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Attempt at Use of the Fluorometric Neuraminidase Assay System for the Enzyme Antibody Inhibition Test*)

Katsuhiro KIYOTANI[1], Naomi TAKEI[2] and Yoshiyasu MATSUO[1]

1) *Department of Bacteriology, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan*
2) *Hiroshima Prefectural Institute of Public Health, 1-5-70, Ujina Kanda, Minami-ku, Hiroshima 734, Japan*

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ABSTRACT

The fluorometric neuraminidase assay method using 4-methylumbelliferyl-N-Ac-α-D-neuraminide as a substrate was investigated for the purpose of ascertaining its validity as a procedure for influenza viral neuraminidase antibody inhibition test.

The enzyme antibody inhibition titer obtained by the fluorometric assay system was far weaker than that obtained by the standard colorimetric neuraminidase assay method using fetuin as a substrate. Although solubilization of viral neuraminidase spikes with Triton X-100 increased the enzyme inhibition of antiserum to some extent, there is little possibility of using the fluorometric assay system for the purpose.

Previously we reported the fluorometric assay method for neuraminidase activity of influenza viruses using 4-methylumbelliferyl (4-MU)-N-Ac-α-D-neuraminide as a substrate[5]. This method is more sensitive and simple, and less timeconsuming in performance than the colorimetric neuraminidase assay method[2] using fetuin as a substrate. The other assay method[1] using a synthetic substrate of low molecular weight such as sialyl lactose (MW=640) has been known to have similar advantages; however, the method can not be used for the viral neuraminidase activity inhibition test by the specific antibody against influenza virus[1,3]. At present, the colorimetric method is exclusively used as the standard one for the neuraminidase antibody inhibition test[2]. Since the viral neuraminidase activity assay is generally performed as the preceding procedure for neuraminidase inhibition test, the fluorometric neuraminidase assay method was investigated so as to inquire whether it is an effective procedure for the enzyme antibody inhibition test.

Influenza virus strains employed were A/Aichi/2/68 (H3N2), A/USSR/92/77 (H1N1) and B/Kanagawa/3/76 (B). Viruses were propagated in MDCK cells according to the method described by Tobita et al[8]. Each culture supernatant was centrifuged at $100,000 \times g$ for 60 min and the precipitate was suspended in PBS (−) at 1:20 volume of the starting mateial. These virus preparations were used as enzyme sources or immune antigens. Anti-influenza virus serum was prepared by three serial injections of each virus preparation into the marginal ear vein of rabbit at one-week-interval. The whole blood was collected 10 days after the final injection.

The neuraminidase antibody inhibition titer was determined according to the method de-

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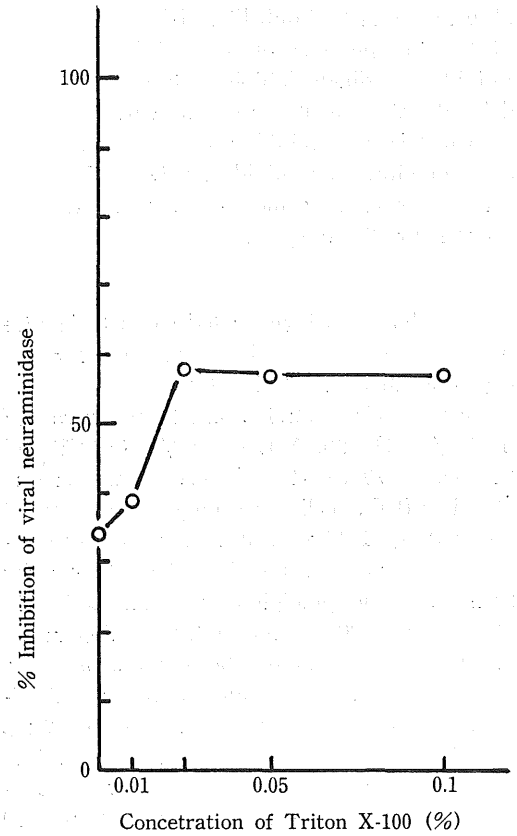
The Table Inhibition of influenza virus neuraminidase activity with specific antisera

