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Isolation and properties of two sialate-*O*-acetylesterases from horse liver with 4- and 9-*O*-acetyl specificities

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Abstract Sialate-*O*-acetylesterase was purified almost 900-fold from particle-free supernatants of horse liver by gel filtration, ion-exchange chromatography and isoelectric focussing. The native enzyme on gel filtration exhibits a molecular weight of 54,000 Da. It was separated by isoelectric focussing into two forms with p*I* values of 4.8 and 5.7, respectively. The esterase with a lower p*I* hydrolyses only 9-*O*-acetyl groups from sialic acids (K_M 1.1 mM), while that with the higher p*I* esterifies both 4- and 9-*O*-acetylated monosaccharides at similar rates (K_M 0.3 M and 1.3 mM, respectively). Both forms are inactive with 7-*O*-acetylated *N*-acetylneuraminic acid. Enzyme assays were carried out at the pH optimum (pH 8.4–8.6) using free *O*-acetylated sialic acids followed by direct analysis of the reaction products by isocratic anion-exchange HPLC. Glycosidically bound sialic acids can also be de-*O*-acetylated. Horse liver esterase seems to be an essential enzyme for the catabolism of 4-*O*-acetylated sialoglycoconjugates, since sialidase from this tissue cannot act on 4-*O*-acetylated sialic acids.

Keywords Sialic acid · 4-*O*-acetylated sialic acid · 9-*O*-acetylated sialic acid · Sialate esterase · Isolation and properties · Horse liver

Introduction

O-Acetylation is a frequent modification of sialic acids (Sia) in microorganisms and in animals of the deuterostome lineage [1–4]. Esterified Sia are involved in manifold biological and pathological processes of which the interaction with microorganisms, especially viruses, their influence on differentiation, malignant growth and immunological reactions including interaction with Siglecs and apoptosis stimulated much interest [1–6]. The ester groups are bound to C-4 or to the side-chain of Sia, preferentially at C-9. They are incorporated by specific *O*-acetyltransferases, which in the mammalian tissues studied are located in the Golgi membranes from where they could only partially be purified so far [7, 8]. Molecular cloning of a sialate *O*-acetyltransferase was only possible from some microorganisms [9–12]. Sia are de-esterified by esterases, frequently found in microorganisms and animal tissues, and which, among the natural *O*-acetylated substances, preferentially hydrolyse *O*-acetylated Sia [1, 3, 13].

The occurrence of sialate-*O*-acetylesterases in frog, rat and cow liver was reported in 1983 [14] and in human erythrocytes in 1985 (A. K. Shukla, Habilitation thesis, University of Kiel, 1985). Such enzymes were isolated and in some cases cloned, for example, from human erythrocytes [15], rat liver [16, 17], mouse tissues and cells [18, 19], bovine brain [20] and hematopoietic cell lines [19, 21, 22]. A gene encoding a Sia-specific 9-*O*-acetylesterase has been found in human adult testis [22]. All these hydrolases are considered to be involved in the regulation of turnover and thus functions of *O*-acetylated Sia [1–4, 13, 23]. For example, transgenic mice expressing a viral Sia-9-*O*-acetylesterase showed developmental abnormalities [24] and similarly transfected hamster melanoma cells, in which the expression of *O*-acetylated GD3 was reduced, changed their differentiation profile [25]. Evidence was obtained that the level of *O*-acetylated Sia present in normal or malignantly diseased human colon mucosa depends on the relative activities of sialate-*O*-acetyltransferase and lysosomal esterase [26].

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Sialate esterases are frequently expressed by viruses, where they seem to be involved in the infection mechanism. The first example was the 9-*O*-acetyl-specific esterase isolated from influenza C virus and considered as a "receptor-destroying" enzyme [27, 28]. Since then besides this orthomyxovirus such esterases were discovered in a variety of corona- and toroviruses, which preferentially hydrolyse either the 4- or 9-mono-*O*-acetylated or the side-chain di-*O*-acetylated Sia [29]. Several of these enzymes were cloned [30, 31].

