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STUDIES ON THE SIALIDOSES

PROPERTIES OF HUMAN LEUCOCYTE NEURAMINIDASES

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Summary

At least two components of neuraminidase (acylneuraminyl hydrolase, EC 3.2.1.18) can be distinguished in human leucocytes on the basis of pH optimum, thermolability at 30°C and the effect of the detergent octyl-β-D-glucoside. With 4-methylumbelliferyl-α-D-*N*-acetylneuraminate as substrate, the A component has a pH optimum of 5.0, is labile at 30°C and is unaffected by 0.2 M octyl-β-glucoside. The B component has a pH optimum of 4.0—4.2, is stable at 30°C but loses most of its activity in the presence of 0.2 M octyl-β-glucoside. Both A and B components are membrane-bound but only the A component is solubilized by octyl-β-glucoside in an active form. Molecular weights of neuraminidases by γ-ray radiation inactivation (a method that does not require solubilization of the enzyme) were found to be 240 000 ± 19 000 for the B component, 203 000 ± 17 000 for the A component and 238 000 ± 8000 for the octyl-β-glucoside-solubilized A component. Gel filtration of soluble A component on Sephacryl S-300, in the presence of octyl-β-glucoside, showed a single peak of activity eluted at or near the void volume suggesting that the enzyme is still in an aggregated form. Profound deficiency of neuraminidase activity was found for both A and B components in leucocytes of patients affected with sialidoses type 1 and 2 (less than 15% normal) and intermediate activity in obligate heterozygotes. These results suggest that the A and

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B components of leucocyte neuraminidase are closely related from the genetic point of view and that rapid diagnosis of sialidoses can be done by fluorimetric assay of neuraminidase in leucocytes.

Introduction

Although the physical and enzymological properties of viral and bacterial neuraminidases (acylneuraminyl hydrolase, EC 3.2.1.18) have been well studied [1,2], less information is available on mammalian and particularly human neuraminidases. Recent interest in human neuraminidases evolved from the discovery of a deficiency of this enzyme in a variety of genetic disorders designated by the term 'sialidosis' [3,4]. Lowden and O'Brien [3] distinguished a normosomatic group (type 1) of patients (the cherry-red spot syndrome with [5—8] or without myoclonus [4]) and a dysmorphic group (type 2) with juvenile (the Goldberg syndrome [9], the cherry-red spot myoclonus syndrome with dementia [10—12]) and infantile onset (mucolipidosis I [13—15]). Other patients who are not classifiable in one of these groups have also been reported [16,17]. All these patients presented with neuraminidase deficiency in cultured skin fibroblasts and excessive excretion of sialyl-oligosaccharides in the urine [5,15—20]. The molecular bases for such differences in clinical expressivity are not clear but could be due to different mutations, or deficiency of different molecular forms of the enzyme [3,15]. Evidence for different mutations was provided by O'Brien and Warner [21] who found lower levels of residual activity in cultured skin fibroblasts of patients with type 2 sialidosis.

Mammalian neuraminidases have been localized in lysosomes [22,23], Golgi apparatus [24], plasma membranes [25] and cytosol [26]. Substrate specificity of the lysosomal and plasma membrane enzyme is directed to both oligosaccharides and gangliosides, whereas the other enzymes hydrolyse only the oligosaccharides. The cytosolic enzyme has a higher pH optimum (pH 5.5) [26] than other neuraminidases (between pH 4 and 4.4). Evidence from studies of neuraminidase deficiency syndromes also indicated that two distinct neuraminidases, one more specific to glycoprotein and another specific to ganglioside substrates, exist in human cultured skin fibroblasts [10]. Evidence for the presence of these two enzymes was also obtained in horse liver [27]. In addition, Strecker and Michalski [20] suggested that cultured human skin fibroblasts and leucocytes contain a neuraminidase specific for $\alpha 2 \rightarrow 6$-linked *N*-acetylneuraminic acid-oligosaccharides and a distinct enzyme specific for $\alpha 2 \rightarrow 3$-linked *N*-acetylneuraminic acid. Only the $\alpha 2 \rightarrow 6$ neuraminidase is deficient in sialidosis patients [20] but others found a deficiency of both $\alpha 2 \rightarrow 3$ and $\alpha 2 \rightarrow 6$ neuraminidase [28,29]. Although more information is needed on the molecular forms of human neuraminidase, actual separations by chromatographic or electrophoretic techniques are hampered by difficulties in solubilizing the enzyme [30]. However, Venerando et al. [31] separated two neuraminidase isoenzymes from pig brain cytosol and Potier et al. suggested the presence of two neuraminidase components with different thermolability in human cultured skin fibroblasts [29] and mouse liver [32]. Srivastava and Abou-Issa [33] solubilized and purified the neuraminidase from rabbit spermatozoal acrosomes and Srivastava and

