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Human-specific evolutionary genetic loss of addition of a single oxygen atom from sialic acids increases hydrophobicity of cells and proteins

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ABSTRACT

Humans, unlike all old world primates studied to date, lack *N*-glycolyl neuraminic acid (Neu5Gc: Gc) due to the evolutionary genomic loss of CMP-N-acetylneuraminic acid hydroxylase (CMAH), leading to accumulation of the *N*-acetyl neuraminic acid (Neu5Ac: Ac). Given the high sialic acid density on cell surfaces, we hypothesized that the ratio of Ac/Gc could influence hydrophobicity. Herein, we employed surface wetting experiments and atomic force microscopy (AFM) to investigate the hydrophobicity of Neu5Ac and Neu5Gc at the surface, protein, and cellular levels. A 5 ± 2° difference in the wetting angle of Ac/Gc-coated surfaces confirmed the greater hydrophilicity of Neu5Ac compared to Neu5Gc. AFM studies using a hydrophobically modified probe and plasma sialoglycoproteins, as well as human lymphoma cells engineered to express varying amounts of Neu5Ac or Neu5Gc, demonstrated that both proteins and cells expressing Neu5Ac exhibit a higher frequency of hydrophobic interactions with the AFM probe than those expressing Neu5Gc. These findings suggest that the loss of a single oxygen atom in sialic acid during human evolution may have significantly influenced hydrophobic properties, contributing to alterations in binding affinity and molecular interactions.

1. Introduction

All living cells, whether they are microbes or part of a multicellular organism, are covered in a coat of glycan molecules commonly known as the glycocalyx. In their position between the cell and its surrounding environment, glycans are involved in a variety of crucial processes that involves cell-cell, cell-matrix, cell-molecule and host-pathogen interactions [1]. Sialic acids (Sias) are a family of derivatives of neuraminic acid that all share a nine-carbon backbone. They are often found as the terminal unit on mammalian glycans, and as a result of this location as well as their diverse structure they carry out multiple biological functions [2]. Roles of Sias include functioning as an epitope for protein binding, masking the underlying glycans from recognition and involvement in cell-cell, pathogen-cell and toxin-cell interactions [3]. Due to an electronegative charge, they also contribute to the net negative charge of the surface, electrostatically based interactions and protein stability. In mammals, the two most abundant Sias are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) [4] (see Table 1)

Around 2–3 million years ago, a deleterious mutation in the *CMAH* gene occurred in our ancestors, resulting in a loss of function mutation in the enzyme cytidine monophospo-N-acetyl neuraminic acid hydroxylase (CMAH). This enzyme hydroxylates Neu5Ac into Neu5Gc. Most other mammals, including our closes genetic relatives, the great apes, retain the function of their CMAH enzyme. As a result, humans show a different glycosylation on our cells, with an abundance of Neu5Ac and no

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Table 1
Contact angle and ellipsometry measurements of compounds 5 and 6 functionalized gold surfaces (Note: error bars represent the standard deviation (SD) of three independent experiments).

Entry	Type of coverage on Slides	Contact angle (°)	Ellipsometry (Å)
1	Au	65 ± 3	
2	Au + Comp **5**	34 ± 2 (0.1 M) 38 ± 2 (0.05 M)	14.2 ± 0.172
3	Au + Comp **6**	29 ± 2 (0.1 M) 37 ± 2 (0.05 M)	14.1 ± 0.232

endogenous expression of Neu5Gc [5].

The evolutionary loss of Neu5Gc on a species-wide scale is thought to have occurred partially in response to pathogens, as several known pathogens are known to bind Neu5Gc specifically but cannot target Neu5Ac [6]. Another relevant mechanism for the loss is sexual selection, where the hypothesis is that detection of Neu5Gc antigen on sperm was recognized in the *cmah*$^{-/-}$ reproductive tract of females [7]. The sexual selection hypothesis is supported by experiments in mice, where a significant drop in fertility was observed when breeding Wild-type (*Cmah*$^{+/+}$ males with *Cmah*$^{-/-}$ females), if the latter had circulating antibodies against Neu5Gc glycans. The current thought is that a combination of pathogen response and sexual selection was required to drive species-wide loss of Neu5Gc [8]. While the biophysical and biochemical ramifications are not well understood, the change in sialylation has been found to have functionally relevant physiological effects [8].

Neu5Gc can be metabolically incorporated into the human body through our consumption of Neu5Gc-rich animal foods, particularly red meat. The form of Neu5Gc-glycans found in meat is bioavailable and can be incorporated into human metabolism. However, the human immune system see Neu5Gc-glycan as a foreign structure (xenoglycan), polyclonal antibodies against Neu5Gc-glycans are produced, resulting in an inflammatory reaction ("xenosialitis") [9,10]. Several studies have indicated a causal relationship between the consumption of red meat and the development of cancer, especially gastro-intestinal cancers [11,12]. One proposed model states that the inflammatory state that can potentially aggravating diverse cancers [13,14], as well as atherosclerosis [8]. of course, the amount of Neu5Gc incorporated into humans from dietary sources is much lower than what might be found in a great ape. But it is sufficient to be detected by the antibodies. On the other hand, it is possible to feed human cells in culture with Neu5Gc, leading to substantial incorporation, which can be controlled by feeding the same amount of Neu5Ac.