Intestinal bacteria were also found to produce sialate-*O*-acetylesterases, which are involved in the degradation of *e.g.* the highly *O*-acetylated human colon mucin and thus may influence their virulence [32–34]. However, these enzymes were not yet purified or cloned. Interestingly, in human meconium no Sia esterase activity was found, whereas the faeces contained an appreciable activity of this enzyme eight days after birth (A. K. Shukla, Habilitation Thesis, University of Kiel, 1985).

Sialate-*O*-acetylesterases are involved in the catabolism of Sia. They act either on glycosidically linked Sia or on free Sia released from glycoconjugates by sialidases which occur in mammalian cells mainly in lysosomes. In animals and bacteria sialidases are active with Sia esterified at C-9, although at reduced rate when compared with unsubstituted *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc), but they are inactive with Sia *O*-acetylated at C-4 [2, 3]. Similarly, the degradation of liberated Sia occurs by the action of the cytosolic aldolase sialate-pyruvate lyase, which is also inactive with Sia esterified at C-4. Since in horse tissues a relatively large proportion of Sia is 4-*O*-acetylated and partially purified sialidase from horse liver was found to be inactive with this Sia derivative [35], we wondered whether the sialate esterase from horse can hydrolyse 4-*O*-acetyl groups from Sia. Such an enzyme is expected to assist the degradation of 4-*O*-acetylated glycoconjugates and the turnover of Sia in this animal and thus represent a "missing link" in glycoconjugate metabolism. We therefore investigated the esterase activity found in horse liver.

Material and methods

Isolation of sialate-*O*-acetylesterase

Preparation of crude enzyme

Sialate-*O*-acetylesterase was isolated from fresh horse liver obtained from the slaughter house or was prepared from tissue stored at −70°C. All isolation steps were performed at 4°C. After the removal of connective tissues and fat, 50 g of liver were cut into small pieces and kept in 50 ml buffer A (50 mM Tris/HCl, pH 7.5, 1 mM 2-mercaptoethanol) containing 1 mM phenylmethylsulfonylfluoride (PMSF). The mixture was homogenised two to three times, each time for 15 s by an ultraturrax, followed by rehomogenisation with a potter (Jahnke & Kunkel, Staufen, Germany). The homogenate was centrifuged for 30 min at 10,000×*g*. The supernatant was centrifuged for 2 h at 100,000×*g* and dialysed against 5 l of buffer A.

The dialysed supernatant containing about 6 g protein was filtrated on a G-200 Sephadex column (27×9 cm; Pharmacia, Freiburg, Germany). As eluent buffer A was used. The enzyme-active fractions were pooled and dialysed against buffer B (the same as buffer A, but at pH 6.9). They were loaded onto a Sephadex-DEAE-A-50 column (12×5.5 cm; Pharmacia), equilibrated with buffer B. The column was washed with this buffer until hemoglobin colour had eluted from the column. The enzyme was then eluted by 0.2 M NaCl in buffer B. The active fractions were concentrated in an Amicon cell using a 10,000 Da exclusion membrane and then dialysed against 1 l of buffer B. Then the enzyme was loaded onto a DEAE-Sephacel column (25×2.5 cm) (Pharmacia). After washing with buffer B it was eluted by a linear gradient of 0–100 mM NaCl (500 ml each) in buffer B. The active fractions were concentrated and dialysed against buffer A (as described in the previous step). The enzyme fraction from this second ion-exchange chromatography step containing about 20 mg of protein was subjected to preparative isoelectric focussing. It was mixed with ampholine (LKB, Brommar, Sweden) of pH 3.5–10 (1 ml), pH 4–6 (4.5 ml), and pH 5–7 (4.5 ml) and applied with a saccharose gradient (440 ml, 43–5% saccharose) to a column without support (no. 8100, LKB, Brommar, Sweden). Phosphoric acid (35 mM) and sodium hydroxide (50 mM) were used as electrode buffers. The isoelectric focussing was performed for 36–48 h at constant power (2 W) until the voltage reached about 1,000 V. Then the column was eluted in 2 ml fractions and monitored for enzyme activity and at 280 nm for protein concentration. The active protein peaks were pooled and dialysed against buffer A to remove ampholine and sucrose, and concentrated to 1–2 ml. These purified enzyme fractions were stored at −70°C.

