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EXPERIMENTS TO DETERMINE NEURAMINIDASE N2 THERMOSENSIBILITY OF SOME STANDARD AND LOCAL INFLUENZA VIRUS TYPE A STRAINS

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Numerous authors obtain various results from the investigations thermosensibility of neuraminidase from different influenza virus strains. Influenza virus A2/57 neuraminidase retains its enzymatic activity after heating at 58 °C but loses it at 60-62 °C while A2/65 - A2/69 starins lose their enzymatic activity already at 56-58 °C. Some starins with thermostable and thermolabile neuraminidase occur among strains isolated during the period from 1959 till 1963 [...,16-19].

This manifested correlation between neuraminidase thermosensibility in various influenza virus type A strains and their antigenis properties enables some authors to suggest first the close relationship between these parameters [7,8,12]. They use the influence of temperature factor on neuraminidase and its enzyme activity in the course of the comparative investigation of this activity of two influenza virus type A2 strains.

Frosner and Gert [22] assume that changes of neuraminidase thermosensibility can alter also the immunologic activity of the strains relating neuraminidase component thermosensibility with inhibitor sensibility, eluating activity, etc.

David-West and Beljavin [21] clarify to a great extent the problem of neuraminidase thermosensibility concerning various viruses concluding that the relation of neuraminidase of various viruses to thermic influence correlates with strain antigenic differences. This argues for the presence of a relation between the molecular architecture of regions with antigenic determinants sensible to this factor. Analogous results are reported by other investigators [8,25,26] who accept that antigenic neuraminidase determinants are closely related to its active centers.

In 1977, Polyakov [14] perfectioned to a considerable extent the method of identification of neuraminidase antigenic profile. He proved that TIN_{50} (temperature at which a 50-per cent enzyme inactivation sets in) presented one of the best informative parameters.

Fridman et al. [17] described the existing relationship between the changes of antigenic structure of virus neuraminidase and its thermosensibility. This relationship was confirmed by other authors, too [1,4,14].

It becomes evident that neuraminidase thermosensibility (in various variants) of influenza virus type A (H2N2) and A (H3N2) strains is insufficiently clarified yet.

The aim of the present work is to determine neuraminidase thermosensibility of both standard and isolated local influenza virus type A (H2N2) and A (H3N2) strains. We use the following approaches: a) Determination of N2 thermosensibility after the so-called "First methodical approach" (Follow-up of enzyme thermosensibility at 56 and 60 °C after heating at various time intervals); b) Determination of total inactivation of enzyme at different temperatures - "Second methodical approach" and determination of TIN_{50}.

MATERIALS AND METHODS

The following standard influenza virus strains were used in our study: A/Singapore /1/57/ (H2N2); A/Hong Kong /1/68 (H3N2); A/Victoria /35/72 (H3N2); A/Texas /1/77 (H3N2). The following local strains isolated in Bulgaria were also used: A/Sofia /1/57 (H2N2) and A/Sofia /142/69 (H3N2) - kindly provided by the Research Institute of Infectious and Parasitic Diseases,

Sofia, as well as A/Varna /123/76 (H3N2) and A/Varna /31/84 (H3N2) - isolated in the Department of Microbiology and Virology, Higher Institute of Medicine, Varna. All strains were of second sero type of neuraminidase.

Examined strains are selected with a view to the (N2) neuraminidase incorporated in them. They are etiological agents of influenza epidemics during a long period of time - from 1957 till 1984.

Virus-containing allantois cultures passed at least threefold by developing 10-day old chick embryos of an infectious titre of 6.5 - 7.5 lg $EID_{50/0.2cm^3}$ were used for indication of virus strains.

Enzyme activity was estimated after Aminoff's thiobarbituric method [20]. Free sialic acid was estimated by means of a thermoinactivation test. Infectious titre was determined by titration of virus-containing allantois fluid of chick embryos with subsequent reading of the infectious titre after Reed and Muench [24]. Ovomycine prepared after Gottschalk-Lind's method [13] was applied as substrate. Enzyme activity was expressed in mkg/mg protein determined after Lowry's method. Two spectrophotometers - "Specord UV-Vis" and "VSU-2p" were used at wave length of 549 nm to read the optical density. Results obtained were statistically processed by the least square method as well as by using of correlation and dispersion analyses [10,13,15].

RESULTS AND DISCUSSION

Our results obtained by using of the first, second, and third methodical approaches are demonstrated on fig. 1,2 and 3.

Data about neuraminidase thermosensibility obtained by using of the first methodical approach (fig. 1) show close temperature values for single examined strains. It is evident that virus enzyme loses almost completely its activity for 10-15 min except for neuraminidase of A/Singapore /1/57 (H2N2). Enzyme activity does not alter practically after a 5-min long heating at 56 °C. It decreases by 1.19 per cent after a 15-min long heating and by 3.46 per cent after a 20-min long one. There is no complete inactivation even after a 30-min long heating.