Farooqui [34] solubilized the enzyme from rabbit endometrium.

Recent synthesis of the fluorigenic substrate 4-methylumbelliferyl-α-D-*N*-acetylneuraminate by several laboratories [9,35—37], allowed rapid and sensitive assay of neuraminidase for diagnosis of the sialidoses with cultured skin fibroblasts [9,36,37] or cultured amniotic fluid cells [38], and for the characterization of this enzyme in leucocytes [36,39]. In this paper, we present evidence for the existence of two neuraminidase components in human leucocytes, the solubilization of one component with the detergent octyl-β-glucoside, the molecular weight of the enzymes and neuraminidase activity in leucocytes of homozygous and heterozygous carriers of sialidoses.

Materials and Methods

Materials. The sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) substrate was synthesized according to Potier et al. [36]. Bovine serum albumin (Cohn's fraction IV), bovine erythrocyte acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) and beef liver β-glucuronidase (β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31) were purchased from Sigma Chemical Co. (St. Louis, MO). Sephacryl S-300, catalase, (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6), thyroglobulin, ferritin, aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13) and Dextran blue were obtained from Pharmacia Fine Chemicals Co. (Montréal) and the detergent octyl-β-glucoside from Calbiochem (La Jolla, CA). All other reagents were obtained from Fisher Scientific Co. (Montréal) and were of the best grade available.

Preparation of leucocytes, lymphocytes, platelets and erythrocyte plasma membranes. Human leucocytes were prepared by Dextran sedimentation of freshly collected heparinized blood (16 ml) from three patients with sialidosis and their parents, all of Italian origin, by the method of Kampine et al. [40].

Control leucocytes were obtained from healthy laboratory personnel and voluntary donors. Two of the sialidosis patients (type 1), a 17-year-old girl and a 24-year-old boy from family 1 have been previously described [4]. They present with bilateral cherry-red spots in the macula and visual loss, but normal intelligence and no evidence of skeletal dysplasia. The other sialidosis patient (type 2, family 2) is a 2-year-old boy with facial dysmorphism, skeletal dysplasia, mild mental retardation and bilateral cherry-red spots. All patients showed highly increased sialyl-oligosaccharidiuria (analysis done by Dr. G. Strecker, Université des Sciences et Techniques de Lille, Lille, France), neuraminidase deficiency in cultured skin fibroblasts but normal activities of other lysosomal enzymes.

Large scale preparation of leucocytes was also undertaken using a modification of this method. Blood was collected (450—500 ml) from male or female volunteers (28—33-years-old) in heparinized standard plastic collection bags and mixed with 125 ml of 60 mg/ml Dextran/0.04 M EDTA. Red blood cells were allowed to settle for 150 min at 37°C and the supernatant was collected. To remove contaminating erythrocytes, leucocytes were centrifuged at 1520 × *g* for 10 min, resuspended in water for 1 min and then mixed with an equal volume of 0.31 M NaCl to restore isotonicity. This procedure was