Quantitative and qualitative protein analysis

Protein concentrations were determined using the Lowry assay [36] with BSA as standard and analysed by PAGE according to Laemmli, using 10–12% gel for separation. Proteins were stained with Coomassie blue. Esterase activity of proteins separated by analytical isoelectric focussing on ampholine of pH 3.5–9.5 was visualised by staining with α-naphthylacetate and diazonium salt (Fast Red).

Esterase assay using HPLC

Samples from the homogenate and the fractionation procedures (10 µl) were tested for esterase activity in 100 µl total volume of 50 mM Tris/HCl buffer, pH 8, containing 1 mM 2-mercaptoethanol and 1 mM of *N*-acetyl-4-*O*-acetylneuraminic acid (Neu4,5Ac_2), *N*-acetyl-7-*O*-acetylneuraminic acid (Neu5,7Ac_2) or *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac_2) as potential substrates. The incubation time routinely was 30 min at 37°C. These conditions, like pH value, incubation time, and substrate concentrations were varied for kinetic measurements accordingly. In some experiments, potential activators and inhibitors (see Results) were added. For blanks heat-denatured enzyme fractions were used. Equine submandibular gland mucin (sialic acid concentration 1 mM) containing mainly Neu4,5Ac_2 was also incubated with the horse liver esterase (1 mU from the DEAE-Sephacel step) in 200 µl for 1 h. Sialic acids were liberated by mild acid hydrolysis [7] before and after enzyme incubation and analysed by HPLC.

For Sia analysis, aliquots of 10 µl were taken from these incubation mixtures at different time intervals and directly injected into an Aminex A-29 column of stainless steel (40×4.5 mm) as described in Ref. 37. If the sample contained more than 10 mg/ml the protein was precipitated by adding an equal volume of 4% phosphotungstic acid and centrifugation at 12,000×*g* for 3 min before HPLC analysis of the supernatant was carried out. When testing Neu5,7Ac_2 as a possible substrate the pH value of the buffer was adjusted to 7 and 0.1 mM 2-mercaptoethanol was added. (Higher concentrations of 2-mercaptoethanol would overlap the Neu5,7Ac_2 peak on HPLC). These conditions were chosen for Neu5,7Ac_2 in order to avoid migration of the ester group to O-9 [38]. It was furthermore ascertained that at pH 7 the enzyme can hydrolyse 9-*O*-acetyl groups.

This anion-exchange HPLC was performed on a Spectra-Physics SP 8000 apparatus equipped with a photometer (Spectroflow monitor SF 770, Schoeffel Instrument Corp.) and a SP 4000 integrator for automatic calculation of peak areas. Standard Sia were prepared from bovine and equine submandibular gland mucins [39]. The Sia nature of the enzymatic products identified by HPLC were furthermore proven by the application of sialate pyruvate lyase which degrades Sia and by alkaline saponification, which destroys the peaks of *O*-acetylated Sia leading to a concomitant increase of the non-*O*-acetylated sialic acid species [40].