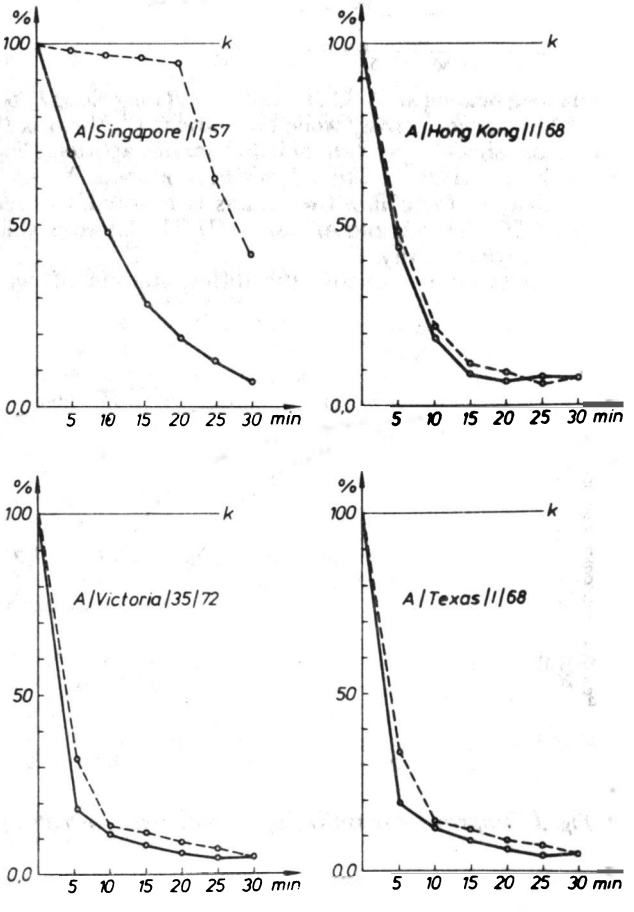

Fig. 1 Changes of enzyme activity in various time intervals for 4 influenza virus strains

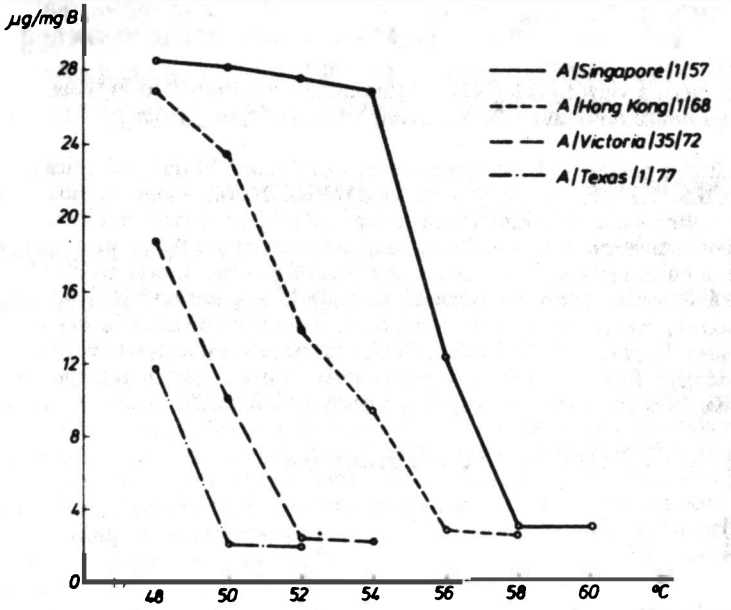

Fig. 2 Changes of the degree of neuraminidase activity of various influenza viruses (N2) at various temperatures - second approach (complete inactivation)

A 5-min long heating at 56 °C of standard A/Hong Kong/1/68 (H3N2) strain eliminates 50 per cent of initial enzyme activity while both strains (A/Victoria/35/72 (H3N2) and A/Texas/1/77 (H3N2) retain about 33 per cent of initial enzyme activity. The same tendency is observed when strains are heated at 60 °C: after a 5-min heating about 70 and 45 per cent, respectively, of initial enzyme activity of these first two strains is restored. Concerning the other two strains (i.e. A/Victoria/35/72 (H3N2) and A/Texas/1/77 (H3N2) we establish a restoration of about 19 - 19.5 per cent of enzyme activity.