While the occurrence of Neu5Gc clearly has an impact on human health, the two Sias Neu5Gc and Neu5Ac have very similar structure, differing only at carbon-5 where Neu5Ac has a methyl group instead of the hydroxymethyl-group present on Neu5Gc [3]. Based on the structural difference, one would therefore expect Neu5Ac to be more hydrophobic. Hypothetically, the loss of millions of hydroxy-groups from Neu5Gc and with accumulation of the precursor Neu5Ac at the cell surface could give rise to changes in membrane hydrophobicity between humans and other species as previously suggested [8]. An example of this effect can be seen in so-called ganglioside patches. Gangliosides are a sialylated form of glycosphingolipid that play an important role in the nervous system. Neighboring gangliosides can express saccharides that form clustered patches called ganglioside patches, that can form ligands for specific antibody interactions or mask antibody recognition of a single ganglioside [15]. Another example can be seen in lipid raft compartments on cell membranes where the formation of compartments could potentially magnify small changes in interactions that involve Neu5Ac versus Neu5Gc by modulating multivalent interactions [16].

The present study aims to investigate whether this structural difference impacts the hydrophobicity of cells and proteins expressing these two Sias in varying ratios. This question is addressed using atomic force microscopy (AFM). The use of AFM for the study of biological samples is already well-established and both mechanical [17–19] and adhesive properties of cells [20,21] have been successfully characterized. In this paper, the technique is used to investigate whether the Sias in question have a measurable effect on the surface hydrophobicity of proteins and cells that express either Neu5Ac or Neu5Gc. The AFM-based approach used in the current study enables the quantification of interactions with a force resolution down to the pN scale [22]. The technique is therefore well suited to investigate the properties and functions of weak glycan-based interactions in biological systems [23,24]. In the current study the interactions between proteins or human lymphoma cells expressing either Neu5Gc or Neu5Ac and a hydrophobically modified AFM probe are quantified. The results obtained reveal to what extent the altered glycosylation influence the strength and frequency of hydrophobic interactions occurring between the protein or cell surfaces and the hydrophobic probe, a parameter reflecting the overall hydrophobicity of the protein or cell surface.

2. Results

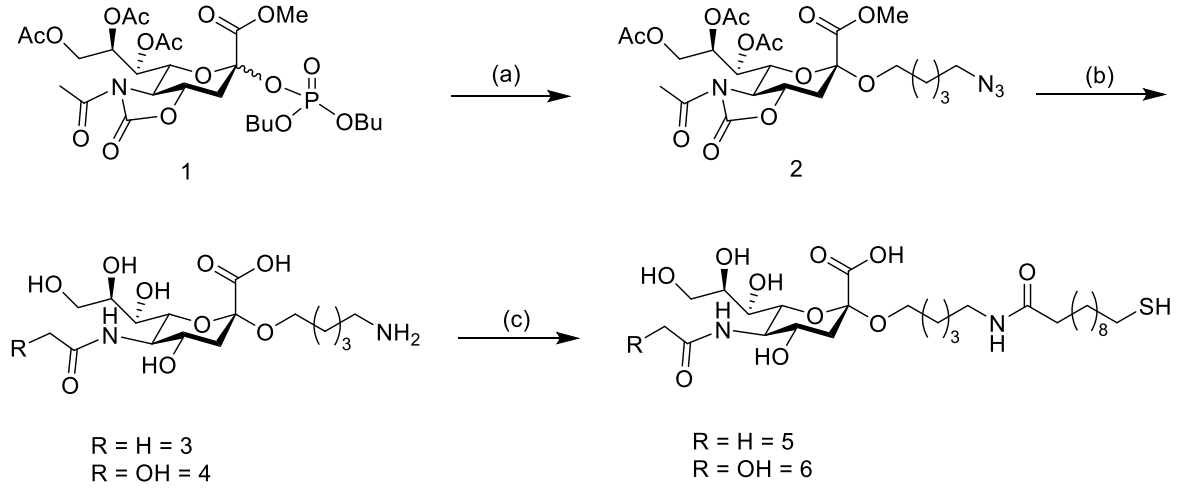
2.1. Wetting process

As a first step to analyze the hydrophobic difference between Ac/Gc, we synthesized Neu5Ac and Neu5Gc ligands with thio-linkers (**5** & **6**) for functionalization onto gold-coated glass slides. The sialic acid dibutyl phosphate donor **1** was used as a precursor to synthesize both the Neu5Ac and Neu5Gc ligands. Donor **1** was synthesized in eight straightforward steps, following a reported procedure [25–28], and was glycosylated with an azido-pentanol linker in the presence of Trimethylsilyl Trifluoromethanesulfonate (TMSOTf) at −45 °C, yielding compound **2**. Compound **2** then subjected to global deprotection, followed by N-acetylation or *N*-glycolylation using acetyl chloride or acetoxyacetyl chloride, and was subsequently hydrogenated to yield Neu5Ac or Neu5Gc with an amine linker (**3** or **4**). Finally, the amine-terminated carbohydrate ligands were reacted with pentafluorophenyl 11-mercaptoundecanoate to produce the respective thio-sugars (**5** & **6**), suitable for gold surface functionalization (Scheme 1).

