Effect of Temperature on the Order of Electrophoretic Migration of Influenza Virus Neuraminidase and Nucleoprotein Genes in Acrylamide Gels Lacking Denaturing Agents

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SUMMARY

When subjected to electrophoresis at 33 °C in 3% polyacrylamide gels with no urea added, the nucleoprotein and neuraminidase genes of an H2N2 and H3N2 virus migrate as RNA bands 5 and 6 respectively. If the temperature of electrophoresis is increased to 46 °C, however, this order of migration is reversed.

The three size classes of RNA in influenza A virus demonstrated by velocity gradient sedimentation (Skehel, 1971) consist of eight species (Pons, 1976). The three largest RNAs (1, 2, and 3) code between them for high mol. wt. virus-specific polypeptides designated P1, P2, P3; the three intermediate-sized RNAs (4, 5, and 6) code between them for HA, NA and NP; the two smallest RNAs (7 and 8) code between them for M and NS protein (Ritchey et al. 1976; Inglis et al. 1977).

We reported previously that electrophoresis in the absence of denaturing agents is a technique which can be used to determine whether RNA 8 in some recombinant viruses is derived from their H2N2 or H3N2 parent, since the electrophoretic mobility of RNA 8 was different for the parent viruses (Kendal et al. 1977). We also observed reproducible differences in the relative migration rates of RNAs 4, 5 and 6 in H2N2 and H3N2 viruses, although we were originally unable to identify clearly the origin of RNAs 5 and 6 in several recombinants.

In further studies we found that the relative migration rates of RNA species 5 and 6 in our H2N2 and H3N2 parent viruses are greatly affected by the temperature of the gel during electrophoresis. By allowing for this, we can now establish suitable experimental conditions to determine the derivation of RNAs 4, 5 and 6 in our recombinants by electrophoresis in the absence of urea.

The viruses studied and the methods used for labelling virion RNAs with 3H-uridine or 32P have been described (Kendal et al. 1977). All electrophoresis was done using 3% polyacrylamide gels cast in glass tubes and an apparatus which has a glass wall between the electrophoresis buffer and the circulating water which regulates temperatures. Approximately 10 cm of the 12-cm-long gels were immersed in the lower (anode) buffer, which had its temperature controlled. The composition of gels and buffers and methods for processing gels were identical to those we used previously (Kendal et al. 1977).

When we co-electrophoresed the RNAs of H2N2 virus A/Ann Arbor/6/60 with the H3N2 virus A/Victoria/3/75, strain-specific differences between the relative mobilities of RNAs 4, 5 and 6 were observed whether electrophoresis was done at 33 °C or 46 °C (Fig. 1a, 2a). At intermediate temperatures of 36 °C, 38 °C or 42 °C, incomplete resolution was observed between RNAs 5 and 6 of A/Ann Arbor/6/60 and/or of A/Victoria/3/75 (not shown).
Fig. 1. Comparison of RNAs in A/Ann Arbor/6/60 (H2N2\textsubscript{60}), A/Victoria/3/75 (H3N2\textsubscript{75}), and their recombinants AA-CR\textsubscript{22} clone 17 (H3N2\textsubscript{60}) and AA-CR\textsubscript{19} (H3N2\textsubscript{75}) by co-electrophoresis in 3\% acrylamide gels at 33 °C. \textsuperscript{3}H-uridine and \textsuperscript{32}P-labelled viruses were co-electrophoresed in a 12 cm gel at 5 mA/gel for 14 h. For clarity, only RNAs 4, 5 and 6 are shown. (a) Co-electrophoresis of \textsuperscript{32}P-A/Victoria/3/75 (---) and \textsuperscript{3}H-A/Ann Arbor/6/60 ( ); (b) \textsuperscript{32}P-AA-CR\textsubscript{22} clone 17 (---) and \textsuperscript{3}H-A/Victoria/3/75 ( ); (c) \textsuperscript{32}P-AA-CR\textsubscript{22} clone 17 (---) and \textsuperscript{3}H-A/Ann Arbor/6/60 ( ); (d) \textsuperscript{32}P-AA-CR\textsubscript{19} (---) and \textsuperscript{3}H-A/Ann Arbor/6/60 (---).