These results do not enable the differentiation of eventually existing neuraminidase

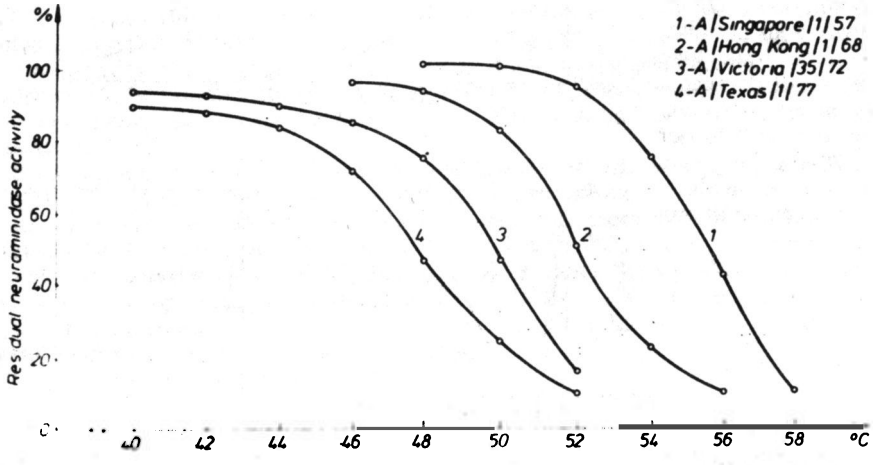

Fig. 3 Temperature sensitivity of neuraminidase activity of various influenza virus strains

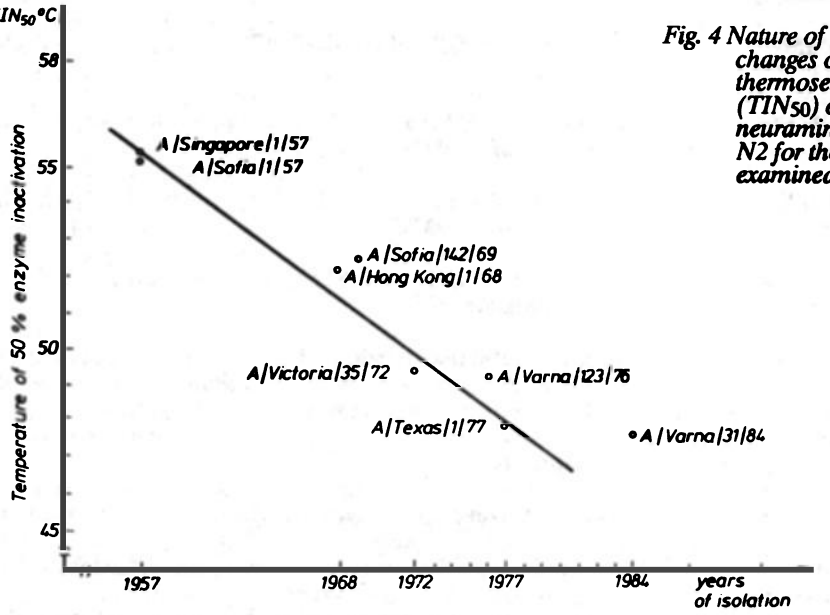

Fig. 4 Nature of the changes of thermosensibility (TIN_{50}) of neuraminidase type N2 for the strains examined

heterogeneity in these various strains examined by us. However, determination of virus neuraminidase thermosensibility by heating at various temperatures for a constant period of time, i.e. for 30 min in the course of the second methodical approach (fig. 2) allowed to establish that neuraminidase of A/Singapore /1/57 (H2N2) loses its initial activity at 58 °C; that of A/Hong Kong /1/68 (H3N2) - at 56 °C; that of A/Victoria /35/72 (H3N2) - at 52 °C, and that of A/Texas /1/77 (H3N2) at 50 °C.

Our results concerning the first three strains coincide completely with literature data available. We first perform investigations of the A/Texas /1/77 (H3N2) strain.

The third methodical approach which is a perfectioned second methodical approach [14] is much more informative concerning the identification of neuraminidase antigenic profile. On the basis of our data a fig. 3 is prepared reflecting the dependance of enzyme activity level on temperature of heating of samples. Experimental points are located as percentages. We judge of enzyme thermosensibility in corresponding strains from the character of enzyme activity changes in samples heated at different temperatures. Processing of empirical results reveals the dependence between the factor consequence and the factor argument which enables us to follow-up this dependence.