repeated once and leucocytes were centrifuged as described above. Leucocyte neuraminidase activity is stable when pelleted leucocytes are kept at —60°C for less than 2 months. The leucocyte preparation contained about 81% granulocytes and the rest were lymphocytes. Lymphocytes (about 96% pure) were isolated from fresh heparinized blood using a Ficoll-Paque (Pharmacia Fine Chemicals Co.) discontinuous gradient as described by Bφyum [41]. Blood platelets were prepared by the method of Marcus et al. [42] and erythrocyte plasma membranes were obtained by repeated washing of fresh human erythrocytes with deionized water and then 0.15 M NaCl, until the pellet was colorless.

When needed, samples were thawed slowly or fresh preparations were homogenized for 2 min in water using a Potter-Elvejhem homogenizer fitted with a Teflon pestle (protein concentration, 3—5 mg/ml). Neuraminidase activity in the homogenate was stable for at least 8 h at 0°C.

Neuraminidase assay. Neuraminidase activity was assayed at 25°C unless otherwise indicated, according to Potier et al. [36] with 0.2 mM sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) as substrate in the presence of 0.2 M acetate buffer (pH 4.2 or 5.0), except that the reaction was stopped with 1 ml absolute ethanol and the fluorescence was read after addition of 0.1 ml 1 M NaOH, as previously described [32]. Due to instability of neuraminidase in presence of the detergent octyl-β-glucoside at 25°C, assays were carried out at 0°C for 18 h at pH 5.0. 1 unit (U) of neuraminidase activity was the amount of enzyme that liberates 1 μmol 4-methylumbelliferone per min.

Heat inactivation of neuraminidase. A leucocyte homogenate in water was made up to 0.02 M with maleate buffer (pH 6) and preincubated at 30°C. At intervals of 15 min, 0.1 ml aliquots were taken and immediately cooled at 0°C. Samples were then equilibrated at 25°C for 3 min and neuraminidase activity was assayed at both pH 4.2 and 5.0 as described above.

Solubilization of neuraminidase with the detergent octyl-β-glucoside. A leucocyte homogenate was made up to 0.2 M in octyl-β-glucoside and allowed to stand at 0°C for 20 min. The preparation was then centrifuged at 105 000 × *g* for 30 min and the supernatant was kept for further studies. Attempts to solubilize leucocyte neuraminidase with Triton X-100, sodium taurocholate, Tween 20, freezing and thawing, chaotropic agents KSCN and $NaClO_4$, or sonication were unsuccessful resulting in loss of enzyme activity.

Sephacryl S-300 chromatography. The octyl-β-glucoside soluble neuraminidase (1 ml) was applied to a Sephacryl S-300 column (0.6 × 53 cm) previously equilibrated with 0.02 M maleate buffer (pH 6.8)/0.1 M NaCl/0.2 M octyl-β-glucoside. Fractions (0.4 ml) were collected and neuraminidase assayed. The column was calibrated with Dextran blue and with the following standard proteins: thyroglobulin, 669 000; ferritin, 440 000; catalase, 232 000 and aldolase, 158 000. The elution buffer was used without octyl-β-glucoside for column calibration with standard proteins.

Molecular weight determination by radiation inactivation. Leucocyte suspensions (3—5 mg protein) in 0.15 M NaCl were centrifuged in 1.5 ml capped plastic tubes at 800 × *g* for 10 min and the supernatant was discarded. Pelleted leucocytes or octyl-β-glucoside-solubilized neuraminidase preparations were then lyophilized for 3 h and the residue was placed under nitrogen atmosphere. More than 82% of neuraminidase activity was recovered after lyophilization.

Lyophilized powders are stable for several days at −60°C or for at least 24 h at room temperature.