Results

The HPLC method using Amino A-29 allows fast separation of *O*-acetylated and non-*O*-acetylated sialic acids, without any intensive purification and derivatisation of the sialic acids [37, 41]. It thus enables monitoring the sialate-*O*-acetylesterase (SOAE) activities in different species and tissues. Figure 1, for example, shows partial saponification of Neu5,9Ac_2 by the esterase which is accompanied by an increase of the Neu5Ac peak. The sialic acid nature of the Neu5Ac and residual Neu5,9Ac_2 peaks could be further demonstrated by the use of the lyase, which degraded these monosaccharides (not shown). This much increases the specificity of the assay, as residual low molecular weight substances other than Sia, which may be derived from the cytosol or from ampholytes or mercaptoethanol, disturb the test. Sialate-*O*-acetylesterase activity was mainly found in the soluble fraction from horse liver. Therefore, the particle-free 100,000×*g* supernatant was used as starting material for the isolation of this enzyme. Steps and factors of the isolation procedure applied are presented in Table 1 showing an almost 900-fold purification. Preparative and analytical isoelectric focussing followed by protein and esterase activity determination in the eluate or protein and esterase localisation in the gels revealed the existence of two different enzyme activities (Figs. 2 and 3) exhibiting various isoelectric points. The esterase with p*I* 4.8 hydrolysed only *O*-acetyl groups from position 9, whereas the esterase with p*I* 5.7 hydrolysed *O*-acetyl groups from C-4 as well as from C-9, although 4-*O*-acetyl groups were slightly preferred (Fig. 4). None of these enzyme fractions can hydrolyse an *O*-acetyl group localized at C-7.

These esterases eluting from DEAE-Sephacel show a molecular weight of about 54,000 Da, as was determined

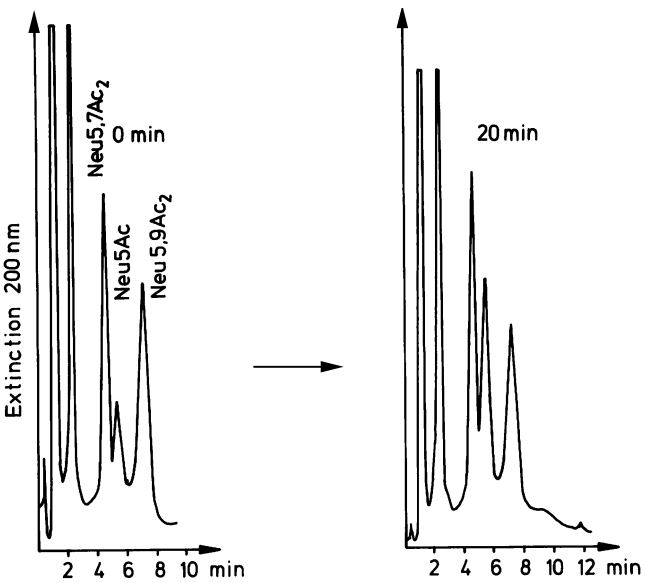

Fig. 1 HPLC on Aminex A29 of Neu5,9Ac_2 and Neu5,7Ac_2 incubated with horse liver esterase from DEAE-Sephacel chromatography step. Only Neu5,9Ac_2 was (partially) hydrolysed, resulting in an increase of the Neu5Ac peak; Neu5,7Ac_2 was inactive with this enzyme (compare also Fig. 4). For details see the text

Table 1 Purification steps of sialate-*O*-acetylesterase from horse liver

Purification step	Specific activity (mU/mg protein)	Purification factor	Yield %
Homogenate	0.12	1	100
100,000×*g* supernatant	0.35	3	85
Sephadex G-200	0.52	4.3	50
DEAE-Sephadex A-50	1.4	11	25
DEAE-Sephacel	12.3	102	14
Preparative isoelectric focussing (p*I* 4.8)	104	866	6

For details see the text

by gelfiltration (Fig. 5). Their optimum activity is at pH 8.4–8.6 when using Tris/HCl or phosphate buffer**.** At pH 7 the activity was 60% lower. The esterases obtained by isoelectric focussing exhibited different K_M-values. The enzyme with p*I* 4.8 hydrolysing only Neu5,9Ac_2 has a K_M-value of 1.1 mM for Neu5,9Ac_2, and that with p*I* 5.7 hydrolysing both 4-*O*-acetyl and 9-*O*-acetyl groups has a K_M-value of 0.3 mM for Neu4,5Ac_2 and 1.3 mM for Neu5,9Ac_2, respectively. These activity values were obtained with free sialic acids. The esterase was also active with glycosidically linked sialic acids. It hydrolysed about 20% of the 4-*O*-acetyl groups of equine submandibular gland under the conditions described. Preliminary, unpublished (A. Shukla) experiments showed susceptibility towards the horse liver esterase of 9-*O*-acetyl sialyllactose, horse serum α_1-acid glycoprotein, and rat or equine erythrocyte membranes containing Neu5,9Ac_2 or Neu4,5Ac_2, respectively.