Functionalization of synthetic Neu5Ac and Neu5Gc ligands was carried out on glass slides (around 1 × 1 cm) coated with chromium (10 nm) and gold (100 nm). The slides were placed in an ethanol solution of compounds **5** and **6** (0.1 and 0.05 M) for 48 h, followed by an ethanol rinse to remove loosely bound materials. Contact angle analysis showed a 62° angle for the clean gold surface, which decreased to 34° and 29° on surfaces with compounds **5** and **6** (0.2 M) respectively, indicating sialic acid ligands' hydrophilicity. The 5° difference highlighted the greater hydrophilicity of Neu5Gc over Neu5Ac. Reducing the ligand concentration led to an increased angle of 38° and 37° (0.05 M of **5** & **6**), suggesting that the hydrophilicity disparity was most evident at high ligand concentrations.

2.2. AFM studies on serum glycoproteins of WT and human-like CMAH null mice

The functionalized AFM probe was brought in contact with the protein-coated mica surfaces and then retracted. Interactions between hydrophobic AFM probes and Mouse serum sialoglycoprotein-coated surfaces were quantified based on force versus distance curves. In order to reveal the potential variation in the results obtained for surfaces and probes prepared using the same protocol, repeated experiments were performed. For each protein type, nine different protein coated surfaces were prepared and investigated on different experimental days and using different AFM probes. A minimum of 900 force-curves were recorded for each protein surface. Surfaces coated with proteins with different structure (WT, CMAH$^{-/-}$), and investigated using the same modified AFM probe is hereafter named an experimental series. If an adhesive interaction is formed between the hydrophobic probe and the



Scheme 1. (a) 5-azido-1pentanol, TMSOTf, CH_2Cl_2, -45 °C, 2 h, 84 %; (b) LiOH, EtOH/H_2O (3/2, v/v), 70 °C, 12 h; ii) acetyl chloride or acetoxyacetyl chloride, $NaHCO_3$, CH_3CN/H_2O (3/2, v/v), 0 °C, 2 h; iii) LiOH, H_2O, 0 °C, 2 h; iv) $Pd(OH)_2/C$, H_2, H_2O/MeOH (3/1, v/v), rt, 24 h, 19 % over four steps; (c) pentaflurophenyl 11-meracaptoundecanoate, DIPEA, DMF, 40 °C, 4 h, 84 %.

protein surfaces, the interaction is ruptured when retracting the cantilever holding the AFM probe, generating a force jump in the force-distance curve. Fig. 1a presents a selection of force-distance curves displaying frequently observed signatures of interactions when retracting the AFM probe from the protein surface.

The frequency of the occurrence of adhesive interactions between the hydrophobic AFM probe and the protein-coated surfaces was determined based on the number of force-distance curves displaying signature of the rupture of adhesive interactions, divided by the total number of force-distance curves obtained. The interaction frequencies were for all the experimental series found to be higher for the $CMAH^{-/-}$ coated surfaces than for the WT protein coated surfaces (Fig. 1b). When preparing the samples investigated in the experimental series iv-ix presented in Fig. 3c, protein samples that were de-O-acetylated prior to experimentation were used. This did not appear to affect the general trend, as interactions with the $CMAH^{-/-}$ protein were more frequent in all 9 series regardless of pre-treatment (Fig. 1c). Based on the totality of the data the frequencies obtained were found to be 34 ± 8 % and 20 ± 6 % for the $CMAH^{-/-}$ proteins and the WT proteins, respectively (Fig. 1d). The deadhesion work is the amount of work that must be performed in order to separate two adherent surfaces to a large separation distance. The deadhesion work was determined with JPKSPM data analysis software based on a quantification of the area under the retraction curve. The deadhesion work was found to be slightly higher for the WT-derived serum protein than the CMAH serum protein (Fig. 1b).

2.3. AFM studies on BJAB human lymphoma cells

The two adhesive chemicals PDA and PEI were assessed for their ability to immobilize BJAB cells on a glass surface. While both chemicals lead to a similar number of cells bound on the glass, cells on PEI appeared to undergo lysis and die whereas most of the cells on PDA were viable after immobilization (Fig. 2). Based on these results, cells were for all subsequent AFM experiments immobilized on PDA functionalized glass surfaces.

BJAB K20 cells as well as BJAB K20 cells fed with either Neu5Ac - or Neu5Gc Sias were immobilized on PDA functionalized glass slides and probed with hydrophobically modified AFM probes. The interaction frequency between the hydrophobic AFM probe and the cell samples was determined as described above for the studies of the protein-coated samples. For the BJAB K20 cells enriched with Neu5Ac or Neu5Gc, the AFM experiments were performed in three parallels and with n ≥ 10 cells of each type per parallel. The BJAB K20 negative control was included in two of the parallels with n = 10 cells per parallel. Fig. 4 presents a selection of representative force-distance curves obtained when retracting the AFM probe from the surface of BJAB-K20 lymphoma cells enriched in Neu5Ac- or Neu5Gc (Fig. 1a). The hydrophobic interaction frequencies were 48 ± 16 % for BJAB K20, 45 ± 11 % for BJAB K20 + Neu5Ac and 68 ± 17 % for BJAB K20 + Neu5Gc (Fig. 3b).

