Salt-Dependent Hemagglutination with *Bunyaviridae* Antigens

BARRY J. BEATY, ROBERT E. SHOPE,* AND DELPHINE H. CLARKE

Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, Connecticut 06510

Received for publication 6 January 1977

Hemagglutination titers of antigens from nine serologic groups of *Bunyaviridae* were increased by hypertonic NaCl. The effect was predominantly on sonically treated antigens and was additive with the increase from sonic treatment alone.

Enhanced hemagglutination (HA) with increased salt concentration in diluents has been demonstrated with measles and rabies viruses (4, 5, 7, 8). Recently, enhanced HA was reported for Akabane and Aino viruses of the Simbu group of the *Bunyaviridae* (6).

Unlike antigens of the *Togaviridae*, which commonly have high HA titers after acetone extraction of infected mouse brain, many *Bunyaviridae* antigens are poor hemagglutinators. Satisfactory HA antigens are often obtained only after additional manipulation, such as sonication or special culture techniques (1, 2). The classic methods of Clarke and Casals (3) for HA and hemagglutination-inhibition (HI) specify a 0.15 M NaCl viral adjusting diluent. After an initial experiment in which we confirmed the report of Inaba (6) with a Simbu group antigen, we hypothesized that many *Bunyaviridae* antigens with low or nondetectable HA titers would be satisfactory antigens when used with higher salt concentrations.

Representative lyophilized antigens from 10 serologic groups of the *Bunyaviridae* and two ungrouped bunyaviruses were obtained as sucrose-acetone extracted mouse brain from the World Health Organization reference center of the Yale Arbovirus Research Unit. Some of these antigens had been sonically treated. Antigens were reconstituted in water and allowed to dissociate 1 h before serial twofold dilutions were prepared in bovine albumin borate saline (pH 9). Two La Crosse virus tissue culture antigens were Tween-80-ether treated by the method of Chappell et al. (2).

HA and HI were carried out according to Clarke and Casals (3) using goose erythrocytes suspended in phosphate buffers which were adjusted to final NaCl molarities of 0.15, 0.25, and 0.4. Titrations were in microtiter plates with antigens diluted either in tubes or by loops. The two dilution techniques yielded

comparable results. Antigens were used at final pH values of 5.75, 6.0, 6.2, and 6.4, except for Congo antigens, which were used at pH 6.8, 7.0, 7.2, and 7.4. Four HA units were employed in HI tests. No obvious differences in sedimentation time and pattern of goose erythrocytes were noted.

Antibody consisting of reference sera or ascitic fluids was from mice immunized with from two to four intraperitoneal injections of infected mouse brain. Antibody preparations were acetone extracted and adsorbed with goose cells before use.

Table 1 shows the effect of increased NaCl concentration on 29 antigens representative of 10 different serologic groups of the *Bunyaviridae* and two ungrouped bunyaviruses. Enhancement of HA titer was typically greatest at pH 5.75. Nine antigens had ≥ 4 -fold increases in HA titer in the hypertonic saline. Four of these (Anopheles A, Bwamba, La Crosse, and Oropouche) had no demonstrable HA at 0.15 M NaCl, but were high titered at higher NaCl molarities.

Table 2 demonstrates the specificity of the enhanced agglutinins by HI for representative antigens. In all cases, the agglutination was inhibited by homologous antiserum, and, in cases of cross-reaction, the homologous antiserum inhibited to higher titer than did the heterologous antisera. Two La Crosse tissue culture antigens had titers of 1:2,048 at 0.4 M NaCl, but specificity could not be proven by the HI test. The source of these nonspecific salt-dependent hemagglutinins has not been determined.

Twenty-two of the 29 antigens had at least twofold enhancement of HA titer with hypertonic saline. Nine of these were ≥ 4 -fold increases. Sonically treated antigens accounted for 89% of those with ≥ 4 -fold increases, whereas none of the seven antigens without a demonstrable HA enhancement had been soni-

cally treated.

