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Low incidence of *N*-glycolylneuraminic acid in birds and reptiles and its absence in the platypus

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Dedicated to Professor Dr. Hans Kamerling on the occasion of this 65th birthday

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ABSTRACT

The sialic acids of the platypus, birds, and reptiles were investigated with regard to the occurrence of *N*-glycolylneuraminic (Neu5Gc) acid. They were released from tissues, eggs, or salivary mucin samples by acid hydrolysis, and purified and analyzed by thin-layer chromatography, high-performance liquid chromatography, and mass spectrometry. In muscle and liver of the platypus only *N*-acetylneuraminic (Neu5Ac) acid was found. The nine bird species studied also did not express *N*-glycolylneuraminic acid with the exception of an egg, but not tissues, from the budgerigar and traces in poultry. Among nine reptiles, including one turtle, *N*-glycolylneuraminic acid was only found in the egg and an adult basilisk, but not in a freshly hatched animal. BLAST analysis of the genomes of the platypus, the chicken, and zebra finch against the CMP-*N*-acetylneuraminic acid hydroxylase did not reveal the existence of a similar protein structure. Apparently monotremes (platypus) and sauropsids (birds and reptiles) cannot synthesize Neu5Gc. The few animals where Neu5Gc was found, especially in eggs, may have acquired this from the diet or by an alternative pathway. Since Neu5Gc is antigenic to man, the observation that this monosaccharide does not or at least only rarely occur in birds and reptiles, may be of nutritional and clinical significance.

1. Introduction

Among the more than 50 species of sialic acids, *N*-glycolylneuraminic acid (Neu5Gc) has been frequently identified in members of the Deuterostome lineage, from echinoderms to mammals.[1–3] However, it was detected neither in microorganisms nor in the few animal species lower than echinoderms, where sialic acids have been found, including insects. There is one exception: *Trypanosoma cruzi*, but this parasite acquires Neu5Gc in addition to *N*-acetylneuraminic acid (Neu5Ac) from the culture medium, since it cannot synthesize this sialic acid itself.[4] Humans and chicken are known exceptions for the regular occurrence of Neu5Gc in higher animals. Man has lost the ability to synthesize this sialic acid during his divergence from the Great Apes about 2–3 million years ago.[5] The reason for this was the loss of a 92 base pair exon of the CMP-Neu5Ac hydroxylase (CMAH) gene, leading to a truncated, enzymatically inactive protein due to a frame shift mutation. CMAH is responsible for the oxidative conversion of Neu5Ac to Neu5Gc requiring the Rieske center, an iron–sulfur cluster, for its activity.[6–8]

The consequences of this defect for human life and development may have been enormous, as discussed by Varki and associates.[9] Microorganisms specifically binding to Neu5Gc on infection were no more hazardous to human life which may have promoted human spreading and development. For example, the major binding protein of *Plasmodium reichenowi*, which causes malaria in Great Apes binds to Neu5Gc and may have threatened ancient man.[10] In contrast, the corresponding protein of *P. falciparum* has a preference for Neu5Ac. Furthermore, an AB_5-toxin secreted by Shiga toxigenic *Escherichia coli* (STEC) and causing gastrointestinal disease, was also found to bind to Neu5Gc.[11]

Although humans can no longer synthesize Neu5Gc, traces of this sialic acid are found in glycans from healthy donors, and higher amounts are found in some tumors.[1,2,12,13] The values vary between less than 1% of the sialic acid fraction and a few percent in some malignant tumors. Evidence for a pathway alternative to the enzymatic oxidation of Neu5Ac by CMAH leading to Neu5Gc has not been found,[14] although metabolic prerequisites exist.[12,15] Different experimental approaches have shown that Neu5Gc can be taken up from orally applied Neu5Gc by humans[16] or rats and mice[17] or from the medium by human cells in culture.[18] It is therefore conceivable that we can acquire Neu5Gc from Neu5Gc-containing foodstuffs, such as red meat, fish, and milk. This is discussed to have pathophysiological consequences and may promote inflammatory diseases including rheumatism and cancer.[9,13,19] The reason is that Neu5Gc is antigenic in humans, leading to the formation of Hanganutziu–Deicher antibodies detectable in variable amounts in blood serum.[12,19]