The hydrophobic interaction frequencies were determined for different experimental series for both the lymphoma cells enriched in Neu5Ac and the cells enriched in Neu5Gc (Fig. 3c). For the cells enriched in Neu5Ac, the interaction frequencies were equal to 72 ± 12 %, 74 ± 13 % and 58 ± 17 % for parallel I, II and III, respectively. For the cells expressing Neu5Gc, the interaction frequencies observed in experimental parallel I, II and III were equal to 48 ± 14 %, 42 ± 6 % and 44 ± 6 %, respectively. The error bars are based on the SD calculated from the fraction of hydrophobic interaction for each of the individual cells investigated in each parallel. Moreover, Fig. 4 presents the variation of the interaction frequency for the individual lymphoma cells grown in a medium containing either Neu5Ac or Neu5Gc, and thus expressing-Neu5Ac (aquamarine symbols) or Neu5Gc (yellow symbols) in experimental parallel I, II and III, respectively.

The deadhesion-work (nN nm) performed when retracting the hydrophobic AFM probe from cell surfaces was determined using the software program developed for the current study as described in the data analysis section. The analysis revealed a higher deadhesion work when retracting the probe from the BJAB K20 cells grown in a medium containing d Neu5Gc compared to the ones grown in the presence of Neu5Ac (Fig. 5a). The histogram distribution observed for the deadhesion work observed for BJAB-K20 resembled this observed for cells grown in the presence of Neu5Gc, whereas this observed for BJAB-K88 cells resembled the distribution observed for cells growing the presence of Neu5Ac (Fig. 5).

3. Discussion

3.1. Contact angle measurement of Neu5Ac/Gc coated surface

Several techniques are available to assess the hydrophobicity of carbohydrates. Common approaches include eluting carbohydrate ligands with water using C18-modified silica gel column chromatography and determining the octanol-water partition coefficient through spectroscopic analysis [29–31]. In this study, we employed a surface wetting experiment to investigate the hydrophobicity of Neu5Ac and Neu5Gc ligands. This method involves functionalizing sialic acid ligands onto

