Interaction Between the Soluble and Particulate Neuraminidases of Chick Liver[1]

D. R. P. Tulsiani[2], V. P. Bhavanandan[3] and R. Carubelli

Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City (Oklahoma 73104, USA), 15 June 1976.

Summary. The sum of the neuraminidase activities found in the isolated soluble and particulate fractions of chick liver was considerably higher than that observed in the cytoplasmic extract from which these fractions were obtained. Addition of increasing amounts of particulate neuraminidase to a constant amount of the soluble preparation resulted in a progressive loss of enzyme activity.

Chick liver, as the liver of other vertebrates, contains both a soluble and a particle-bound neuraminidase[4]. Unlike the situation observed with rat liver[5], the chick liver neuraminidases have rather close pH optima: 3.8 for the particulate and 4.4 for the soluble enzyme[4].

While investigating the intracellular distribution of this enzyme, it was observed that the sum of the neuraminidase activities found in the isolated soluble and particulate fractions was more than 50% higher than that of the cytoplasmic extract from which these fractions were derived. In order to further investigate this observation, chick liver homogenates (1:4 w/v) were prepared in 0.154 *M* KCl and nuclei and debris were removed by low speed centrifugation (10 min at 800 *g*). Neuraminidase activity was then measured in this cytoplasmic extract as well as in the soluble fraction and in the sedimentable fraction (resuspended in 0.154 KCl) obtained from it by centrifuging for 1 h at 105,000 *g*. All neuraminidase assays were conducted at pH 4.0; at this pH, about 95% of the maximum activity for the soluble and particulate fractions can be detected[4]. The *N*-acetylneuraminic acid (NANA) released by the action of the enzyme was measured by the thiobarbituric acid method[6].

In a series of determinations conducted using as substrates various sialyltrisaccharides isolated from rat mammary gland[7] and from cow colostrum[8], the neuraminidase activity in the cytoplasmic extract was consistently lower than the sum of the values obtained for the soluble and particulate fractions (see table).

Addition of increasing amounts of the particulate neuraminidase to a constant amount of the soluble preparation resulted in a progressive loss of neuraminidase activity (see figure). When the two enzymes are present in the mixture in the same proportion found in the original tissue, the activity of the mixture is usually 50–70% of that given by the soluble neuraminidase by itself. Heat inactivated (15 min at 100 °C) particulate neuraminidase was less effective but, nevertheless, clearly inhibitory.

Several possible mechanisms that could account for this inhibition were investigated and the results are summarized below.

Addition of ashes from 1 g of whole fresh liver to the soluble fraction from 1 g of liver caused much less inhibition (less than 20%); at least one half of the inhibition caused by the ashes is probably due to the inhibitory effect of chlorides on the soluble neuraminidase[4].

[1] This investigation was supported in part by U. S. Public Health Service Grant NS09176 from the National Institutes of Health.

[2] Present address: Department of Molecular Biology, Vanderbilt University, Nashville (Tennessee 37232, USA).

[3] Present address: Department of Biological Chemistry, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey (Pennsylvania 17033, USA).

[4] D. R. P. Tulsiani and R. Carubelli, Biochim. Biophys. Acta *284*, 257 (1972).

[5] R. Carubelli and D. R. P. Tulsiani, Biochim. Biophys. Acta *237*, 78 (1971).

[6] L. Warren, J. Biol. Chem. *234*, 1971 (1959).

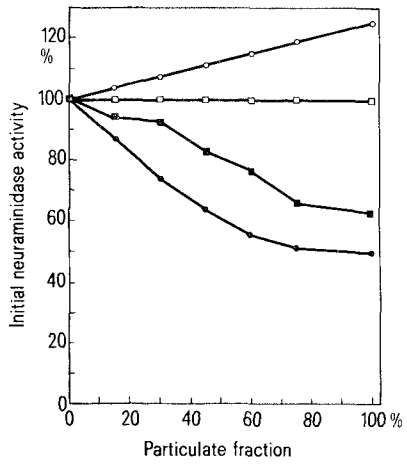
[7] H. U. Choi and R. Carubelli, Biochemistry *7*, 4423 (1968).

[8] M. L. Schneir and M. E. Rafelson, Jr, Biochim. Biophys. Acta *130*, 1 (1966).

Neuraminidase activity in the soluble and particulate fractions and in the unfractionated cytoplasmic extract prepared from the livers of 8-day-old white leghorn chicks

Substrate: type of linkage and source	Enzyme preparation	Neuraminidase activity			
		nmoles NANA/h · g of liver			
Neuramin-lactose	Cytoplasmic extract	247			205 ± 33
α2→3′	Soluble fraction	318	(396)	292 ± 61	(395 ± 65)
Rat mammary gland	Particulate fraction	78		103 ± 21	
Neuramin-lactose sulfate	Cytoplasmic extract	499			613 ± 99
α2→3′	Soluble fraction	597	(752)	583 ± 57	(820 ± 129)
Rat mammary gland	Particulate fraction	155		237 ± 92	
Neuramin-lactose	Cytoplasmic extract	225			
α2→3′	Soluble fraction	308	(372)		
Cow colostrum	Particulate fraction	64			
Neuramin-lactose	Cytoplasmic extract	112			
α2→6′	Soluble fraction	149	(184)		
Cow colostrum	Particulate fraction	35			

The set of values on the left corresponds to the results obtained from a single representative experiment conducted with the various substrates listed. The mean ± S. D. from seven different experiments with neuramin-lactose, and three experiments with neuramin-lactose sulfate, are shown on the right side of the table. Figures in parentheses represent the sum of the activities of the soluble and particulate fractions.



