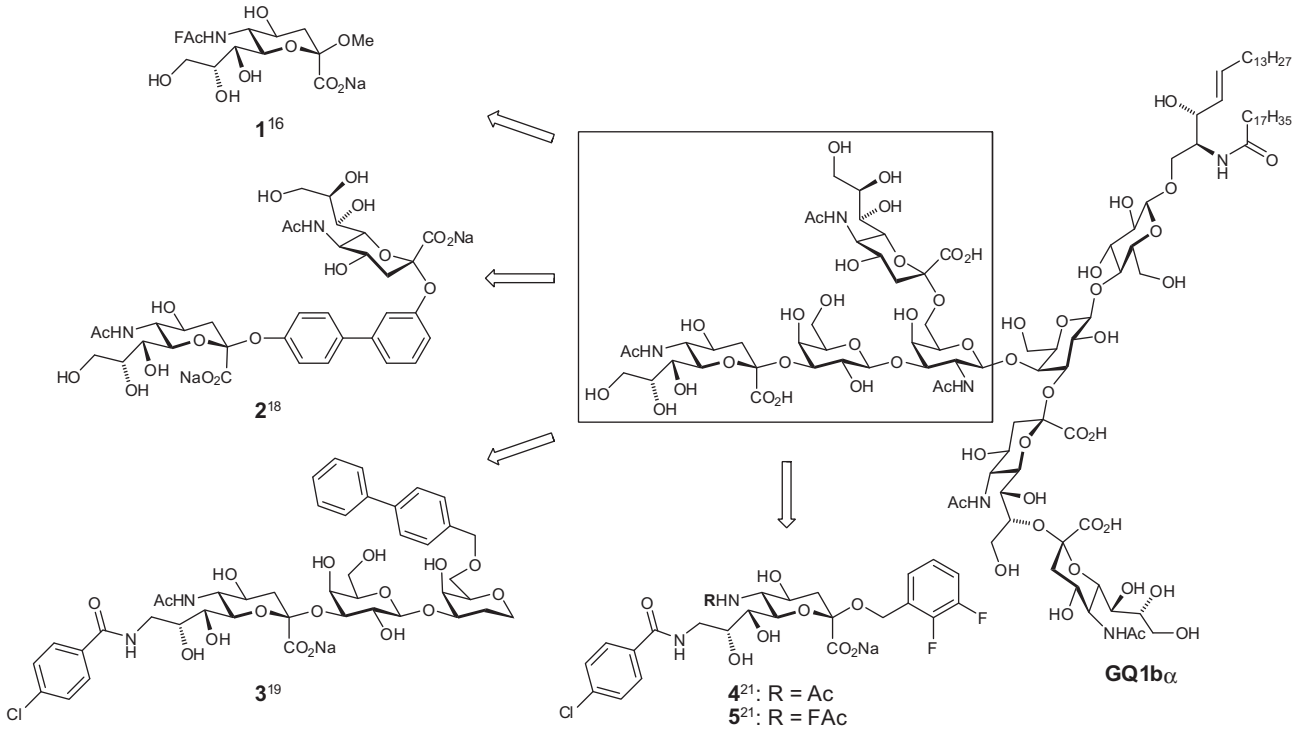

Figure 1. MAG antagonists **1**[16], **2**[18], **3**[19] and **4**, **5**[21]derived from the tetrasaccharide core structure (highlighted in box) of GQ1bα.

Table 1
Carbohydrate–protein interactions: thermodynamic and kinetic binding parameters

Protein	Ligand	K_D (μM)	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	$t_{1/2}$ (s)
P-Selectin[24]	PSGL-1	0.3	$4{\cdot}10^6$	1.4	0.5
E-Selectin[25]	ESL-1	62	$4{\cdot}10^4$	3.0	0.2
GSLA-2 mAb[26]	Sialyl Lewis[a]	4.3	$1.1{\cdot}10^5$	0.48	1.5
MAG[19]	Neu5Ac derivative **3**[19]	2.8	$3.5{\cdot}10^5$	0.8	0.9

of thermodynamic and kinetic parameters for carbohydrate–protein interactions are summarized in Table 1.

For medical applications, an improved $t_{1/2}$ of the drug–protein complex is beneficial, because the therapeutic effect can be reached with a lower dose. Zanamivir is one of the prominent examples, where a carbohydrate-based lead was optimized to yield a drug with a dramatically improved kinetic behavior, showing a half-life of 33 min of its complex with the B/Memphis/3/89 (H3N2) influenza virus.[27] In this communication, we present various MAG antagonists modified at the 4- and 5-position with the aim to modulate their kinetic properties. In general, lead optimization is often achieved by additional lipophilic contacts and thereby improving the binding entropy. As a result of the increased lipophilicity, the dissociation half-life ($t_{1/2}$) of the drug–target complex is extended.[28,29] The starting point for our investigation was MAG antagonist **5**,[21] a result of an extended optimization program focusing exclusively on the improvement of its thermodynamic binding properties.[17–19,21]

2. Results and discussion

Recently, we reported the synthesis and biological evaluation of a series of MAG antagonists with affinities in the low micromolar range.[21] Furthermore, pharmacokinetic parameters such as stability and membrane penetration indicated that the antagonists **4** and **5** (Fig. 1) fulfill the basic requirements for lead compounds. As halogenated acetates at the 5-position led to a drastic improvement of the binding affinity,[16,21] we investigated the impact of this position on the thermodynamic properties and also examined its influence on the dissociation half-life time. Molecular modeling studies with a homology model of MAG[30] suggested that the hydroxy group in the 4-position is not directly involved in the binding process[19] and therefore provides a possibility for derivatization. Because additional hydrophobic contacts based on the 4-position and an inverted configuration at C-4 are expected to alter the thermodynamic and kinetic behavior, we synthesized a small library of antagonists and analyzed their binding properties by surface plasmon resonance.

2.1. A MAG antagonist modified in the 5-position of the Neu5Ac scaffold

With isothermal titration calorimetry, we determined the thermodynamic parameters ΔH, ΔS, and ΔG[31] of antagonist **5** interacting with a recombinant protein consisting of the three N-terminal domains of MAG and the Fc part of human IgG ($MAG_{d1\text{-}3}$-Fc).[32] For the ITC experiment, a solution of **5** (500 μM, HBS-E buffer) was injected into a solution of $MAG_{d1\text{-}3}$-Fc (48.35 μM, HBS-E buffer) at 25 °C (Fig. 2).

The experimental data were fitted to a theoretical titration curve (one site binding model) using *Origin version 7* software (MicroCal) and the thermodynamic parameters calculated according to the equation shown in Table 2. The ITC experiment confirmed the high potency of **5**, having a K_D in the nanomolar