THE MOLECULAR WEIGHT DETERMINATION OF PROTEINS AND GLYCOPROTEINS OF RNA ENVELOPED VIRUSES BY POLYACRYLAMIDE GEL ELECTROPHORESIS IN SDS

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SUMMARY. The apparent molecular weights for glycoproteins of four RNA enveloped viruses - influenza virus, NDV, VSV and AMV, calculated relative to protein standards depend upon the percent of acrylamide used. Such anomaly is not observed for other proteins of these viruses. The irregular behaviour of glycoproteins resulted from their lesser capacity to bind SDS.

Polyacrylamide gel electrophoresis of proteins treated with SDS is at present the most frequently used method for their molecular weight determination (1). The electrophoretic mobility of simple reduced proteins in these gels depends on the molecular weight only (2,3). Nevertheless, several pro - teins have been found with anomalous electrophoretic behaviour in SDS (4-6). Furthermore, the apparent molecular weights estimated by this method for more complex proteins e.g. glyco-

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Abbreviations: NDV-Newcastle disease virus, VSV-Vesicular stomatitis virus, AMV-Avian myeloblastosis virus.

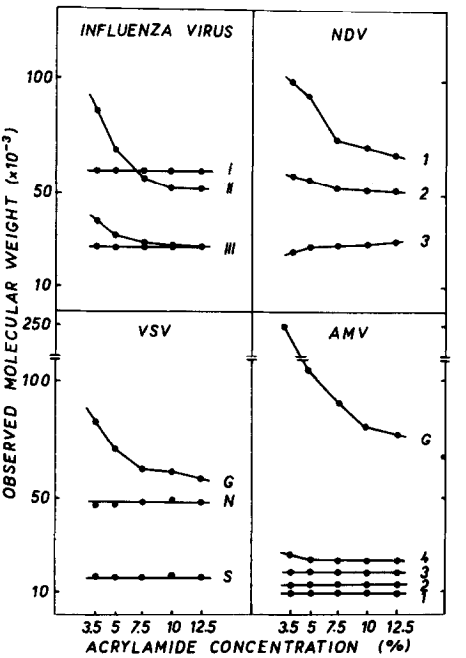

Fig.1. Observed molecular weights of glycoproteins and proteins of influenza virus (two glycoproteins: II and III-upper curve, two proteins: I and III-lower curve), NDV (glycoprotein: 1, two proteins: 2 and 3), VSV (glycoprotein: G, two proteins: N and S) and AMV (glycoprotein: G and four proteins: 1,2,3 and 4) versus acrylamide gel concentration.

proteins are accepted with some doubts (7). Therefore the molecular weights of proteins and glycoproteins from influenza virus (proteins I and III, glycoproteins II and III), NDV (proteins 2 and 3, glycoprotein 1), VSV (proteins N and S, glycoprotein G) and AMV (proteins 1, 2, 3 and 4, glycoprotein G) (8-11), were reinvestigated. The principle of varying concentration of acrylamide which provides a reliable approach to detect anomalies, had been used in this study (12).

MATERIALS AND METHODS. The following viruses were used :

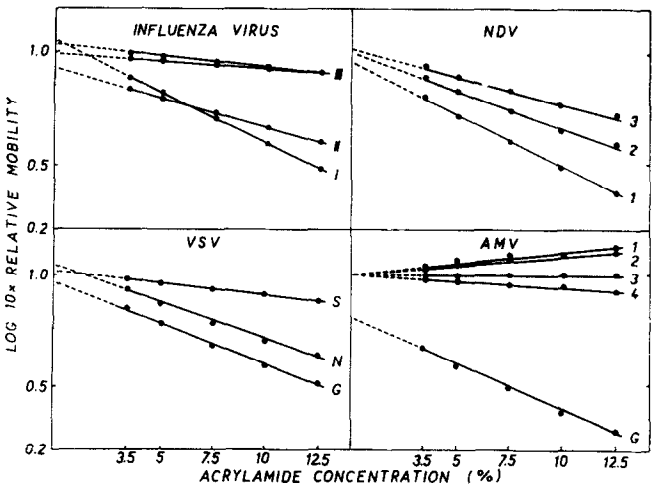

Fig.2. The effect of different gel concentration on the relative mobilities of the SDS complexes of glycoproteins and proteins. For explanation the symbols see Fig.1., except influenza virus (two glycoproteins: II and III-lower line, two proteins: I and III-upper line).

influenza virus A/Singapore(H2N2), NDV, VSV (all propagated in chick embryos) and AMV (isolated from chick plasma). The viruses were purified by differential centrifugation, followed by equilibrium centrifugation in a 15-55% sucrose gradient. Samples for electrophoresis were prepared by adding 10% SDS and solid dithiothreitol to final concentration 1% , followed by heating at 100°C for 1 minute. The following standard proteins were used to construct standard plots of log molecular weight versus relative mobility in the diffe - rent acrylamide concentrations: bovine serum albumin (BSA) - m.w. 66,000; structural protein of influenza virus ribonucleoprotein (RNP-P) - m.w. 61,000; ovalbumin (OVA) - m.w.