Inhibition of soluble chick-liver neuraminidase by increasing concentrations of active and heat-inactivated particulate neuraminidase. A constant amount of soluble enzyme was present in all incubation mixtures. Ordinate values represent the activity of the mixtures expressed as percent of the activity given by the soluble fraction by itself. Abcissa values indicate percent of active (●) and heat inactivated (■) particulate fraction added to the mixture; at 100%, soluble and particulate fractions are present in the same proportion found in the original fresh tissue. All assays were conducted using neuramin-lactose (2→3′) as the substrate; the data shown corresponds to the average of two separate experiments. Theoretical values, calculated by adding the activities of the soluble and particulate enzymes assayed separately, are included for mixtures containing active (○) and heat-inactivated (□) particulate preparations.

No destruction of free NANA (NANA-aldolase activity), or interference with the thiobarbituric acid assay, by the particulate fraction could be detected.

No marked differences were observed in the pH-versus activity curves of the cytoplasmic extract, soluble and particulate enzymes.

No abnormal behavior was observed upon determination of neuraminidase activity with increasing substrate concentrations. The K_m values for neuramin-lactose (2→3′) were of the same order of magnitude: soluble neuraminidase 1.20×10^{-3} *M*; particulate neuraminidase 2.72×10^{-3} *M* and cytoplasmic extract 1.97×10^{-3} *M*.

Experiments with [^{14}C]-U-neuramin-lactose[9] indicated absence of irreversible binding of the substrate by the particulate fraction and ruled out possible transfer (transglycosylation) of NANA (cleaved from the substrate by neuraminidase action) to endogenous or exogenous acceptors.

Since the cytosolic neuraminidase of chick liver exhibits very low activity towards macromolecules[4], our data suggests the possibility that binding of this enzyme to sialyl groups on the surface of the native, and of the heat-inactivated, particulate fraction may account for the loss of activity observed in these experiments. It is tempting to speculate that a similar phenomenon may also play a role in the regulation of neuraminidase activity in vivo.

[9] R. CARUBELLI, B. TAHA, R. E. TRUCCO and R. CAPUTTO, Biochim. Biophys. Acta *83*, 224 (1964).

On the Nature of Protein Benzoquinone Complexes[1]

K. LORENTZ

Medizinische Hochschule Lübeck, Medizinische Klinik, Kronsforder Allee 71–73, D-24 Lübeck (Federal Republic of Germany, BRD), 20 November 1975.

Summary. Proteins form monosubstituted 1,4-benzoquinone derivatives with an excess of reagent via uniformly coloured charge transfer complexes. Some properties of these compounds are reported.

Several investigations have been made on the reaction of amino acids and proteins with 1,4-benzoquinone (pBQ) purporting the formation of mono- and disubstituted quinones in buffered aqueous solutions of organic solvents[2–6], whereas the nature of these products remains controversial[7,8]. Therefore the present work describes some properties of protein pBQ complexes, analogously prepared to the reaction of amino acids[9].

Materials and methods. Human serum albumin and fibrinogen (Behringwerke), α-casein, gelatin, porcine pepsin (Sigma), bovine insulin (generous gift from Hoechst), and bovine pancreas ribonuclease (Boehringer) were purified from low molecular and protein contaminations by repeated gel filtration on Sephadex G-25 (Pharmacia) and G-75 or G-200 resp., dialyzed exhaustively and lyophilized. Acetylated, alkali denatured, and performic acid oxidized proteins[10] were treated in the same manner. 1.0 ml of protein solution, containing 5–20 mg protein in 10 mmol/l NH_4OH, was mixed with 1.0 ml of 200 mmol/l phosphate pH 6.5 and 1.0 ml of 150 mmol/l pBQ in dimethylsulfoxide (DMSO), leading to a final pH value of 7.9 in 50 mmol/l pBQ, 4.7 mol/l DMSO, and 67 mmol/l phosphate. The reaction was allowed to proceed at 25 °C and terminated by double extraction with diethylether after diverse times. The substituted proteins were purified as mentioned above and compared with the native ones with regard to their solubility (A_{280} and A_{250} nm), spectrum (PMQ II Zeiss), molecular weight (gel filtration on Sephadex G-75 or G-200), and charge (paper electrophoresis 8 h with 7 V/cm in 50 mmol/l phosphate pH 6.5 and barbital pH 8.6 (equipment LKB 3276) and ion ex-

[1] Dedicated to Prof. J. Kühnau in commemoration of his 75th birthday.

[2] M. MORRISON, W. STEELE and D. J. DANNER, Arch. Biochem. Biophys. *134*, 515 (1969).

[3] K. HAIDER, L. R. FREDERICK and W. FLAIG, Pl. Soil *22*, 49 (1965).

[4] J. BRANDT, L.-O. ANDERSSON and J. PORATH, Biochim. Biophys. Acta *386*, 196 (1975).

[5] S. ÅKERFELDT, Ark. Kemi *7*, 75 (1954).

[6] I. S. JOFFE and Z. Ya. KHAVIN, Zh. Obsjch. Khim. *24*, 521 (1954).

[7] G. H. MOXON and M. A. SLIFKIN, J. Chem. Soc. Perkin II, *1972*, 1159.

[8] R. FOSTER, N. KULEVSKY and D. S. WANIGASEKERA, J. Chem. Soc. Perkin I, *1974*, 1318.

[9] K. LORENTZ, Z. analyt. Chem. *269*, 182 (1974).

[10] T. DÉVENYI und J. GERGELY, in *Analytische Methoden zur Untersuchung von Aminosäuren, Peptiden und Proteinen* (Akad. Verlagsgesellschaft, Frankfurt a. M. 1968).