Amino Acid Composition of Polypeptides from Influenza Virus Particles

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SUMMARY

The neuraminidase, the membrane protein, the ribonucleoprotein (nucleocapsid protein) and the light and heavy polypeptide chains of the haemagglutinin subunits were isolated from particles of influenza virus type A₀ (strain BEL) and type B (strain LEE). Each of these substances migrated as a single component during electrophoresis on SDS-polyacrylamide gels. The neuraminidase of type A₂ influenza was isolated from the A₀–A₂ recombinant virus, X-7F₁. This enzyme contained two polypeptides differing slightly in electrophoretic mobility.

This paper gives the amino acid composition of the individual polypeptides from the type A₀ and type B influenza viruses and the neuraminidase protein from X-7F₁ virus.

INTRODUCTION

Particles of influenza viruses have been reported to contain from 5 to 8 different polypeptide chains separable by SDS-polyacrylamide gel electrophoresis (Compans et al. 1970; Schulze, 1970; Skehel & Schild, 1971; Laver, 1971). Reports have also claimed that some influenza virus polypeptides are relatively rich in arginine, methionine and cysteine (Haslam et al. 1970, Taylor et al. 1970; White et al. 1970). However, these latter reports were based on studies of the incorporation of labelled amino acids, which gave no information about the total amino acid composition of the polypeptides.

In this paper we describe the isolation of polypeptides from particles of type A and type B influenza viruses and their amino acid analysis using conventional techniques; the isolation and amino acid composition of the light and heavy polypeptide chains from the haemagglutinin subunits of a type A₀ influenza virus (strain BEL) has already been described (Laver, 1971). The amino acid composition of particles of influenza types A and B (Knight, 1947) and of three protein fractions released from influenza virus particles by treatment with ether (Hoyle & Davies, 1961) have also been reported previously.

METHODS

Viruses. The BEL strain of A₀ influenza (Burnet et al. 1942), the LEE strain of influenza type B (Francis, 1940) and the A₀–A₂ recombinant virus, X-7F₁ (Kilbourne et al. 1967) were grown in the allantoic sac of 11-day-old chick embryos. The virus particles were purified by adsorption to and elution from chicken erythrocytes followed by differential centrifuging and sedimentation through a 10 to 40 % sucrose gradient in 0·15 M-NaCl (Laver, 1969).
Isolation of the polypeptide chains. The virus particles were disrupted with 1% SDS at room temperature (20 °C) and the virus proteins were separated by electrophoresis on cellulose acetate strips in tris-boric acid-EDTA buffer, pH 9, containing 0.4% SDS (Laver, 1964). The proteins were eluted from the strips into water and filtered to remove pieces of cellulose acetate. The proteins were then precipitated with cold (−20 °C) ethanol (3 vol.) resuspended in water and stored frozen at −20 °C. The light and heavy polypeptides of the haemagglutinin subunits were separated by centrifuging on guanidine hydrochloride-dithiothreitol density gradients (Laver, 1971).

Acrylamide gel electrophoresis. The proteins were electrophoresed in 7.5% polyacrylamide gels containing 0.2% N,N'-bismethylene acrylamide in tris-boric acid-EDTA buffer, pH 9 with 0.1% SDS and 0.1 mg/ml dithiothreitol (Laver, Wrigley & Pereira, 1969).

Amino acid analyses. Protein samples containing 200 to 300 µg were hydrolysed with 6 N-HCl (2.0 ml) in sealed evacuated tubes at 110 °C for 22 h and the hydrolysates were analysed by the method of Moore, Spackman & Stein (1958) using the Model B Beckman amino acid analyser with an expanded scale (Spackman, 1960). No corrections were made for losses occurring during hydrolysis.

