Acta virol. **13** : 26—33, 1969

PLAQUE PRODUCTION BY GROUP A ARBOVIRUSES. II. ENHANCING EFFECT OF DEAE-DEXTRAN ON PLAQUE NUMBERS IN CHICK EMBRYO CELLS

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Received July 5, 1968

Summary. — Diethylaminoethyl- (DEAE-) dextran added either to the virus inoculum or to chick embryo cell (CEC) monolayers prior to infection doubled or trebled the number of plaques produced by Sindbis (large plaque type) virus. The mode of action of DEAE-dextran is on the cell surface influencing the adsorption phase of the infectious cycle. A similar enhancing effect was also found for Middelburg-l and Getah viruses. An opposite effect was observed with the small plaque producing strains of Midelburg Sindbis and Western equine encephalomyelitis (WEE) viruses.

Introduction

In a previous paper (Pattyn and de Vleesschauwer, 1967) we mentioned that DEAE-dextran influenced the number of plaques produced by Getah virus and the large plaque variant of Sindbis virus (Si-l). We now present the results of our observations on the latter system showing that DEAE-dextran acts on the attachment phase of the infecting cycle.

Materials and Methods

These were previously described (Pattyn and de Vleeschauwer, 1967).

Results

If DEAE-dextran is added to a Si-l virus inoculum during the adsorption period, the number of plaques may be doubled or trebled as compared with an inoculum not containing the additive. That this effect is not due to some action of the DEAE-dextran on the virus was shown by the following observation:

Virus suspensions were mixed with DEAE-dextran to a final concentration of 500 μg/ml; controls were mixed with the same volume of buffered-gelatin-tris (BGT). After incubation for several time lapses at room temperature and at 37° C, these mixtures were diluted 1 : 100 or 1 : 1000 and 1 ml amounts inoculated in CEC monolayers. Thus the virus was in contact with identical amounts of DEAE-dextran, but adsorption took place in the presence of 5 or 0.5 μg DEAE-dextran/ml. Virus amounts had been chosen in such a way as to produce countable numbers of plaques after dilution.

The results showed that a higher number of plaques was produced only when 5 μg DEAE-dextran was present during the adsorption period. This

Table 1. Dose relationship of plaque enhancing effect of DEAE-dextran on Si-1 virus under agar, agarose and carboxymethyl-cellulose overlays

µg/ml DEAE-dextran in inoculum	Number of plaques produced under		
	Agar overlay	Agarose overlay	CMC overlay
0	11	12	12
5	25	24	20
25	32	37	35
125	37	28	33
250	33	27	28

The data are mean numbers of plaques counted on 3 Petri dishes.

effect was absent when only 0.5 µg were present. Incubation of the virus with DEAE-dextran before inoculation had no influence on plaque numbers, nor did it protect the virus against heat inactivation.

The effect of increasing amounts of DEAE-dextran added to the virus inoculum can be seen in the first two columns of Table 1. Maximum effect was reached at a concentration of 25 µg DEAE-dextran, while at the higher

Table 2. Influence of pretreatment of CEC monolayers for 4 hours before inoculation with different amounts of DEAE-dextran, protamine sulfate or heparin

Pretreatment	Plaque numbers
Controls	18
DEAE-dextran 5 µg/ml	42
25 µg/ml	97
125 µg/ml	70
Protamin sulfate 5 µg/ml	43
25 µg/ml	44
125 µg/ml	73
Heparin 40 µg/ml	22
200 µg/ml	20
1000 µg/ml	20

All monolayers rinsed twice with BGT before inoculation. Mean numbers of plaques counted on 3 Petri dishes.

concentrations the number of plaques levelled off again. The enhancing effect could also be observed if the cells were pretreated with DEAE-dextran for 4 hours before inoculation and rinsed twice with BGT before addition of the virus inoculum (in BGT without dextran). The upper part of Table 2 shows the enhancing effect to be more pronounced if the monolayer was pretreated with DEAE-dextran at 25 µg/ml than at 5 µg/ml. At 125 µg/ml the effect levelled off again. Table 3 shows that combination of the two meth-