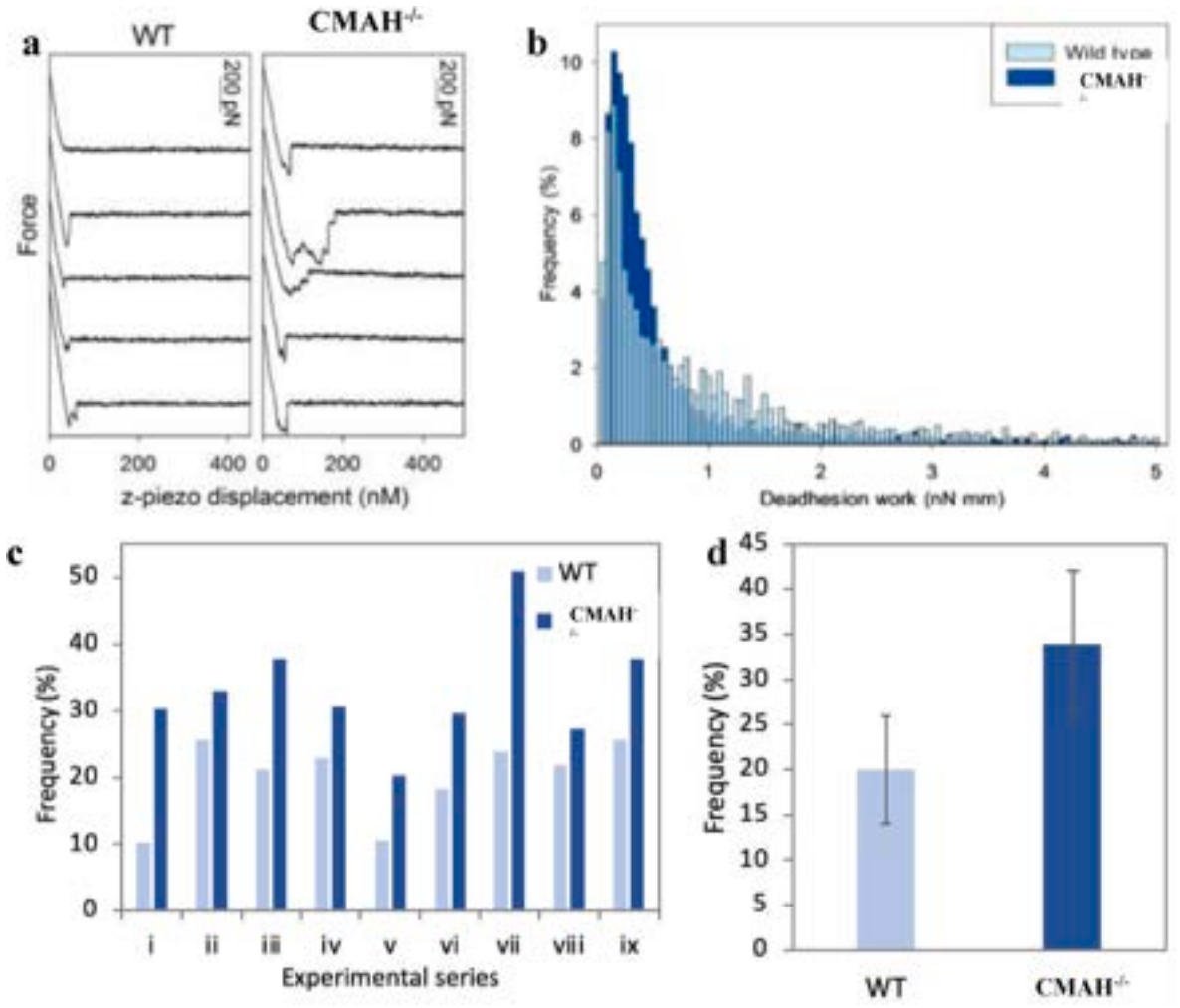


Fig. 1. Hydrophobic interactions between CH_3-modified AFM probes and mouse serum proteins recorded on a ForceRobot 300 SPM. Wild type (WT) Mouse serum sialoglycoproteins express high Neu5Gc glycosylation, $CMAH^{-/-}$ serum proteins express only Neu5Ac glycosylation. a) Gallery of representative force-distance curves obtained when retracting the hydrophobic AFM probe from surfaces coated with either WT or $CMAH^{-/-}$ proteins. b) Histogram presenting the distribution of deadhesion work (nN mm) determined based on force-distance curves of the type presented in a. c) The frequency of adhesive events observed between the hydrophobic probe and protein-coated surfaces. The results obtained for the nine (i-ix) different experimental series are presented. For each experimental series, the WT and $CMAH^{-/-}$ coated surfaces were probed with the same AFM probe. Series v-ix were de-O-acetylated prior to the experiment. d) Average frequency of adhesive events calculated based on the force versus distance curves obtained for WT and $CMAH^{-/-}$ protein-coated surfaces. The error bars represent the standard deviation.

gold-coated glass slides and assessing the difference in hydrophobic/hydrophilic properties by measuring the contact angle of a water droplet on the surface. The wetting process exhibited a 5° difference on the Ac/Gc coated surfaces at higher concentrations, establishing a platform for investigating the influence of sialic acid ligands at the cellular and protein level.

3.2. Hydrophobicity of surfaces coated with different serum proteins

All nine separate experimental series of protein coated surfaces (parallels) showed a higher frequency of interaction for the $CMAH^{-/-}$ proteins compared to the WT protein, with frequencies of 34 ± 8 % and 20 ± 6 % for the WT and $CMAH^{-/-}$ proteins, respectively (Fig. 1d). These results indicate that the $CMAH^{-/-}$ coated surfaces are more hydrophobic than the surfaces coated with WT proteins. This observation is consistent with the structure of the Sias, as the WT serum protein expressing Neu5Gc has an OH-group on carbon 5 where Neu5Ac has a hydroxymethyl-group. The interaction frequencies thus indicate that the Sia expression has a significant impact on the hydrophobicity of the serum proteins. The samples contained in six of the experimental series were de-O-glycosylated due to a higher occurrence of 9-O-acetylation in the $CMAH^{-/-}$ proteins used when preparing these samples. Experimental series where the proteins were de-O-glycosylated gave rise to interaction frequencies in the same range and the non-treated proteins, and also showed the same difference between WT and $CMAH^{-/-}$ protein as the non-treated proteins (Fig. 1c). This indicates that the de-O-acetylation treatment did not affect the surface hydrophobicity of the proteins. The deadhesion work was found to be in the range of 0–5 nN mm for both serum protein types. This value is consistent with values measured between hydrophobic AFM probes and hydrophobic surfaces investigated in previous studies, such as the interaction between hydrophobic probes and b-peptides studied by Ma and colleagues [32]. No significant difference in the size of the deadhesion work was observed between WT and $CMAH^{-/-}$ proteins, indicating that the interactions occurring were similar in strength, but less likely to occur with the $CMAH^{-/-}$ proteins.

The results obtained for the protein-coated surfaces indicate that the $CMAH^{-/-}$ protein-coated surfaces are more hydrophobic than the WT protein-coated surfaces. It would therefore be interesting to investigate if this difference in hydrophobicity can also be observed on cell surfaces expressing either of these two Sias in the glycans coating the cell surface. Cell surfaces are considerably more complex compared to the protein