Equation of these curves has the appearance:

$y = a + b.Ln(x) + c.x$

For single influenza virus strains examined by us there are the following equations:

A/Singapore /1/57 (H2N2) - $y = -450.83 + 428.42*Ln(x) - 43.15*xTIN_{50} = 55.79 +/- 0.25$ °C; translation: 40 °C

A/Hong Kong /1/68 (H3N2) - $y = 34.75 + 103.54*Ln(x) - 20.07*xTIN_{50} = 52.10 +/- 0.10$ °C; translation: 40 °C

A/Victoria /35/72 (H3N2) - $y = 87.86 + 64.14*Ln(x) - 19.39*xTIN_{50} = 49.35 +/- 0.10$ °C; translation: 40 °C

A/Texas /1/77 (H3N2) - $y = 116.97 + 2.65*Ln(x) - 9.56*xTIN_{50} = 47.56 +/- 0.10$ °C; translation: 40 °C

A/Sofia /1/57 - $y = -319.255 + 333.08*Ln(x) - 35.12*x$ $TIN_{50} = 55.45 +/- 0.13$ °C; translation: 40 °C

A/Sofia /142/68 - $y = -40.96 + 158.38*Ln(x) - 25.27*x$ $TIN_{50} = 51.95 +/- 0.34$ °C; translation: 40 °C

A/Varna /123/76 - $y = 62.42 + 97.26\ 4*Ln(x) - 24.78*x$ $TIN_{50} = 49.22 +/- 0.12$ °C; translation: 40 °C

A/Varna /31/84 - $y = 101.59 + 46.19*Ln(x) - 19.91*x$ $TIN_{50} = 47.16 +/- 0.2$ °C; translation: 40 °C

Our results argue for a complete and definite dependence between virus strain enzyme activity and heating temperature. Graphic interpretation of these S-like data demonstrate that temperature inactivation is based on co-operative process of protein denaturation.

Differences in TIN_{50} values for these strains are statistically significant ($p < 0.05$) which indicates N2 neuraminidase heterogeneity.

This is the most informative method. It enables a much better comparison of data obtained. Further studies show that TIN_{50} reflects completely thermosensibility of this enzyme. It ascribes an analytical appearance of the curves and thus enables a much more precise TIN_{50} estimation. It proves the correlation between factor-argument and factor-consequence. Our results indicate that the degree of neuraminidase activity level reduction in the samples heated at different temperatures depends on the initial enzyme activity. However, TIN_{50} does not depend on this initial activity. That is why in our further investigations of neuraminidase thermosensibility we can judge from the temperature of enzyme activity reduction by 50 per cent (TIN_{50}).

We can draw the conclusion that N2 neuraminidase varies significantly among different influenza virus types in relation to sensibility towards thermic influence. Our data coincide with these reported by other investigators [1,5,6,9,11,14]. As indicated on fig. 4, TIN_{50} dependence towards the years of circulation of corresponding influenza virus strains is practically of linear nature, i.e. TIN_{50} continuously decreases, or strains become more thermolabile. It can be supposed that probably a new variant of second sero type neuraminidase appears.

CONCLUSIONS

1. Thermostability of neuraminidase of some standard and local virus strains type A (H2N2) and type A (H3N2) which neuraminidase is of type N2 demonstrates significant differences when estimated after two different methodical approaches.

2. Determinations of N2 antigenic profile of standard and local strains by TIN_{50} assessment show statistically reliable differences in enzyme thermosensibility. It seems probable that this parameters can be applied as genetic marker g N2 antigenic differences.

3. These differences in N2 thermosensibility of the examined influenza virus strains allow the presumption of a probable heterogeneity of this antigenic and enzymatic component of influenza viruses.

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ЭКСПЕРИМЕНТЫ ДЛЯ ОПРЕДЕЛЕНИЯ ТЕРМОЧУВСТВИТЕЛЬНОСТИ НЕВРАМИНИДАЗЫ N2 ПРИ НЕКОТОРЫХ ЭТАЛОННЫХ И МЕСТНЫХ ШТАММАХ ВИРУСОВ ГРИППА ТИПА А

М. Чивиджиян, Г. Капрелян, В. Русев, М. Хайг

РЕЗЮМЕ

При исследовании были использованы эталонные штаммы вирусов гриппа типа А со вторым серотипом невраминидазы : А/Сингапур/1/57 /H2N2/; А/Хохг Конг/1/68 /H3N2/; А/Виктория/35/72 /H3N2/; А/техсас/1/77 /H3N2/ и некоторые штаммы вирусов гриппа, изолированные у нас в стране: А/София/1/142/69 /H3N2/; А/София/1/57 /H12N2/; А/Варна/123/76 /H3N2/ и А/Варна/31/84 /H3N2/.

Отбор исследованных штаммов проводился с учетом включенной в них невраминидазы N2.

Исследования проводились по трем методическим подходам, что позволило сделать следующие выводы:

Результаты экспериментальных исследований для определения антигенного профиля N2 при эталонных и местных штаммах посредством определения ТИН50 показали достоверно выраженные различия в термочувствительности энзима. По всей вероятности эти показатели могли бы послужить генетическим маркером, характеризующим антигенные различия N2. Различия в термочувствительности N2 исследованых штаммов вирусов гриппа позволяют предполагать вероятную гетерогенность этого антигенного и энзимного компонента вирусов гриппа.