Table 3. Effect of DEAE-dextran in inoculum on normal cells and cells pretreated with DEAE-dextran

Pretreatment of cells	Treatment before inoculation	Number of plaques*	
		Control inoculum	Inoculum with 25 µg/ml DEAE-dextran
None	No rinse	19	69
	Rinse	15	71
DEAE-dextran 25 µg/ml	No rinse	35	71
	Rinse	28	68

* Means of 3 Petri dishes.

ods, pretreatment of the monolayers and addition of DEAE-dextran to the virus inoculum, was not additive.

The time relationship of pretreatment was investigated in experiment summarized in Table 4. It appears that the action of DEAE-dextran was very rapid since the effect was manifest if cells were treated for only 5 minutes

Table 4. Effect of DEAE-dextran (25 µg/ml) pretreatment of CEC monolayers for different time intervals

Kind of treatment	Numbers of plaques* produced
Controls	44
Treatment for 24 hours	106
Treatment for 1 hr at −24 hr before inoculation[1])	42
Treatment for 4 hr at −24 hr before inoculation[1])	65
Treatment for 4 hr before inoculation	118
Treatment for 1 hr at −4 hr before inoculation[2])	74
Treatment for 30 min before inoculation	107
Treatment for 5 min before inoculation	116

* Means of 3 Petri dishes.

[1]) DEAE-dextran added at 24 hr before inoculation to CEC cultures which were rinsed respectively 1 and 3 hr later and covered with normal growth medium until time of inoculation.

[2]) DEAE-dextran added at 4 hr before inoculation to CEC cultures which were rinsed 1 hr later and covered with normal growth medium until time of inoculation.

All Petri dishes rinsed twice before inoculation.

before inoculation. Treatment for longer periods did not result in higher plaque numbers. Furthermore, the cells could revert more or less to their original level of sensitivity when, after treatment with DEAE-dextran for 1 to 4 hours, they were kept without DEAE-dextran for 20—24 hours.

Table 5. Plaque numbers of Si-1 virus obtained after addition of DEAE-dextran to the agar overlay

Kind of culture	Plaque numbers*
Controls without DEAE-dextran	25
25 µg/ml DEAE-dextran in inoculum	70
5 µg/ml DEAE-dextran in agar overlay	25
25 µg/ml DEAE-dextran in agar overlay	35
125 µg/ml DEAE-dextran in agar overlay	40

* Means of 3 Petri dishes.

The following findings offered evidence that the plaque enhancing effect of DEAE-dextran was not due to interaction of DEAE-dextran with the agar overlay:

a) the effect, as shown in Table 1, was also observed when agarose or carboxymethyl-cellulose overlays were applied instead of agar;

b) although the effect was also observed if DEAE-dextran was added to the agar overlay (Table 5), it was of a lesser degree.

The influence of DEAE-dextran on the velocity of adsorption was determined through addition of the agar overlay after 15, 30, 60, 90 minutes of contact of a) virus inoculum in BGT with normal monolayers (control);

