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SEROTYPES OF RED CLOVER NECROTIC MOSAIC VIRUS. III. IMMUNOELECTROPHORESIS UNDER DIFFERENT CONDITIONS

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Summary. — The effects of 4 buffers at different pH and of different molarities was tested on the immunoelectrophoresis of representatives of three serotypes and their natural mixtures of red clover necrotic mosaic virus (RCNMV). No substantial differences were observed with buffers at pH 7.2, 8.0 and 8.6. The representatives of the three RCNMV serotypes showed different mobilities in 0.1 and 0.01 mol/l ionic buffers in the agarose gel from cathode to anode. In nonionic 0.01—0.1 mol/l N-2-hydroxyethylpiperazine-N′-ethanesulphonic acid (HEPES) buffer, the RCNMV isolates moved from the anode to the cathode. Isolate TpM48 (serotype B) moved the most rapidly, while isolates No. 6 (serotype C) and TpM34 (serotype A) showed lower mobilities distinct from one another. This made possible a reliable differentiation of serotype B from serotypes A and C as well as of serotype A from serotype C. Agarose gels in 0.001 mol/l buffers proved to be unsuitable for the immunoelectrophoresis of RCNMV.

Key words: red clover necrotic mosaic virus; serotypes; immunoelectrophoresis

Introduction

Studies on serological differences between serotypes of red clover necrotic mosaic virus (RCNMV) revealed certain differences in their electrophoretic mobilities also in dependence of the type of buffer used for immunoelectrophoresis (Hollings and Stone, 1977; Musil and Gallo, 1982; Musil *et al.*, 1982). In the present experiments we investigated the effects of pH and molarity of buffers used in immunoelectrophoresis on the mobility of RCNMV serc. types. The results suggesting the possibility of mutual differentiation of the serotypes are presented below.

Materials and Methods

The following RCNMV isolates were used: Tp34 (serotype A); TpM48 (serotype B); No. 6 (Anín; serotype C); No. 11 (Horní Lhota; mixture of serotypes A + C); No. 29 (Krušetnica;

mixture of serotypes A + B); and No. 3 (Kostelec; mixture of serotypes A + C) — see Musil *et al.*, 1982. The virus isolates were propagated in bean plants and used in the form of purified suspensions (Musil and Gallo, 1982) for immunoelectrophoresis.

Immunoelectrophoresis. One per cent agarose gels prepared as described (Musil and Gallo, 1982) were buffered with 0.1, 0.01 and 0.001 mol/l phosphate at pH 7.2 and 8.0; 0.1, 0.01 and 0.001 mol/l Tris-HCl at pH 7.2 and 8.6; 0.1 0.01 and 0.001 mol/l sodium diethyl barbiturate-HCl at pH 7.2 and 8.6; and 0.1, 0.05, 0.01 and 0.001 mol/l N-2-dihydroxyethylpiperazine-N′-ethanesulphonic acid (HEPES) at pH 7.2. The vessel buffer always was the same as used for buffering of the gel. Electrophoresis lasted for 18 hr. A constant current (4 mA per gel) was used with gels prepared in 0.1 mol/l gels; no drying of the gels or their deformation occured during electrophoresis under these conditions, as distinct from buffers of lower molarities. To prevent such effects, with gel prepared in buffers of molarities lower than 0.1 mol/l we used rather a constant voltage (25 V per gel). Under the latter conditions the current intensity decreased, e.g. with gel in 0.01 mol/l HEPES buffer at pH 7.2 to 1 mA per gel and with 0.001 mol/l HEPES buffer at pH 7.2 to 0.5 mA per gel. Similar differences in current intensities occured also with 0.01 and 0.001 mol/l ionic buffers. After the end of electrophoresis the longitudinal throughs were filled with antiserum TpM50 diluted 1 : 5 (Musil, 1969) containing antibodes to serotypes A, B and C. The gels were stained and preserved as described (Musil and Gallo, 1982).

Results

In agarose gels prepared in 0.1 mol/l ionic buffers we found the following differences in the electrophoretic mobilities of the RCNMV isolates tested:

a) Agarose gels prepared in phosphate buffer at pH 7.2 and 8.0. All isolates moved to the anode. The highest mobility was shown by isolate TpM34 (serotype A), followed by isolate No. 6 (serotype C), while isolate TpM48 (serotype B) moved the most slowly (part of the purified virus suspension diffused around the well). RCNMV isolates representing mixtures of 2 serotypes showed the same mobilities. These isolates separated on immunoelectrophoresis into two components. One of them migrated like a serotype A isolate while the other component reached a distance corresponding to that of a serotype C isolate (isolate No. 11), or one component remained located around the well like a serotype B isolate and the other migrated to a distance corresponding to that reached by serotype A or C isolates (cf. isolates Nos. 29 and 3) (Fig. 1).