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Due to the increasing biological and pathological significance of Neu5Gc, which was also shown by inactivation of the CMAH gene in mice,[14] it is important to know whether more vertebrates are unable to synthesize Neu5Gc and whether there is a developmental reason for this. During the early years of analytical determination of sialic acids from a variety of animals, it was found that one of the most important sources for sialic acids, especially Neu5Ac, the mucin glycoproteins from the nests of the swiftlet (*Collocalia fuciphaga* = *Aerodramus fuciphagus*, 'edible bird nest substance, collocalia mucoid') does not contain Neu5Gc.[20–22] It has been known for a long time that glycoproteins from the chicken or other poultry contain only Neu5Ac or only insignificant quantities of Neu5Gc.[23] When, by chance, the analysis of milk of the Australian spiny anteater echidna (*Tachyglossus aculeatus*), which is an early mammal and belongs to the monotremes, proved to lack Neu5Gc,[1] we were prompted to study the platypus (*Ornithorhynchus anatinus*), another still living monotreme, as well as more bird species. The studies were extended to some reptiles and a turtle, because, together with the birds, they belong to the taxon *Sauropsida*. A hypothesis is forwarded that, based on sialic acid and BLAST analysis of the currently available genomic sequence databases, both the sauropsids and the evolutionarily closely related monotremes, which represent ancient mammals, may have lost the capability to synthesize Neu5Gc. Evolutionary aspects and nutritive consequences of this finding will be discussed.

2. Results and discussion

The results of these studies are summarized in Table 1. All taxonomic names are listed in Section 3 ('Animals') and in the Tables. They demonstrate that Neu5Gc is absent in the biological materials analyzed from platypus, most bird and reptile species, as well as from turtle (Figs. 1–4). In two animal species, however, in the egg of the bird, the budgerigar, and in both the egg and tissues of an adult green basilisk, appreciable amounts of these sialic acids were detected by HPLC and confirmed by mass spectrometry. Traces of Neu5Gc, below 1%, were found in some poultry species. In duck intestine, small amounts (about 2%) of the sialic acid fraction were also reported to consist of Neu5Gc.[24] Screening the literature for sialic acid analyses of bird and reptile materials (Table 2) showed, to

Table 1
Sialic acids in tissues, eggs, or mucins from platypus, birds, reptiles, and turtle

Animal	Material or tissue	Neu5Gc	Total sialic acid quantity
Platypus (*Ornithorhynchus anatinus*)	Liver	n.d.[a]	6.4[b]
	Muscle	n.d.	3.5[b]
Chicken (*Gallus gallus*)	Liver	Trace[c]	n.a.[d]
	Muscle	n.d.	n.a.
	Embryonic brain	Trace	n.a.
	Ovomucin	n.d.	n.a.
Duck (*Anatidae*)	Liver	n.d.	n.a..
	Ovomucin	n.d.	n.a.
Turkey (*Meleagrididae*)	Liver	Trace	n.a.
Goose (*Anatidae*)	Liver	Trace	n.a.
	Ovomucin	n.d.	n.a.
Ostrich (*Struthio camelus*)	Liver	n.d.	n.a.
	Ovomucin	n.d.	n.a.
Emu (*Dromaius novae-hollandiae*)	Egg	n.d.	44.3[b]
	Muscle	n.d.	n.a.
Scarlet macaw (*Ara macao*)	Egg	n.d.[*]	6[b]
Budgerigar (*Melopsittacus undulatus*)	Egg[f]	18.8[e,*]	
	Liver[f]	n.d.	3.3[g]
	Muscle[f]	n.d.	5.8[g]
Oriental swiftlet (*Aerodramus/Collocalia species*)	Nest glycoproteins[h]	n.d.	210[g,i]
Swallow (*Hirundo urbica urbica* L.)	Nest glycoproteins	n.d.	n.a.
Green iguana (*Iguana iguana*)	Egg	n.d.[*]	10.3[b]
Agama (*Pogona vitticeps*)	Egg	n.d.[*]	n.a.
Green basilisk (*Basiliscus plumifrons*)	Egg	73[e]	30[b]
	Total freshly hatched animal[j]	n.d.[*]	13[b]
	Liver	33[e,*]	3.4[g]
	Muscle	33[e]	1.6[g]
Anaconda (*Eunectes murinus*)	Liver	n.d.	6.0[g]
Hundred pace viper[j] (*Deinagkistrodon acutus*)	Liver	n.d.[*]	6.0[g]
	Muscle	n.d.	14.7[g]
Taiwan sting snake[j] (*Elaphe carinata*)	Liver	n.d.[*]	20
	Muscle	n.d.[*]	10
Taiwan beauty snake[j] (*Elaphe taeniura*)	Muscle	n.d.[*]	2
Crocodile (*Crocodilus niloticus*)	Muscle	n.d.	n.a.
Turtle (*Cuora amboinensis*)	Liver	n.d.	6.2
	Muscle	n.d.	3.8

[a] Not detectable.
[b] μg sialic acid/mg protein.
[c] Below 0.7% of total sialic acid fraction.
[d] Not analyzed.
[e] Means % total sialic acid fraction.
[f] Egg and tissues were from different animals.
[g] μmoles/g dry tissue.
[h] Three different samples (see Section 3).
[i] Value from Chinese collocalia mucoid.[20]
[j] Relatively large amounts (26–66%) of 7(9)-mono-*O*-acetyl-Neu5Ac were found.
[*] Samples also analyzed by mass spectrometry.