Identification of RNA 4 as the gene coding for the influenza virus H2 or H3 haemagglutinin was done by co-electrophoresis of the RNAs of A/Victoria/3/75 with a recombinant AA-CR\textsubscript{22} clone 17. This recombinant had been shown by haemagglutination inhibition and neuraminidase inhibition tests to contain A/Victoria/3/75 haemagglutinin but the neuraminidase of A/Ann Arbor/6/60. AA-CR\textsubscript{22} clone 17 derived its RNA 4, but not RNA 5 or 6, from A/Victoria/3/75 whether RNAs were compared by electrophoresis at 33 °C or 46 °C (Fig. 1b, 2b). The reciprocal experiment confirmed that only RNAs 5 and 6 of this recombinant co-migrated with RNAs of A/Ann Arbor/6/60 at 33 °C (Fig. 1c) or at 46 °C (not shown). Thus, RNA 4 must be the gene coding for the HA of these viruses.

Identification of the RNA species coding for the neuraminidase and nucleoprotein genes of the viruses was obtained by co-electrophoresis of the RNAs of A/Victoria/3/75 with recombinant AA-CR\textsubscript{19} that derives both its haemagglutinin and neuraminidase from A/Victoria/3/75. AA-CR\textsubscript{19} had only one intermediate-sized RNA that co-migrated with an RNA from A/Ann Arbor/6/60 (and must therefore be the nucleoprotein gene), whereas the other two intermediate-sized RNAs co-migrated with RNAs from A/Victoria/3/75. When electrophoresis was done at 33 °C, the RNA common to AA-CR\textsubscript{19} and A/Ann Arbor/6/60 was RNA 5 (Fig. 1d) whereas when electrophoresis was done at 46 °C the common RNA
was RNA 6 (Fig. 2c). Corresponding experiments with AA-CR19 and A/Victoria/3/75 showed that when electrophoresed at 33 °C RNAs 4 and 6 (but not 5) of AA-CR19 and A/Victoria/3/75 co-migrated (not shown), whereas when electrophoresed at 46 °C RNAs 4 and 5 (but not 6) of AA-CR19 and A/Victoria/3/75 co-migrated (Fig. 2d). Since the HA gene of A/Ann Arbor/6/60 and A/Victoria/3/75 migrates as RNA 4 under either condition of electrophoresis used (see above), and since AA-CR19 derives its HA and NA from A/Victoria/3/75, we conclude that when electrophoresis is carried out at 33 °C the NP and NA genes migrate as RNAs 5 and 6 respectively and that this order of migration is reversed when electrophoresis is performed at 46 °C. Our findings therefore show that the order of migration of the NA and NP genes is not necessarily dependent solely upon the virus strains studied (Almond et al. 1977; Palese & Schulman, 1976; Ritchey et al. 1976; Rohde et al. 1977), but in our urea-free electrophoresis system the order is dependent on the temperature of electrophoresis.

By performing tube gel electrophoresis at 46 °C (or analogous slab-gel electrophoresis techniques) when studying additional cold-adapted viruses AA-CR6, -CR12, and -CR18 obtained by recombination between mutant A/Ann Arbor/6/60 and wild-type H3N2 strains A/Queensland/6/72, A/Ann Arbor/9/73, or A/Scotland/840/74 (Kendal et al. 1977;
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Spring et al. (1977) we found that each cold-adapted recombinant derives its NP gene from the mutant parent as is also shown here for recombinants AA-CR19 and -CR22. The possible significance of the consistent transfer of the NP gene, in addition to the previously reported transfer of M protein gene, of cold-adapted mutant A/Ann Arbor/6/60 during recombination at 25 °C is being studied further.

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