Table 3 shows the cumulative effect of increased NaCl and sonic treatment on the same antigen. There was an additive effect of the two treatments, resulting in increased HA titer. The

TABLE 1. *Effects of increased NaCl molarity on HA titers of 29 Bunyaviridae antigens*

Antigen	Sonically treated	Reciprocal of HA titer at NaCl molarity of:		
		0.15	0.25	0.4
Anopheles A	Yes	<4 ^a	128	256
Apeu	No	<4	<4	<4
Bunyamwera	No	<4	8	8
Buttonwillow	Yes	16	32	64
Bwamba I ^b	Yes	<4	64	128
Bwamba II ^b	No	<2	<2	<2
Bwamba II	Yes	<2	<2	2
California	Yes	2	<2	16
Capim	Yes	8	16	16
Congo I ^b	Yes	64	32	128
Congo II ^b	Yes	<2	<2	4
Guajara	Yes	16	64	64
Itaqui	Yes	64	64	128
Jurona	No	<2	<2	<2
Keystone	No	<4	<4	<4
La Crosse I	No	<2	<2	2
La Crosse I	Yes	<2	4	32
Manzanilla	No	<2	<2	<2
Oropouche	Yes	<4	64	128
Sicilian I ^b	No	<4	8	8
Sicilian II ^b	No	32	64	512
Sicilian II	Yes	64	512	≥1,024
Snowshoe hare	No	4	8	8
Tahyna I ^b	Yes	4	8	8
Tahyna II ^b	No	16	32	32
Tahyna II	Yes	256	128	512
Turlock	No	4	4	2
Trivittatus	No	2	<2	<2
Witwatersrand	No	16	16	32

^a Viral adjusting diluents were pH 5.75, 6.0, 6.2, and 6.4 for all antigens except Congo, with which diluents of pH 6.8, 7.0, 7.2, and 7.4 were used. Optimal HA pH is not listed, but enhancement was typically greatest at 5.75.

^b Different antigen preparations.

La Crosse mouse brain antigen was unusable at 0.15 M NaCl, but titered 1:32 at 0.4 M NaCl after sonic treatment. The Sicilian and Tahyna antigens were functional at 0.15 M NaCl without sonic treatment, but the combination of techniques resulted in much higher HA titers.

The mechanism by which high salt concentration increases *Bunyaviridae* HA titer is not yet known. That some *Bunyaviridae* antigens in our experiments did not increase in titer at 0.4 M NaCl does not mean lack of capability of enhancement. Optimum conditions of antigen preparation, virus substrate, molarity, pH, and erythrocyte type have not yet been established

TABLE 3. *Effects of sonic treatment and increased NaCl molarity on HA titers of three Bunyaviridae antigens*

Antigen	Sonically treated	NaCl molarity	HA titer (reciprocal)
La Crosse	No	0.15	0
	No	0.25	0
	No	0.4	2
	Yes	0.15	0
	Yes	0.25	4
Sicilian	Yes	0.4	32
	No	0.15	32
	No	0.25	64
	No	0.4	512
	Yes	0.15	64
Tahyna	Yes	0.25	512
	Yes	0.4	≥1,024
	No	0.15	16
	No	0.25	32
	No	0.4	32
	Yes	0.15	256
	Yes	0.25	128
	Yes	0.4	512

TABLE 2. *Specific inhibition of enhanced hemagglutination by homologous antibodies*

Antigen ^a	NaCl molarity	Antiserum				
		Homologous	California (BFS-283)	Pongola	Lukuni	Normal
Anopheles A	0.25	320 ^b	0	0	0	0
Anopheles A	0.4	320	0	0	0	0
Bwamba	0.25	160	0	0	0	0
Bwamba	0.4	320	0	0	0	0
Guajara	0.4	80	0	0	0	0
La Crosse	0.4	40	0	0	ND	ND
Sicilian	0.15	80	0	0	ND	ND
Sicilian	0.4	80	0	0	ND	ND
Tahyna	0.15	80	20	0	ND	ND
Tahyna	0.4	160	40	0	ND	ND

^a All antigens were sonically treated.

^b Reciprocal of highest serum dilution inhibition agglutination by 4 U of antigen. 0, No inhibition at 1:10 serum dilutions. ND, Not done.

and may not have been achieved in these experiments. The results do, however, indicate the broad occurrence of the salt-dependent hemagglutinin among *Bunyaviridae* and the wide applicability of this simple enhancing technique in diagnostic virology.

The technical expertise of Robert W. Kowalski is gratefully acknowledged.

This research was supported by the United States Army Medical Research and Development Command DADA-17-72-C2170; by Public Health Service grant R01-AI 10984 from the National Institute of Allergy and Infectious Diseases and grant T32-NS 07036 from the National Institute of Neurological Diseases and Stroke; and by the World Health Organization.

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