b) Agarose gels prepared in Tris-HCl buffers at pH 7.2 and 8.6. Isolate TpM34 showed the highest electrophoresis mobility. Isolates TpM48 and No. 6 moved much more slowly, diffusing around the well. Isolates representing mixtures of the individual serotypes separated in this environment according to the serotype components involved, i.e. serotype component A migrated from components B and C to the anode.

c) Agarose gels prepared in sodium diethylbarbiturate-HCl buffers. At pH 7.2, the mobility of all three isolates (TpM34, TpM48, No. 6) was low. Isolate TpM34 moved to the anode, TpM48 diffused around the well and No. 6 showed a tendency to migrate to the cathode. In this environment there resulted no clear-cut separation of isolates representing natural mixtures of the serotypes. At pH 8.6, isolates TpM34 and No. 6 showed the same mobility towards the anode, while isclate TpM48 remained located around the well. In this way a differentiation of isolates representing mixtures of

serotypes B + C and B + A was possible. No separation occurred with the mixture of serotypes A + C (Fig. 2).

In agarose gels prepared in 0.01 mol/l ionic buffers the mobility to the anode of all isolates was lower, but the possibility to separate the isolates according to the serotypes was preserved (Fig. 1). In agarose gels prepared in 0.001 mol/l ionic buffers all isolates showed a marked mobility to the cathode of about the same rate so that isolates representing mixtures of serotypes were not separated into the individual components (Fig. 1).

In 0.1 mol/l nonionic HEPES buffer at pH 7.2 isolate TpM34 moved to the cathode much more slowly than isolates TpM48 and No. 6, the migration rate of TpM48 having been much higher than that of No. 6. Due to the different migration rates of RCNMV serotypes, isolates representing serotype mixtures were clearly separated into the individual serotype components. These components reached the same distance as the type isolates of the corresponding serotypes (Fig. 3).

In gels prepared in 0.05 and 0.01 mol/l HEPES buffers the RCNMV isolates (especially TpM48 and No. 6) moved more rapidly to the cathode than in 0.1 mol/l buffer. The migration rate increased with decreasing molarity of the buffer. Gels prepared in 0.001 ml/l HEPES buffer proved to be unsuitable for immunoelectrophoresis of RCNMV isolates tested with the exception of TpM34. In this environment the migration of the isolates to the cathode was diffuse which had an adverse effect on immunoprecipitation and thus on the differentiation of the isolates according to their serotypes (Fig. 3).

Discussion

With ionic buffers (with the exception of sodium diethylbarbiturate-HCl buffer), the pH had no substantial effect on the character of the separation of RCNMV serotypes and natural mixtures of these serotypes. Gels prepared in 0.1 to 0.01 mol/l buffers proved to be the most suitable. This molarity range has also been used for buffering agarose gels in immunoelectrophoresis by others (Hollings and Stone, 1977; Koenig, 1970; Ferenčík, 1980; Ferenčík *et al.*, 1981; Musil and Gallo, 1982; etc.). Gels prepared in 0.001 mol/l buffers proved to be unsuitable for separation of RCNMV serotypes and their natural mixtures. Some differences in the separation of RCNMV serotypes in agarose buffers depending on the nature of buffer (anionic or cationic) have been pointed out previously (Musil and Gallo, 1982).

HEPES buffer at pH 7.2 in the concentration range from 0.1 to 0.01 mol/l proved to be the most suitable for immunoelectrophoresis of RCNMV. The separation of its serotypes and their mixtures became more marked with decreasing molarity of the buffer. In gels prepared in 0.001 mol/l HEPES buffer the migration of isolate TpM34 (serotype A) to the cathode was the most marked but at this molarity the natural serotype mixtures did not separate into the individual components. The best separation of the mixtures was obtained with 0.01 mol/l HEPES buffer.

The results obtained concerning immunoelectrophoresis of isolates representing natural serotype mixtures showed that in comparative experi-

ments with the use of ionic buffers reference virus isolates have to be included. By contrast, with the nonionic HEPES buffer the separation into serotype components was so clear-cut that the use of reference virus isolates could be avoided.

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Explanation of Figures (Plates XI—XII):

Fig. 1. Immunoelectrophoresis of RCNMV isolates in agarose gels prepared in phosphate buffers at different pH and of different molarities. A — isolate TpM34, B — isolate TpM48, C — isolate No. 6, AC — isolate No. 11, AB — isolate No. 29, BC — isolate No. 3.

Fig. 2. Immunoelectrophoresis of RCNMV isolates in agarose gels prepared in 0.1 mol/l sodium diethylbarbiturate-HCl buffer at different pH. Designation of isolates as in Fig. 1.

Fig. 3. Immunoelectrophoresis of RCNMV isolates in agarose gels prepared in HEPES buffers of different molarities at pH 7.2. Designation of isolates as in Fig. 1.