Antiserum	Dilution	Neuraminidase inhibition (%)* against A/Aichi/2/68 (1)		A/Aichi/2/68 (2)		A/USSR/92/77 (1)		A/USSR/92/77 (2)		B/Kanagawa/3/76 (1)		B/Kanagawa/3/76 (2)	
A/Aichi/2/68	10^{-1}	95		37		94		39		2		1	
	10^{-2}	89	794**	30	<10	87	631	27	<10	0	<10	0	<10
	10^{-3}	45		10		41		13		2		1	
A/USSR/92/77	10^{-1}	92		25		91		18		5		1	
	10^{-2}	91	1,260	18	<10	89	3,900	12	<10	0	<10	1	<10
	10^{-3}	53		6		70		6		1		1	
B/Kanagawa/3/76	10^{-1}	0		0		2		3		94		20	
	10^{-2}	0	<10	2	<10	0	<10	0	<10	90	1,780	15	<10
	10^{-3}	0		3		9		8		65		10	

* Percent inhibition of viral neuraminidase was determined colorimetrically using fetuin (1) and fluorometrically using 4-methylumbelliferyl-N-Ac-α-D-neuraminide (2) as substrates.

** Neuraminidase inhibition antibody titer was expressed as the maximum dilution of antiserum showing 50% inhibition of the enzyme activity.



The Figure Influenza A/Aichi/2/68 virus preparation was incubated with 1:10 diluted specific antiserum in the presence or absence of Triton X-100 and the neuraminidase activity was assayed fluorometrically.

scribed by Aymard-Henry et al.[2] with some modification. In brief, after the incubation of 50 μl of the virus preparation with an equal volume of the antiserum at 37°C for 60 min in water bath, the remaining neuraminidase activity was assayed colorimetrically using fetuin (Type III, Sigma)[2] or fluorometrically using 4-MU-N-Ac-α-D-neuraminide (Koch Light Laboratories)[5] as substrates. Viral neuraminidase activities used were OD 0.5 at 549 nm in the colorimetric assay method and 1 nmole/ hr/ 50 μl of sample in the fluorometric assay one, respectively. Neuraminidase antibody inhibition titer was expressed as the maximum dilution of antiserum showing 50% inhibition of the enzyme. The results are shown in The Table.

Strong inhibition of each viral neuraminidase activity was observed with the respective antiserum in the colorimetric assay method. In the fluorometric assay, the inhibition was very weak even in homologous combination and the titer was below 1 : 10 in every combination. It has been considered that antibody inhibition of enzymatic activity occurs by a steric process and that antibody is not always directed to the active site, and moreover, the inhibition of neuramindase activity by antibody was found to be dependent upon the size of the substrate employed[3].

In order to combine more effectively the antibodies to viral neraminidase spikes, influ-

enza A/Aichi/2/68 virus preparation was incubated with 1:10 diluted specific immune serum in the presence or absence of Triton X-100 and the remaining neuraminidase activity was assayed fluorometrically. The results obtained are shown in The Figure. The percent enzyme inhibition with the antiserum increased from 34% (control) to 57% by the addition of 0.025% Triton X-100, possibly due to solubilization of aggregates of virion by the detergent. Thus, the neuraminidase antibody inhibition titer was calculated at 1:15.6 to 1:20 with the fluorometric assay method, but the values were still lower than those obtained with the colorimetric one.

The molecular weight of the substrate 4-MU-N-Ac-α-D-neuraminide is 556.52 and is much lower than that of fetuin (more than 44,000). The failure of the fluorometric assay in neuraminidase antibody inhibition test may be attributed to low molecular weight of the substrate as stated by Bucher and Palese[3].

There were strong cross reactions between the two influenza A viruses regarding neuraminidase antibody inhibition. Strong cross reactions were also observed between the two antisera in hemagglutination inhibition test even with RDE-KIO_4 treated antisera (data were not shown). There should not exist any essential cross antigenicities between the two viral strains in both hemagglutinin and neuraminidase. It has been known that antibodies to hemagglutinin can inhibit neuraminidase activity[6,9] and vice versa among some viral strains[4,7]. The observed cross reactions are presumably related to nonspecific inhibitors in the rabbit immune serum.

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