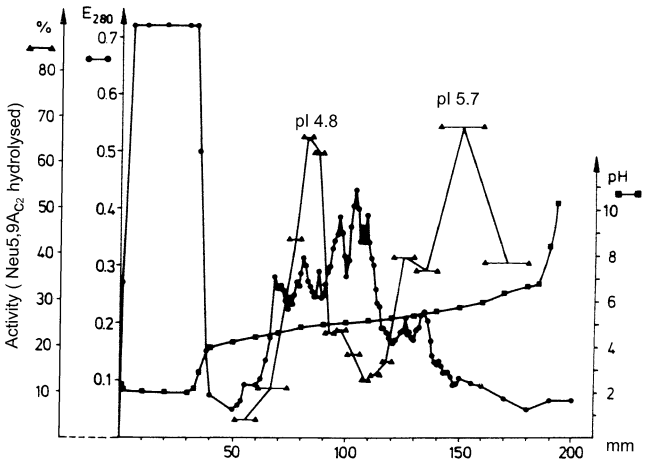

Fig. 2 Preparative isoelectric focussing of sialate-*O*-acetylesterase from horse liver. Activity peaks were found at p*I* 4.8 and 5.7, respectively. Focussing of the active fraction obtained from DEAE-Sephacel chromatography was carried out at 2 W up to 1,000 V using a pH gradient as indicated. Fractions of 2 ml each were collected and hydrolysis rates determined with Neu5,9Ac_2. The activities de-*O*-acetylating Sia could also saponify α-naphthylacetate. Protein was measured at 280 nm. Note that the protein amount of the active fractions at p*I* 5.7 is lower than that at p*I* 4.8; compare also Fig. 3. The horizontally positioned bars of the activity plot represent variations of double estimations. For further details see "Material and methods"

The esterases remained active for 1–2 years in frozen liver. The enzymes were quite stable up to the DEAE-Sephacel step. Repeated freezing and thawing did not lead to significant loss of activity of the partially purified enzymes from DEAE-Sephacel. However, after preparative isoelectric focussing they were not very stable even at −20°C and lost their activity within a few weeks.

Preincubation of the esterases with 1 mM 4-hydroxymercuribenzoate at 25°C for 15 min led to a total loss of enzyme activity, which allows the conclusion that these enzymes contain essential SH-groups. Correspondingly, Hg^{2+}, Cu^{2+}, and Zn^{2+} ions at 1 mM concentration also inhibited enzyme activity totally. This also explains the necessity of the presence of mercaptoethanol during the purification procedure, especially on isoelectric focussing in order to preserve esterase activity. Furthermore, preincubation with 1 mM diisopropylfluorophosphate or 1 mM paraoxon (diethyl-4-nitrophenylphosphate, E 600) at 25°C for 15 min resulted in a total loss of enzyme activity. In contrast, the negatively charged bis-(4-nitrophenyl)phosphate is no inhibitor. 1 mM Ca^{2+} and 1 mM ethylene diaminotetraacetate (EDTA) also showed no influence on enzyme activity. As all other known esterases, equine SOAE also hydrolyses α-naphthylacetate.