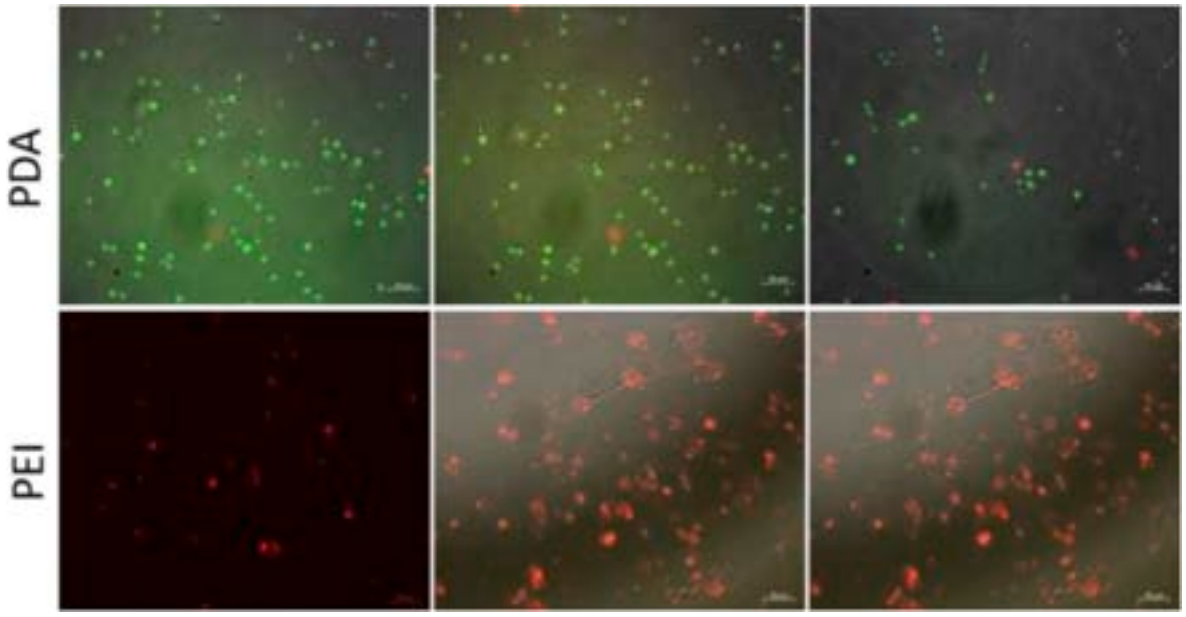


Fig. 2. BJAB K20 lymphoma cells immobilized on PDA (upper row) or PEI (lower row) functionalized glass surfaces. Images were recorded on a ZeissAxioObserver. A1 microscope with 20x magnification, excitation (483 nm/305 nm for green/red). Cells were treated with a live/dead assay (L3224, Invitrogen), green stain indicates live cells and red stain indicates dead cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

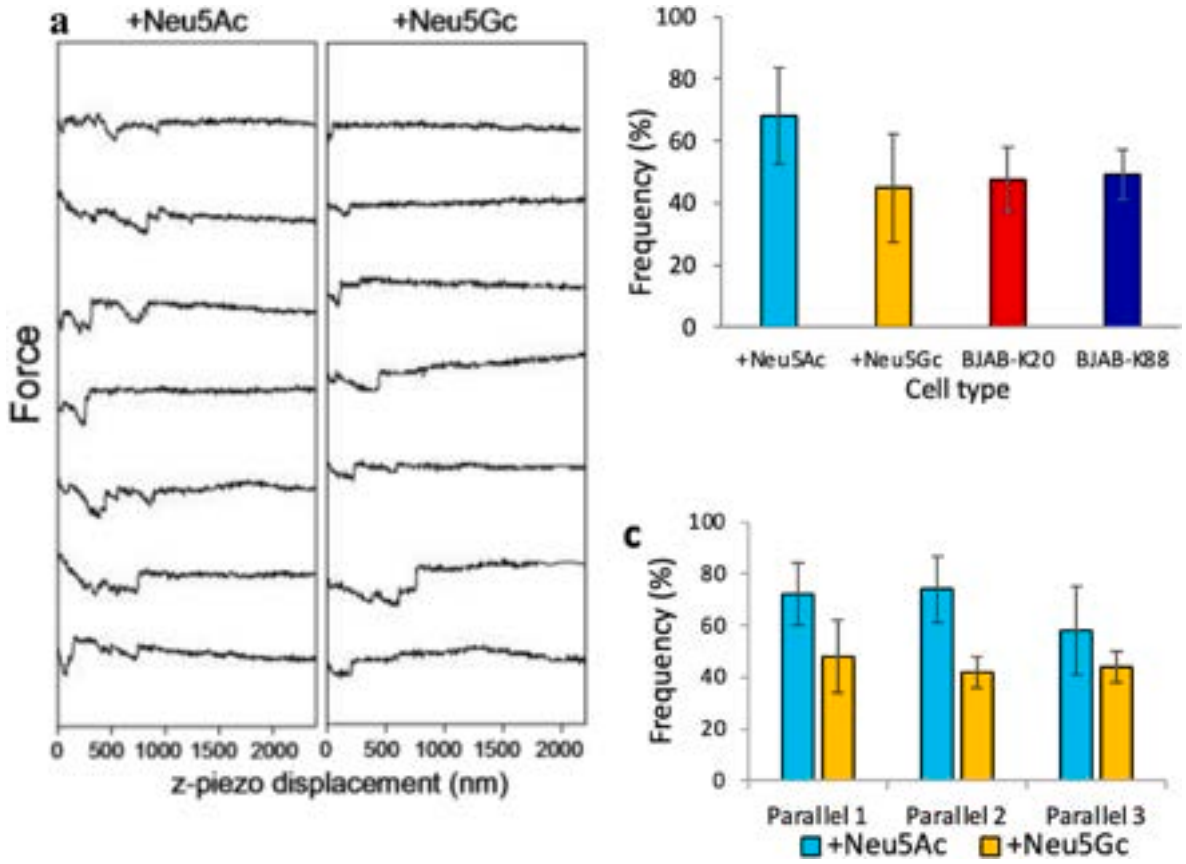


Fig. 3. Experimental data obtained when retracting a hydrophobic CH_3-functionalized AFM probe from the surface of a BJAB lymphoma cell. A: Typical force versus distance curves obtained when retracting a hydrophobic AFM probe from BJAB lymphoma cells expressing Neu5Ac (referred to as + Neu5Ac, left panel) or Neu5Gc (referred to as + Neu5Gc right panel). Only the retract curves are presented. b: Frequency of hydrophobic interaction events observed when retracting a hydrophobic AFM probe from immobilized BJAB K20 or BJAB K88 lymphoma cell surfaces displaying different Sias. The error bars indicate the standard deviation (SD) calculated based on the fraction of hydrophobic interaction as determined in different experimental series. C: Frequency of hydrophobic interaction as determined for the different experimental parallels for the BJAB-K20 lymphoma cells fed with and expressing Neu5Ac or Neu5Gc.