Radiation inactivation was carried out in a ^{60}Co Gamma Cell, Model 220 (Atomic Energy of Canada Ltd., Ottawa) at room temperature (22°C) for different intervals of time up to 6 h. Control tubes were left for the same intervals at room temperature but were not irradiated. Quintuplicates of irradiated and control tubes were then analysed for enzyme activity at each irradiation period (corresponding to doses). The lyophilized powder was resuspended in 0.1 ml of deionized water and allowed to stand at 0°C for 20 min. Neuraminidase activity was assayed at 0°C for 24 h as described above.

The logarithm of percent residual neuraminidase activity was plotted against radiation dose and the dose (D_{37}), in megarad, giving 37% of initial neuraminidase activity was determined. The molecular weight (M_r) was obtained from the equation [43]:

$$M_r = \frac{6.4 \cdot 10^5}{D_{37}}$$

Beef liver catalase (230 000, Ref. 44), bovine erythrocyte acetylcholinesterase (72 000, Ref. 45), beef liver β-glucuronidase (81 000, Ref. 46) and rat liver cytosolic neuraminidase (55 000 as determined by gel filtration on Sephacryl S-200) were used as molecular weight standards to determine the dose rate of the apparatus and test the method. At the time of the analyses the apparatus contained 4925 Ci of ^{60}Co and the dose rate was 0.612 Mrad/h. Enzyme preparations were lyophilized as described above. Catalase was assayed by the method of Beers and Sizer [47], acetylcholinesterase according to Ellman et al. [48] as modified by Wermuth and Boodbeck [49], β-glucuronidase according to Gianetto and de Duve [50], and neuraminidase according to Potier et al. [36], except that a 0.1 M sodium cacodylate buffer (pH 6) was used in the incubation medium.

Determination of apparent Michaëlis-Menten constant. $K_{m,app}$ was determined at 0°C under standard assay conditions with sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) as substrate except that substrate concentration was varied between 0.08 and 4 mM. A Lineweaver-Burk plot was used for graphical determination of $K_{m,app}$ [51].

Determination of protein concentration. Protein concentration was determined by the method of Bradford [52] with bovine serum albumin as standard. Before assay, proteins were dissolved in 0.1 M NaOH.

Results

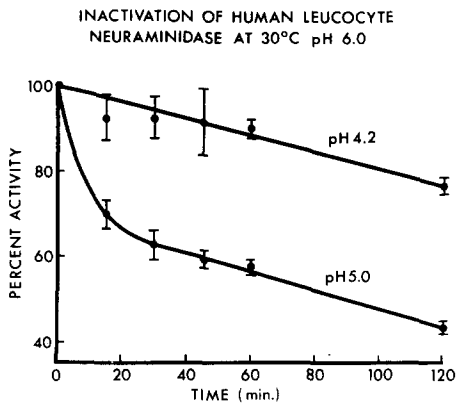
Neuraminidase activity in various blood fractions. The highest neuraminidase activity was found in lymphocytes (0.0244 ± 0.005 U/g protein at pH 4.2 and 0.0142 ± 0.003 U/g protein at pH 5.0, $n = 5$). From the known composition of our leucocyte preparations obtained by Dextran sedimentation (about 81% granulocytes and 19% lymphocytes), we calculated a neuraminidase activity of 0.0108 ± 0.0035 U/g protein at pH 4.2 and 0.0047 ± 0.0013 U/g protein at pH 5.0 for pure granulocytes. The ratio between neuraminidase activity at pH 4.2 and 5.0 was similar in granulocyte and leucocyte preparations. The neuramini-

dase activity in blood platelets and erythrocyte plasma membranes was not detected with our assay conditions (<0.0002 U/g protein) at both pH 4.2 and 5.0.

Thermolability of neuraminidase. When preincubated at pH 6 and 30°C for different intervals of time without substrate and then assayed at pH 4.2 and 5.0, neuraminidase activity at pH 5.0 was found to be more labile than that at pH 4.2 (Fig. 1). The denaturation kinetics were not first-order, as indicated by the shape of the curve but were of a more complex nature, probably reflecting the fact that neuraminidase is membrane-bound and that its denaturation kinetics may be influenced by membrane components or membrane interaction.