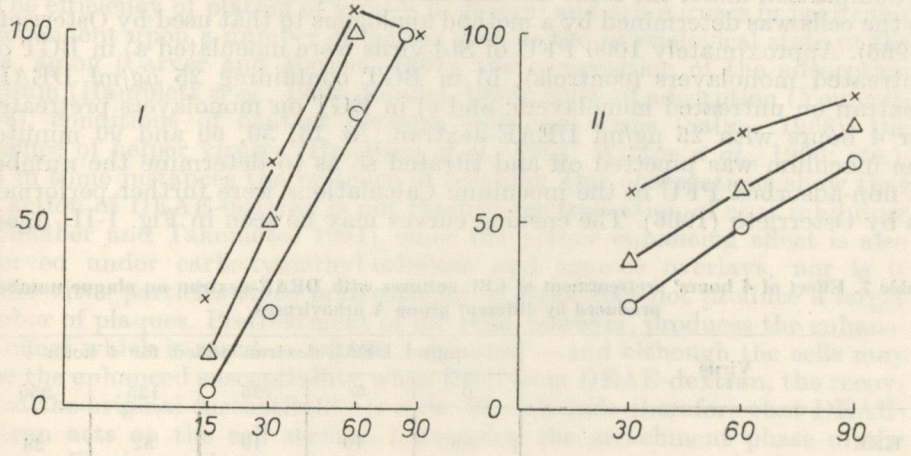

Fig. 1.

Influence of DEAE-dextran on the velocity of adsorption of Sindbis (Si-1) virus

I — Abscissa: square root of time (in minutes); ordinate: number of plaques as percentage of the number of plaques at 90 minutes.

II — Abscissa: time in minutes; ordinate: per cent virus adsorbed.

○ Controls.

△ Pretreatment for 4 hours with 25 µg/ml DEAE-dextran.

× 25 µg/ml DEAE-dextran in inoculum.

b) virus inoculum in BGT with monolayers pretreated for 4 hours with 25 μg DEAE-dextran per ml and rinsed twice before inoculation; and c) virus inoculum containing 25 μg/ml DEAE-dextran with normal monolayers. In each instance (Fig. 1-I) adsorption was proportional to the square root of time but from the start more virus was adsorbed when DEAE-dextran

Table 6. Influence on plaque numbers in CEC of the addition of DEAE-dextran in different concentrations to the virus inoculum of several group A arboviruses

Virus strains	Concentration of DEAE-dextran in virus inoculum (μg/ml)					
	0	5	25	125	200	300
EEE	68	55	50	52	23	21
Getah	12	17	30	21	20	20
Middelburg-l	62	88	95	95	63	68
Middelburg-s	44	29	27	31	35	50
Sindbis-s	73	71	70	79	64	50
Semliki Forest	46	42	27	12	7	6
WEE-l	62	75	57	70	81	90
WEE-s	120	54	30	44	0	32

had been used and this figure was higher when DEAE-dextran had been added in the inoculum, as compared with the 4 hours' pretreatment of the cells.

Comparison under the same circumstances of the amount of virus attached to the cells was determined by a method analogous to that used by Osterrieth (1966). Approximately 1000 PFU of Si-l virus were inoculated a) in BGT on untreated monolayers (controls); b) in BGT containing 25 μg/ml DEAE-dextran on untreated monolayers; and c) in BGT on monolayers pretreated for 4 hours with 25 μg/ml DEAE-dextran. At 15, 30, 60 and 90 minutes the inoculum was pipetted off and titrated so as to determine the number of non-adsorbed PFU in the inoculum. Calculations were further performed as by Osterrieth (1966). The ensuing curves may be seen in Fig. 1-II. Again

Table 7. Effect of 4 hours' pretreatment of CEC cultures with DEAE-dextran on plaque numbers produced by different group A arboviruses

Virus	μg/ml DEAE-dextran added for 4 hours				
	0	5	25	125	250
EEE	88	49	19	32	28
Middelburg-l	57	73	50	56	45
Middelburg-s	33	39	18	16	34
Sindbis-s	220	185	86	67	91
Semliki Forest	33	33	27	15	13
WEE-s	124	108	40	38	56
WEE-l	53	45	43	NT	NT

NT = not tested.

the curves are parallel, the shapes being identical, but the amount of virus adsorbed when DEAE-dextran was mixed with the inoculum was higher from the start of the experiment.

Experiments aimed to show an enhanced interferon production or sensitivity under the influence of DEAE-dextran failed to show such an effect.

As shown in Table 2, pretreatment of CEC monolayers with protamine sulfate also enhanced plaque numbers produced by Sindbis-l virus. In contrast, heparin remained without effect.