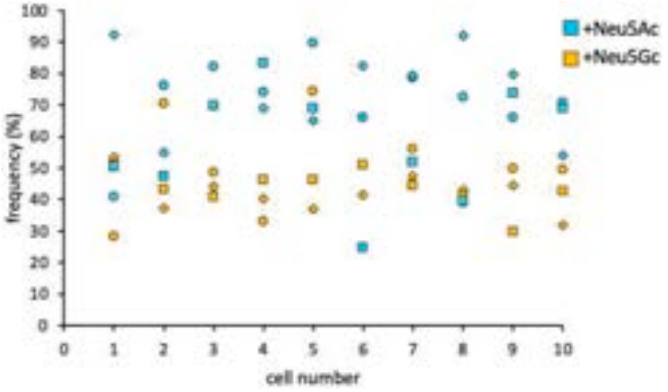


Fig. 4. Interaction frequencies between hydrophobic AFM probes and different BJAB-K20 cells (cell number 1 to 10) expressing Neu5Ac (aquamarine symbols) or Neu5Gc (yellow symbols) in experimental parallel I (circle), II (diamond) and III (square), respectively. Each experimental parallel contained 10 separate cells probed on three different days. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

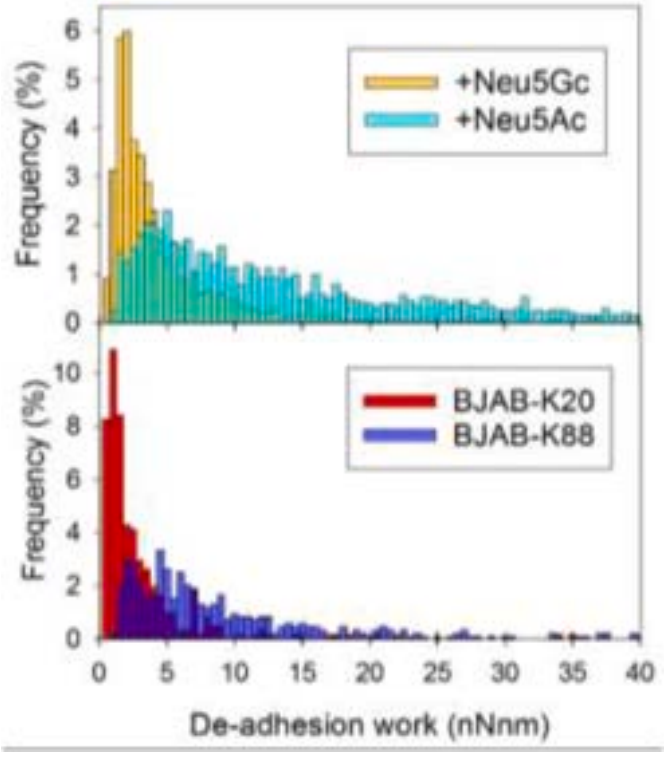


Fig. 5. Histograms showing deadhesion work between a hydrophobic AFM probe and BJAB lymphoma cells. All values are based on force-distance curves recorded on a BioScope Catalyst. (a) BJAB-K20 lymphoma cells, a hyposialic mutant cell line cultivated in a medium containing Neu5Ac (turquoise) or Neu5Gc (yellow) and (b) BJAB K20 and BJAB K88 (parent cell line) with normal sialic acid expression pattern lymphoma cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

coated surfaces, as a multitude of different molecules are present on the cell surfaces. Therefore, the difference in hydrophobicity resulting from variation in the structure of the Sias, might be insignificant and undetectable on these more complex surfaces.

3.3. BJAB cell hydrophobicity

BJAB cells were successfully immobilized and remained viable on PDA functionalized glass surfaces (Fig. 4). The strength and frequencies of hydrophobic interactions were quantified between the surface of these immobilized cells and a hydrophobically modified AFM probe. The same AFM probe was used during the AFM force measurements for all cells belonging to the same experimental series. Therefore, the variation in the interaction frequency observed for the different cell types is assumed to be due to variations in the hydrophobicity of the glycan coverage on the cell surfaces rather than variations in the hydrophobicity of the AFM probe. The results obtained revealed that the lymphoma cells predominantly displaying Neu5Ac deviated in behavior from the other three cell types included in this study, in the sense that a higher frequency of hydrophobic interactions was observed between the AFM probe and these cells compared to the other cell types (Fig. 3). This indicates that an increased expression of Neu5Ac leads to an increased hydrophobic character of the cell surface.