46,000; α-chymotrypsinogen (CHY) - m.w. 25,741 and structural protein of tobacco mosaic virus (TMV-P) - m.w. 17,400. Five concentrations of acrylamide gels (3.5, 5.0, 7.5, 10.0 and 12.5%) were used. A constant proportion of acrylamide to N,N'-methylene-bis-acrylamide was maintained in all gels (50:1) . Continuous buffer system 0.1M phosphate pH 7.0 with 0.1% SDS had been used. After electrophoresis the gels were stained with either coomassie brilliant blue or periodic acid Schiff . Relative mobilities were calculated as a ratio of apparent mobility to mobility of the fastes moving marker i.e. structural protein of tobacco mosaic virus. Relative free mobilities were obtained by extrapolation of relative mobilities to zero gel concentration.

RESULTS AND DISCUSSION. The electrophoretic mobilities of the standard proteins (BSA, RNP-P, OVA, CHY and TMV-P) when plotted against the log of their molecular weights were linear for each of the five acrylamide gel concentration studied (Fig. not given). The apparent molecular weights for viral proteins calculated relative to protein standards were inde - pendent upon the percentage of acrylamide used (Fig.1). Unlike the proteins, the glycoproteins behaved anomalously in SDS-polyacrylamide gel electrophoresis; their observed molecular weights varied with acrylamide concentration (Fig.1). With increasing gel concentration the apparent molecular weights of

glycoproteins approach assymptotically constant values (probably the real molecular weights) (6). The shape of curves indicates that the irregular behaviour of glycoproteins is due to a decreased binding of SDS. If the amount of bound SDS is insufficient to cause the glycoprotein migration in the same manner as the standard proteins, increasing acrylamide concentration will cause the apparent shift of molecular weights to lower values. This results from the fact that at higher acrylamide concentrations the charge of the SDS-protein complex becomes less important relative to the sieving effect of the gel (5,6). This explanation of anomalous glycoprotein behaviour is further confirmed by finding the relative free mobilities of glycoproteins always lower than those of proteins (Fig.2). Moreover, the dependence of molecular weights on acrylamide concentration was more pronounced for those glycoproteins, the relative free mobilities of which were lower. As a consequence of the anomalous behaviour of glycoproteins on SDS gels, the recently reported molecular weights for the studied glycoproteins were in many cases considerably higher than author's estimates in the most concentrated gel (9,11,13).

An interesting finding was the more pronounced separation of glycoprotein III and protein III of influenza virus in the gels with low acrylamide concentrations (Fig.1).Generally, one electrophoretic zone in SDS-polyacrylamide gel electrophoresis

may contain several different proteins of similar sizes. The complex character of such zone could be ascertained if these proteins differ in their capacity of SDS binding. As shown in the case of influenza virus (Fig.1) this is possible rather by decreasing than by increasing the acrylamide concentration. In addition, on Fig.1 is shown, that in 6% acrylamide gel the protein I and glycoprotein II have the same mobility. These two examples again illustrate the possible sources of errors if only a single concentration of acrylamide is used.

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REFERENCES

1. Shapiro, A.L., Viňuela, E., and Maizel, J.V., Biochem. Biophys. Res. Commun., 28, 815 (1967).
2. Reynolds, J.A., and Tanford, C., Proc. Natl. Acad. Sci. U.S.A., 66, 1002 (1970).
3. Reynolds, J.A., and Tanford, C., J. Biol. Chem., 245, 5161 (1970).
4. Koenig, R., Virology, 50, 263 (1972).
5. Mitchell, E.D., Riquetti, P., Loring, R.H., and Carraway, K.L., Biochim. Biophys. Acta, 295, 314 (1973).
6. Segrest, J.P., Jackson, R.L., Andrews, E.P., and Marchesi, V.T., Biochem. Biophys. Res. Commun., 44, 390 (1971).
7. Compans, R.W., Klenk, H.D., Caliguiri, L.A., and Choppin, P.W., Virology, 42, 880 (1970).
8. Russ, G., Thesis, Prague, (1973).
9. Evans, M.J., and Kingsbury, D.W., Virology, 37, 597 (1969).

10. Wagner, R.R., Prevec, L., Brown, F., Summers, D.F., Sokol, F., and MacLeod, R., J.Virol., 10, 1228 (1972).
11. Bolognesi, D.P., and Bauer, H., Virology, 42, 1097 (1970).
12. Neville, D.M., J. Biol. Chem., 246, 6328 (1971).
13. Wagner R.R., Schnaitman, T.A., and Snyder, R.M., J. Virol., 3, 395 (1969).