The enhancing effect of DEAE-dextran on plaque production by group A arboviruses reported previously (Pattyn and De Vleesschauwer, 1967) was studied further by adding graded amounts of DEAE-dextran to the inoculum and by pretreating monolayers for 4 hours with graded amounts of DEAE-dextran prior to inoculation. Table 6 shows that DEAE-dextran had an enhancing effect on plaque numbers produced by Getah virus (optimal at 25 μg/ml) and by Middelburg-l plaque type virus (at 25—125 μg/ml). With the small plaque type strains of MB and WEE viruses, DEAE-dextran inhibited plaque production; this was the case for most other viruses tested when the dextran concentration was rather high. Pretreatment of monolayers for 4 hours with different amounts of DEAE-dextran had a comparable effect, Si-s also being inhibited in this case (Table 7). MB-l virus plaque production was enhanced, but only at 5 μg/ml DEAE-dextran.

Discussion

The efficiency of plating of viruses in general and arboviruses in particular is dependent upon a number of factors such as the physiological state of the cells, aging (Carver and Marcus, 1967), the composition of the adsorption medium (Hamblett *et al.*, 1967; Mandel, 1958), time of adsorption (Younger, 1956), conditions of contact between cells and virus (Salim, 1968), the presence of helper viruses (Hanafusa *et al.*, 1963; Wiktor *et al.*, 1966; etc.), and in some instances the presence of polyions. Our observations show that the action of DEAE-dextran is not through neutralisation of agar inhibitors (Liebhaber and Takemoto, 1961), since the plaque enhancing effect is also observed under carboxymethyl-cellulose and agarose overlays, nor is it on the virus particles since pretreatment of virus does not produce a larger number of plaques. Pretreatment of the cells, however, produces the enhancing effect which is rapid — within 5 minutes — and although the cells may loose the enhanced susceptibility when freed from DEAE-dextran, the recovery of the original susceptibility is slow. We conclude therefore that DEAE-dextran acts on the cell surface, influencing the attachment phase of the virus replicative cycle.

The fact, however, that only Sindbis-l, Getah and Middelburg-l viruses were enhanced, other viruses unaffected and some even inhibited implies that some factor on the virus particle surface — in terms of attachment — is different from one virus strain to another. Allison and Valentine (1960) were the first to study the enhancing effect of poly-cations, including protamine, on the adsorption of fowl plaque virus on cell monolayers. They

explained the effect through action of the ions on the electrostatic forces of the cell surface.

The enhancing effect of DEAE-dextran on virus infectivity was first described by Pagano and Vaheri (1965) and Pagano *et al.* (1967) for both intact poliovirus and RNA prepared from it. These authors found "that the effect leading to the increased efficiency of plating with mature virus was apparently exerted early in the post adsorption period". In the case of poliovirus RNA the mechanisms is complex but at least partially due to action of DEAE-dextran on the cell surface. Arnos and Kearns (1963) also found that protamine acts on the cell membranes either electrostatically or through alteration in sensitivity. DEAE-dextran was later found to enhance infectivity of rubella (Vaheri *et al.*, 1967) and rabies viruses (Kaplan *et al.*, 1967) on BHK21 cells; in the latter case also protamine sulfate had a similar effect. These authors, not excluding a direct action of DEAE-dextran on the virus particle, also conclude that the action of the enhancing effect of DEAE-dextran must occur during the early phase of infection: either the adsorption or the penetration phase.

Plaque production by arboviruses then may be dependent upon the removal of inhibitors from the overlaying agar through addition of optimal amounts of DEAE-dextran to the agar (Pattyn and De Vleesschauwer, 1967; Miles and Austin, 1963); the use of other types of overlay such as agarose, methylcellulose or carboxymethylcellulose; the addition of DEAE-dextran to the virus inoculum; the addition of cortisol in the overlay medium (Bergold and Mazzali, 1968); or the technique of infection of the cells (Austin, 1963; Salim, 1968).

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