Discussion

The application of HPLC for the analysis of underivatised Sia, as described here, or using DMB-derivatives [42], allows a sensitive and specific qualitative and quantitative analysis of sialate esterase activities without too extensive purification of the samples. The use of methylumbelliferyl acetate or *para*-nitrophenyl acetate, which are also substrates of sialate esterases, can only be used for primary orientation or gel staining. These substances are hydrolysed by a variety of other esterases not attacking Sia, for example acetylcholine hydrolase or carboxyl esterases [43]. On HPLC, the Sia nature of the individual peaks can indirectly be proven by saponification of the *O*-acetylated

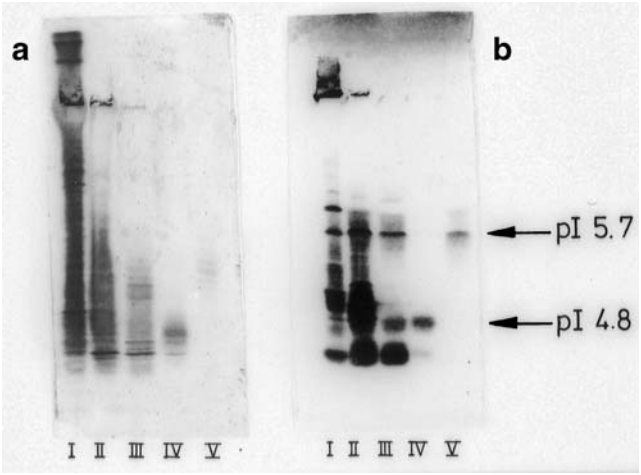

Fig. 3 Analytical isoelectric focussing of sialate-*O*-acetylesterases isolated from horse liver on polyacrylamide gel (ampholyte pH 3.5–9.5). Staining was done with Coomassie blue (**a**) and α-naphthylacetate with diazonium salt (Fast Red) (**b**). *I* Active enzyme fraction from Sephadex G-200 purification step, *II* from DEAE-Sephacel, *III* from preparative native electrophoresis (not described here), *IV* and *V* the two activity peaks obtained from preparative isoelectric focussing (see Fig. 2). For details see the text

Sia peaks or by their destruction using sialate lyase, when running the untreated probe in parallel.

The discovery of two esterase forms in horse liver, one hydrolysing only 9-*O*-acetyl groups from Sia and the other, exhibiting a higher p*I*-value of pH 5.7, also saponifying 4-*O*-acetyl esters, is remarkable. Especially the latter enzyme thus may enable desialylation of glycoconjugates in horse by assisting the sialidase, which otherwise could not start the catabolism of these glycoconjugates. By prior action of this esterase the sialic acid catabolism can be completed by sialate lyase finally cleaving Sia in pyruvate and acylmannosamine in the cytosol. The existence of an esterase with high activity on 4-*O*-acetylated Neu5Ac and Neu5Gc, frequently occurring in horse, seems to be an adaptation to the metabolic requirement of glycoconjugates from this animal. 4-*O*-Acetylated Sia were detected in horse submandibular gland mucins (over 50% Neu4,5Ac_2 and *N*-glycolyl-4-*O*-acetylneuraminic acid (Neu4Ac5Gc) [3, 44], in knee cartilage (unpublished), erythrocytes [45], and in serum acidic α_1-glycoprotein (M. Sander, Doctoral Thesis, University of Kiel, 1987). In the latter glycoprotein, which is produced in liver, over 30% of the Sia were found after acid hydrolysis and by HPLC analysis to be *O*-acetylated, mainly Neu4,5Ac_2 and a small portion of Neu4Ac5Gc. The other mammalian esterases studied, from rat and mouse cells [16–19], human testis [22], and frog (*Rana esculenta*) liver (A. K. Shukla, Habilitation Thesis, University of Kiel, 1985) were reported to act on Neu5,9Ac_2. However, bovine brain esterase hydrolyses Neu4,5Ac_2 at about half the rate to Neu5,9Ac_2 [20] and rat liver esterase at about 10% [46]. In both tissues, 4-*O*-acetylated sialic acids are unknown [1, 3].

In contrast to these eukaryotic enzymes a variety of viruses were found to exhibit sialate esterase activities strongly specific for either 4-*O*-acetyl or 9-*O*-acetyl groups, respectively [28–31]. These Sia have receptor functions for these viruses. The best investigated esterase in this respect is that from influenza C virus, which is practically inactive with Neu4,5Ac_2, showing only 3% hydrolytic rate when compared with Neu5,9Ac_2 [28]. On the other hand, the 4-*O*-acetylesterases from infectious salmon anemia virus (ISAV) and mouse hepatitis virus (MHV) are inactive with 9-*O*-acetylated Sia [47, 48].