Mercaptoethanol had no effect on the stability of neuraminidase, but Triton X-100 and octyl-β-glucoside increased thermolability. Neuraminidase active at pH 4.2 loses 47% of its activity at 30°C after 60 min in presence of 0.008% Triton X-100 and retains only 21% in presence of 0.2 M octyl-β-glucoside.

Effect of octyl-β-glucoside. The pH-activity curve of neuraminidase was wide with a maximum activity at pH 4—4.2 when assays were carried out at 0 or 25°C (Fig. 2). A previously published curve obtained at an incubation temperature of 37°C showed relatively less activity at pH 5 [39]. This is consistent with the finding that some activity at pH 5 is thermolabile (Fig. 1). When 0.2 M octyl-β-glucoside was added, most of the activity at acidic pH was lost and maximum activity shifted to pH 5 (Fig. 2). The leucocyte neuraminidase is labile in presence of octyl-β-glucoside at 25°C and thus, all assays with octyl-β-



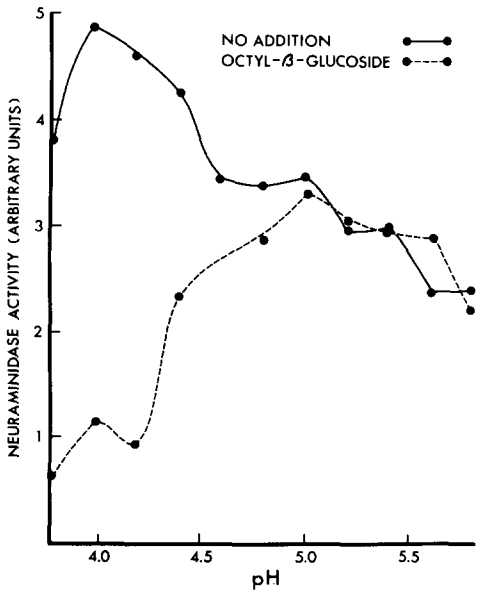


Fig. 1. Effect of preincubation time at 30°C and pH 6 on neuraminidase activity assayed at pH 4.2 and pH 5.0.

Fig. 2. pH-activity curve of leucocyte neuraminidase in 0.2 M acetate buffer at an assay temperature of 0°C. No addition (●———●); with 0.2 M octyl-β-glucoside (●- - - - ●).

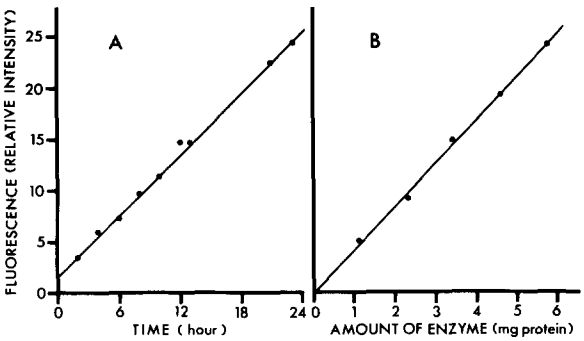


Fig. 3. Linearity of fluorescence intensity with time (A) and amount of enzyme (B) in the assay of octyl-β-glucoside-solubilized neuraminidase at 0°C and pH 5.

glucoside were carried out at 0°C and pH 5. Linearity with incubation time and amount of enzyme was obtained in these conditions (Fig. 3).

The addition of octyl-β-glucoside to a leucocyte homogenate previously treated at 30°C (pH 6), for 60 min, to destroy neuraminidase activity at pH 5, did not give more activity at pH 5. This result suggests that the neuraminidase active at pH 5 in presence of octyl-β-glucoside is not produced from the enzyme active at pH 4.2 but is apparently a distinct enzyme.