The deadhesion work observed when retracting the AFM probe from cells expressing Neu5Gc was in the range of 0–20 nN mm, whereas for cells expressing Neu5Ac the values of the deadhesion work ranged up to 40 nN mm (Fig. 5, upper histogram). As the deadhesion work is a measure of how strongly the hydrophobic AFM probe adheres to the cell surface, the result indicates a more hydrophobic surface of the cells expressing Neu5Ac, consistent with the observations from protein-coated surfaces. In the present study, the BJAB K88 parent cell line as well as the BJAB K20 cell line without sialic acid expression [33] but capable of metabolic incorporation of free sialic acids added to the medium were included as controls. Here, the deadhesion work was higher when investigating the BJAB K88 cell line. The deadhesion work observed for this cell line was comparable to the values obtained for the cells expressing the sialic acid Neu5Gc, whereas the BJAB K20 cells gave rise to the lowest values of the deadhesion work (Fig. 5, lower histogram).

In addition to the deadhesion work, the probability of observing an adhesive interaction when retracting the AFM probe from the cell surfaces was also quantified. A higher interaction frequency was observed between the hydrophobic surface of the AFM probe and the lymphoma cell surfaces predominantly expressing Neu5Ac (68 ± 17 %), compared to the lymphoma cells predominantly expressing Neu5Gc (45 ± 11 %) (Fig. 5b). However, no significant difference was observed in the interaction frequency between the BJAB-K20 (48 ± 16 %), BJAB-K88 (49 ± 8 %) and the Neu5Gc lymphoma cells (Fig. 5b). This suggests that there is no detectable alteration in the surface hydrophobicity between cells when feeding the BJAB-K20 cells with Neu5Ac (BJAB K88 and +Neu5AC) compared to those expressing no Sias (BJAB K20). The parent cell line K88 also expresses Neu5Ac on the cell surface but shows a lower interaction frequency than the cells enriched with Neu5Ac. A potential explanation is that the parent cell line has a more complex glycocalyx with a varied sialic acid expression, whereas the K20 cells only express the sialic acid that has been added in the growth medium. This lack of Sia diversity could mean that the enriched K20 cells are more influenced by the Neu5Ac expression because this will constitute a larger proportion of the total Sia population on the cell surface. In all of the experimental parallels, an increased frequency of hydrophobic interactions was observed for the Neu5Ac expressing lymphoma cells compared to the lymphoma cells predominantly expressing Neu5Gc (Fig. 5b). This indicates a difference in cell surface hydrophobicity between these cells, where the cells displaying Neu5Ac are characterized by a more hydrophobic surface.

Our results show a clear effect on the surface hydrophobicity of both proteins and cells as a result of differential sialylation. This altered hydrophobicity has potential physiological effects. Sialic acids have well-characterized effects on cell repulsion and adhesion, but knowledge related to how Neu5Ac and Neu5Gc specifically influence these processes through their structural differences is lacking [6]. The loss of

Neu5Gc entails loss of a high number of hydroxyethyl-groups. The results presented in the current study indicate that this loss leads to an increase hydrophobicity of the surface of the proteins or cells. Our findings thus support the hypothesis that loss of Neu5Gc and concomittent increase in Neu5Ac expression leads to changes in membrane hydrophobicity between humans and other species. Cohen and Varki have previously outlined how clustering of saccharides can change ligand interactions significantly with only small numbers of glycans [15]. Our results indicate that the difference in biophysical properties between Neu5Ac and Neu5Gc could modulate ligand interactions through hydrophobic interactions. Overall, the findings in the present study suggest the that the change from Neu5Gc to Neu5Ac expression on human cells can have a wide range of scientifically interesting and physiologically important effects which should be investigated further. In this regard a recent study showed that spin-labeled sialic acid residues attached to the 6-O-position of galactose/*N*-acetyl-galactosamine would experience less steric hindrance and show more flexibility than that attached to the 3-O-position, possibly reflecting differences in local crowding/packing [34]. It would be interesting to study combinations and ratios of Neu5Ac/Gc with α2-3/6 linkages. The reality of the natural situation is of course likely to be even more complex.

4. Conclusion

The experimental data presented in the present paper demonstrate that sialic acid expression influences the surface hydrophobicity on surfaces, serum proteins and human lymphoma cells. For proteins, a higher frequency of hydrophobic interactions was observed between a hydrophobic AFM probe and $CMAH^{-/-}$ protein, i.e., protein expressing only Neu5Ac, compared to WT proteins expressing predominant Neu5Gc. For the BJAB cells, higher deadhesion work and higher frequency of hydrophobic interactions was observed when retracting the hydropobic AFM probe from BJAB K20 + Neu5Ac cells compared to cells with Neu5Gc. The cells with added Neu5Gc were similar to BJAB K20 cells, characterized by an absence of sialic acids, whereas cells with added Neu5Ac behaved more similar to K88. By revealing biophysical consequences of the loss of CMAH function our results support previous findings and hypotheses concerning the significance of the change in sialylation from Neu5Gc to Neu5Ac, but also suggest that they may be many more changes in many types.

CRediT authorship contribution statement

Karen Dunker: Writing – review & editing, Writing – original draft, Investigation. **Kristine Mathingsdal Pedersen:** Investigation. **Suraj Toraskar:** Investigation. **Sandra Diaz:** Investigation. **Ajit Varki:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Marit Sletmoen:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Raghavendra Kikkeri:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2025.109469.

Data availability

Data will be made available on request.

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