Strikingly, the 9-*O*-acetylesterases, as far as investigated in this respect, are unable to saponify 7-*O*-acetylated Sia, best studied with influenza C virus [28], bovine brain [20] and rat liver [46] esterases. They are therefore most suitable analytical tools to discriminate between 7- and 9-*O*-acetylated Sia, *e.g.* on HPLC. Similarly, both horse liver esterases described here, are completely inactive with Neu5,7Ac_2 (Figs. 1 and 4). This has also been reported from the rat liver esterase [46]. Only the esterase from bovine toroviruses hydrolyses a Sia with a 7-*O*-acetyl group, since it seems to prefer Sia di-*O*-acetylated at C-7 or C-8 and at C-9 of its side chain [29]. An explanation for

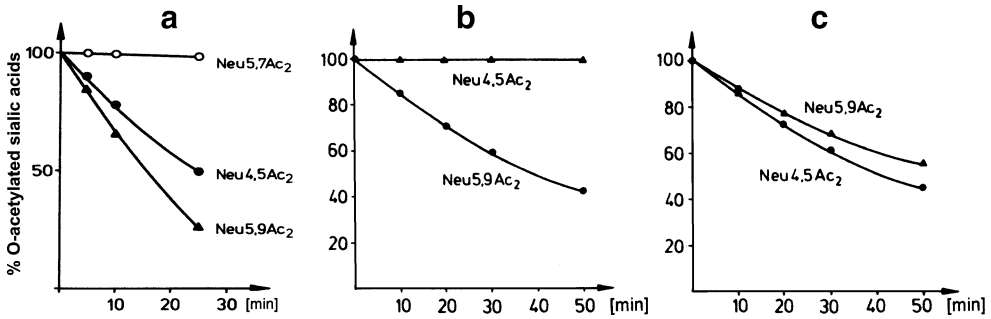

Fig. 4 Substrate specificity of esterases from horse liver. **a** Hydrolysis rate of various *O*-acetylated Sia by horse liver sialate-*O*-acetylesterase enriched on a DEAE-Sephacel column. Note that Neu5,7Ac_2 is not saponified. **b** and **c** Substrate specificity of the two esterases separated by isoelectric focussing at different pH values. Their isoelectric points are 4.8 (**b**) and 5.7 (**c**). Both enzyme fractions are inactive with Neu5,7Ac_2 (not shown). For more details see the text

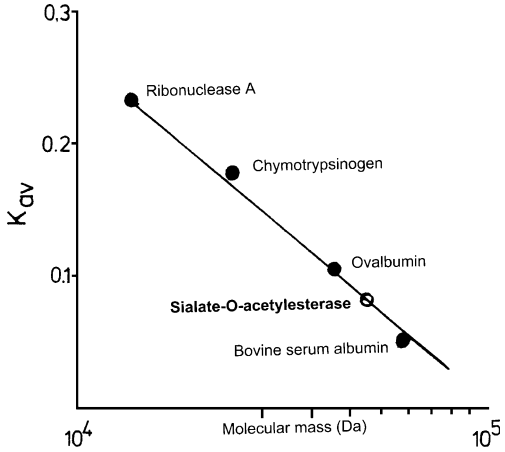

Fig. 5 Molecular mass determination of sialate-*O*-acetylesterase of horse liver from ion-exchange chromatography by gelfiltration on Sephacryl S-200

these specificities is not yet available and structural analyses of esterase–substrate interactions are necessary.

There is, however, no absolute requirement for the existence of a 7-*O*-acetylesterase activity, since the 7-*O*-acetyl groups can migrate under physiological conditions spontaneously to O-9 [14, 38]. Although some evidence was obtained for the existence of an isomerase rapidly catalysing this reaction [49], no enzymatic proof was obtained so far [8]. At position C-9, the 9-*O*-acetyl group can then be hydrolysed by the esterase.