Neuraminidase activity in leucocyte homogenate is membrane-bound. More than 86% of the activity at both pH 4.2 and 5 is recovered in the sedimentable fraction at 105 000 × *g* for 30 min. Fig. 4 shows the effect of increasing concentrations of octyl-β-glucoside on total and soluble neuraminidase activity. Best conditions for solubilization were obtained with 0.2 M octyl-β-glucoside at a protein concentration of 3—5 mg/ml, where 51—78% of total neuraminidase activity and about 75% of the total protein were solubilized.

The $K_{m,app}$ of neuraminidase measured at pH 4.2 and 5.0 at 0°C was 0.07 and 0.09 mM, respectively. The presence of octyl-β-glucoside did not significantly change the $K_{m,app}$ of neuraminidase, determined at pH 5.0 (0.10 mM).

Soluble leucocyte neuraminidase was applied to a Sephacryl S-300 column

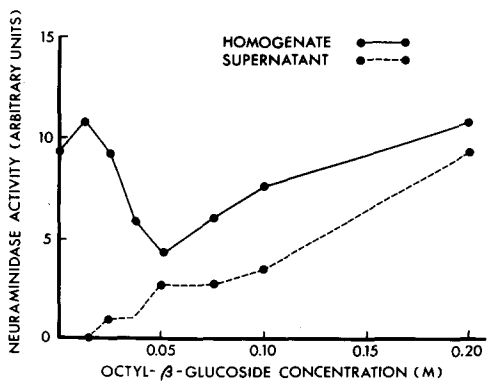


Fig. 4. Effect of increasing concentrations of octyl-β-glucoside on leucocyte neuraminidase activity at pH 5 and 0°C, on whole homogenate (●——●) and supernatant fraction (●- - - -●).

equilibrated with 0.02 M maleate buffer (pH 6.8)/0.1 M NaCl/0.2 M octyl-β-glucoside. All neuraminidase activity was eluted as a single peak near or at the void volume (data not shown). These results suggested that octyl-β-glucoside soluble neuraminidase exists in an aggregated form or still bound to high-molecular weight membrane components.

Octyl-β-glucoside can easily be dialysed out from the soluble neuraminidase preparation against deionized water, but this causes precipitation of protein and loss of almost all activity. Similar results were obtained when either 0.1 M acetate (pH 5) or 0.05 M maleate (pH 6) buffer were used for dialysis.

Determination of molecular weight of membrane-bound and soluble neuraminidase by radiation inactivation. Radiation inactivation curves of neuraminidases are shown in Fig. 5 and molecular weights of the enzymes are given in Table I. The molecular weight for the neuraminidase active at pH 4.2 was slightly higher than that of the enzyme active at pH 5. However, the difference in slope of the least-squares lines was not statistically significant ($P > 0.1$) when compared by analysis of covariance [53]. The octyl-β-glucoside-solubilized enzyme had a molecular weight higher than that of the neuraminidase active at pH 5 (Table I).

Neuraminidase activity in leucocytes of homozygous and heterozygous carriers of the sialidosis. Table II gives neuraminidase activity at both pH 4.2 and 5.0 in leucocyte homogenates of controls, heterozygous and homozygous carriers of type 1 and type 2 sialidosis patients. Neuraminidase activity was deficient at both pH 4.2 and 5.0, being less than 13 and 8% of normal, respectively, in homozygous carriers. Intermediate activities were found at both pH values in heterozygotes (58% of normal at pH 4.2 and 46% of normal at pH 5). The level of residual activity for homozygous carriers of the two different types of sialidosis was not very different and hence these data were pooled. O'Brien and

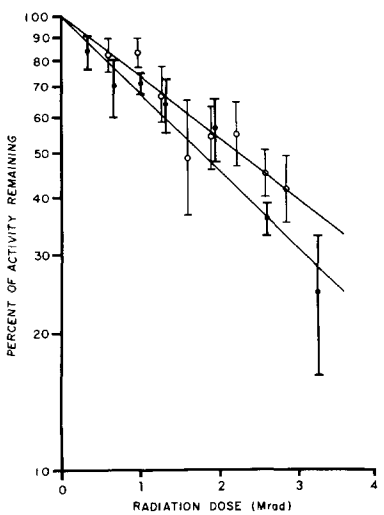


Fig. 5. Radiation inactivation of leucocyte neuraminidase. Neuraminidase assays were carried out at both pH 4.2 (●) and pH 5 (○). Bars indicate range of quintuplicate determinations and straight lines were fitted by the least-squares method.

TABLE I

MOLECULAR WEIGHT OF LEUCOCYTE NEURAMINIDASE AND STANDARD ENZYMES AS DETERMINED BY RADIATION INACTIVATION

Enzyme	D_{37} (Mrad)	Molecular weight		
		Obtained	Literature	Reference
Leucocyte neuraminidase, pH 4.2	2.67 ± 0.21 *	240 000 ± 19 000	—	
Leucocyte neuraminidase, pH 5.0	3.16 ± 0.23	203 000 ± 17 000	—	
Octyl-β-glucoside-soluble leucocyte neuraminidase, pH 5.0	2.69 ± 0.09	238 000 ± 8 000	—	
Rat-liver cytosolic neuraminidase	11.4	56 000	55 000 **	
Catalase	2.68	238 000	230 000	44
Acetylcholinesterase	8.77	73 000	72 000	45
β-Glucuronidase	7.53	85 000	81 000	46

* Mean ± S.D. of three determinations.
** Determined by gel filtration on Sephacryl S-200.

Warner [21] reported much lower residual activity in cultured skin fibroblasts of type 2 patients. There was enough activity in leucocytes of sialidosis patients at pH 4.2 (but not at pH 5.0) to study the effect of octyl-β-glucoside and heat treatment at 30°C. Neuraminidase activity was lost completely in the presence of 0.2 M octyl-β-glucoside as for the normal enzyme, but residual activity was very thermolabile in contrast to the neuraminidase in control leucocytes.

TABLE II

NEURAMINIDASE ACTIVITY IN LEUCOCYTES OF HETEROZYGOUS AND HOMOZYGOUS CARRIERS OF THE SIALIDOSES

	Neuraminidase activity (U/g protein) ($\times 10^3$)			
	pH 4.2	%	pH 5.0	%
Controls (n = 12)				
Mean ± S.D.	13.5 ± 4.5	100	6.5 ± 1.8	100
Range	(9.8—18.5)		(4.0—8.5)	
Heterozygotes (n = 4)				
Family 1 (sialidosis type 1)				
father	9.2		4.0	
mother	6.6		2.5	
Family 2 (sialidosis type 2)				
father	7.7		2.8	
mother	7.5		2.3	
Mean ± S.D.	7.8 ± 0.8	58	3.0 ± 0.7	46
Homozygotes (n = 3)				
Family 1 (sialidosis type 1)				
boy	1.8		0.2	
girl	1.8		0.2	
Family 2 (sialidosis type 2)				
boy	1.3		1.2	
Mean ± S.D.	1.6 ± 0.2	12	0.5 ± 0.3	8

Discussion

Neuraminidase activity was first reported in human leucocytes by Yeh et al. [54]. In this paper, we report the presence of two neuraminidase components, A and B, which can be distinguished on the basis of pH optimum, thermolability at 30°C, the effect of the detergent octyl-β-glucoside and by their molecular weight. The A component has a pH optimum of 5, is labile at 30°C but is unaffected by octyl-β-glucoside at 0°C and has a slightly lower molecular weight than the B component. In contrast, the B component has a pH optimum of 4—4.2, is more stable at 30°C but loses most of its activity in the presence of 0.2 M octyl-β-glucoside. The A and B components are present in similar proportions in lymphocytes and granulocytes.