Although the studies described here were carried out mainly with free sialic acids, it was shown with equine submandibular gland mucin, 9-*O*-acetylated sialyllactose, as well as equine and rat erythrocyte membranes, that glycosidically linked 4- and 9-*O*-acetylated sialic acids can also be saponified by the enzymes studied here. However, relative hydrolysis rates and other kinetic experiments were not yet carried out. Esterases from influenza C virus [28], rat liver [46] and bovine brain [20] were also shown to saponify both free and glycosidically linked *O*-acetylated sialic acids and relative hydrolysis rates were presented. These observations allow the conclusion that the esterases of horse liver are involved in glycoconjugate de-*O*-acetylation prior to sialidase action.

The equine liver esterases seem to contain essential sulfhydryl groups which must be protected on isolation, especially during isoelectric focussing by the addition of *e.g.* mercaptoethanol. Correspondingly, heavy metal ions or 4-hydroxymercuribenzoate inhibit enzyme activity. The strong and irreversible inhibitory potency of diisopropyl-fluorophosphate and diethyl-4-nitrophenylphosphate point to the participation of a serine residue in the active centre of the esterases. This seems to be a general property of sialate-*O*-acetylesterases, as was observed with human erythrocytes [15], murine [16, 50], bovine brain [20], and influenza C virus [28] esterases. Thus, according to Heymann [43] the sialate esterases can be classified as B-type esterases. The presence of an arginine residue seems also to be essential for the hydrolysis of anionic substrates [51].

Concluding remarks and outlook

The discovery of sialate-4-*O*-acetylesterase activity in a mammal expressing 4-*O*-acetylated sialic acids bridges a gap in the enzyme chain involved in sialic acid metabolism by assisting sialidases, which are unable to act on the 4-*O*-acetylated form. Even a sialidase highly active with *O*-acetylated Sia from *Streptococcus sanguis* was found not to be able to release 4-*O*-acetylated Sia from equine submandibular gland mucin [50]. Only fowl plague virus sialidase can slowly release Neu4,5Ac_2 from glycosidic linkage [52].

Of great interest will be the investigation of the structural difference between esterase proteins acting either on 4-*O*-acetyl- or/and 9-*O*-acetyl groups. The large family of sialate esterases shows a broad spectrum of substrate specificities varying between almost exclusive hydrolysis of only one of the two *O*-acetylated Sia studied here or being able to attack both 4- and 9-*O*-acetylated substances. The two esterase forms found in horse liver and exhibiting a similar molecular weight may be isoenzymes which are expected to reveal such differences of the active centre after further purification, molecular cloning and structural elucidation.

Sialate-*O*-acetylesterases are considered to be key enzymes in Sia catabolism [13]. Their localisation in the cytosol, lysosomes and Golgi membranes [16, 18, 21, 53] indicate versatile functions. They are involved in the regulation of the quantity and turnover of *O*-acetylated Sia. In lysosomes and probably also the cytosol they are responsible for the de-*O*-acetylation of engulfed sialoglycoconjugates and liberated Sia. In Golgi membranes, which are also the site of Sia *O*-acetylation, they may be involved in regulation of the steady state level of Sia *O*-acetylation [16]. In the mucosa of normal colon and colorectal carcinoma we found a positive correlation of the level of *O*-acetylated Sia to the relative activities of the sialate *O*-acetyltransferase and esterase activities [26], thus confirming this assumption. The level of free cytosolic Neu5,9Ac_2 in this tissue seems to be regulated by the activity of cytosolic esterase. Also in microorganisms evidence was obtained that Sia esterases are involved in regulation of the expression of *O*-acetylated Sia in capsular polysaccharides. Esterase activities were discovered as C-terminal parts of bifunctional CMP-Sia synthetases (NeuA) of *Escherichia coli* and group B *Streptococcus* [54, 55]. Since presently most work is done on viral and bacterial esterases, attention should again be shifted to structural and

functional aspects of vertebrate esterases, since Sia *O*-acetylation is gaining more attention.

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