Mammalian neuraminidases have been localized in lysosomes [22,23], Golgi apparatus [24], plasma membranes [25] and cytosol [26]. The intracellular distribution of A and B components in leucocytes is unknown but both components were found to be membrane-bound. The detergent octyl-β-glucoside solubilized the A component but the activity of the B component was lost. However, the octyl-β-glucoside-solubilized neuraminidase was still in an aggregated form, or bound to debris of membranes, or asymmetric since all enzyme activity was excluded from a Sephacryl S-300 column (exclusion limit about $1.5 \cdot 10^6$ for globular protein), whereas the molecular weight obtained by radiation inactivation in presence of octyl-β-glucoside was 238 000 (Table I).

To the best of our knowledge, this is the first report on molecular weight of a mammalian neuraminidase. It was not possible to use conventional methods for molecular weight determination due to difficulties in obtaining the enzyme in an active, soluble and unaggregated form. Similar difficulties were encountered by other authors with brain neuraminidase [30]. The method of radiation inactivation does not require solubilization or purification of the enzyme and was applied directly to lyophilized leucocyte preparations. Slightly different curves were obtained when inactivation was followed at assay pH 4.2 and 5.0 (Fig. 5), reflecting different molecular weights of A and B forms of neuraminidase (Table I). The A component had a higher apparent molecular weight in the presence of octyl-β-glucoside, presumably the result of direct addition of detergent to the neuraminidase molecule or to a conformational change in the molecule induced by octyl-β-glucoside. However, the $K_{m,app}$ of the enzyme at pH 5 was similar in the presence or absence of octyl-β-glucoside indicating that the affinity of neuraminidase for the fluorigenic substrate was not substantially affected by octyl-β-glucoside. It is not known whether the molecular weight of neuraminidase obtained by radiation inactivation represents that of the whole molecule or that of a subunit. In a recent review of literature dealing with a large number of multimeric enzymes, Kempner and Schlegel [55] reported that the molecular weight obtained by radiation inactivation corresponded to the subunit for some enzymes, whereas for several others the molecular weight corresponded to that of the whole molecules. They concluded that the molecular weight of a given enzyme obtained by this method reflects the size of the complete functional unit (subunit or oligomer), including the membrane-associated forms [43,55]. The molecular weight found for human leucocyte neuraminidase was higher than that previously reported for

the bacterial enzymes (56 000 for *Clostridium perfringens* neuraminidase [56], 90 000 for *Vibrio cholerae* [57] and 88 000 for *Arthrobacter sialophilus* [58]) and viral neuraminidase (130 000 for influenza virus A_2 neuraminidase [59]).

The two neuraminidase components are closely related from the genetic point of view, since both were deficient in leucocytes of sialidosis patients and enzyme activities were intermediate in obligate heterozygotes (Table II). These findings confirm those of Durand et al. [4], and of Strecker and Michalski [20] who used natural sialyl-oligosaccharides purified from urine as substrates for leucocyte neuraminidase. However, our method is more simple and rapid using a fluorigenic substrate and suitable for routine use. Similar results were also obtained with cultured skin fibroblasts, where the two neuraminidase components (distinguished by their different thermolability) in control fibroblast lines were both deficient in fibroblasts from sialidosis patients [29]. It is possible that the two components are conformers, or that one component is a precursor or a hydrolytic degradation product of the other. No experimental evidence exists in favor of any of these possibilities at present.

The low levels of neuraminidase activity present in human leucocytes (Table II), relatively unsensitive to natural substrates [29], prevented us from determining the specificity of each neuraminidase form towards neuraminyl-lactose, fetuin and gangliosides. Leucocytes are more readily obtained than cultured skin fibroblasts, and are thus very useful for rapid diagnosis of sialidosis and perhaps for detection of heterozygous carriers. It is interesting to note that the residual activity in leucocytes of sialidosis patients was more thermolabile at 30°C than the normal enzyme. This could correspond to a minor neuraminidase isoenzyme or to an altered enzyme molecule. A more detailed structural study of human neuraminidases will require solubilization and purification of each component in an active and unaggregated form.

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