RESULTS

Isolation of proteins from type A0 influenza virus particles

The proteins of the BEL strain of influenza A0 virus were isolated by electrophoresis on cellulose acetate strips as previously described (Laver, 1971). This paper also described the separation and amino acid analysis of the light and heavy polypeptide chains of the haemagglutinin subunits of BEL virus. The other proteins which were isolated from the strips, the neuraminidase, the ribonucleoprotein and the internal (or membrane) protein migrated as single polypeptides during SDS-polyacrylamide gel electrophoresis (Fig. 1; Laver, 1971). The biological activity of each of the three latter proteins was destroyed during electrophoresis on cellulose acetate; they were identified by their mobilities during SDS-acrylamide gel electrophoresis (Compans et al. 1970; Schulze, 1970).

Isolation of proteins from type B influenza virus particles

Particles of type B influenza (strain LEE) were disrupted with SDS and the proteins were separated by electrophoresis on cellulose acetate strips. The pattern of proteins obtained from LEE virus differed from that obtained with the BEL strain (Fig. 1) (Laver, 1964, 1971). The neuraminidase of BEL virus was denatured during electrophoresis whereas the haemagglutinin subunits were not and could be eluted from the strips without loss of biological activity. By contrast, LEE virus haemagglutinin was denatured during electrophoresis while its neuraminidase subunits were stable and could be isolated pure and without loss of biological activity (Laver, 1964).

The proteins of LEE virus, separated by cellulose acetate electrophoresis, were examined by polyacrylamide gel electrophoresis (Fig. 1). Four fractions were isolated from the strips.

NA fraction

This fraction was identified, by its enzyme activity, as the neuraminidase of LEE virus. The protein in this fraction migrated during SDS-polyacrylamide gel electrophoresis as a single component with a mol. wt. of about 60,000 suggesting that the neuraminidase subunit of LEE virus consists of a single species of polypeptide of this size (Fig. 1).
Amino acids of influenza polypeptides

Fig. 1. Stained cellulose acetate strips and polyacrylamide gels showing the electrophoretic separation of the proteins of the A0/BEL and B/LEE strains of influenza virus. The purified virus particles were first disrupted with cold (20 °C) SDS and the proteins were separated by electrophoresis on cellulose acetate strips. The bands of proteins were eluted individually and concentrated by precipitation with cold (−20 °C) ethanol. Samples of the isolated proteins were dissolved in hot (100 °C) SDS and dithiothreitol and electrophoresed on polyacrylamide gels. (HA = haemagglutinin; HA I = haemagglutinin heavy polypeptide; HA 2 = haemagglutinin light polypeptide; NA = neuraminidase; NP = ribonucleoprotein antigen; M = membrane protein of virus envelope.) ('Influenza virus polypeptides and antigens – Summary of influenza virus workshop 1', *Journal of Infectious Diseases* **125**, 447–455 (1972).)
HA fraction

This had no biological activity but, because of its polypeptide composition, we thought it contained the haemagglutinin subunits of LEE virus, denatured by the SDS.

SDS-polyacrylamide gel electrophoresis in the presence of dithiothreitol showed that the protein in this fraction contained two different polypeptides with mol. wt. of about 60,000 and 25,000. These two polypeptides were presumed to be the heavy and light polypeptides of the haemagglutinin subunits similar to those previously described for A0/BEL influenza virus (Laver, 1970). The two polypeptides from the HA fraction of LEE virus were separated by centrifuging in guanidine hydrochloride-dithiothreitol density gradients (Laver, 1971). SDS-polyacrylamide gel electrophoresis of the separated heavy and light polypeptides (HA 1 and HA 2) showed that pure preparations were obtained in each case.

NP and M fractions

The mobilities of the polypeptides in these fractions during polyacrylamide gel electrophoresis suggested that fraction NP contained the ribonucleoprotein (nucleocapsid protein) and fraction M the small mol. wt. internal or membrane protein of LEE virus. Fraction NP was not completely pure (traces of the other proteins were present) but fraction M (the membrane protein) was obtained free from all of the other virus proteins except for a trace of the nucleocapsid protein which remained in the preparation (Fig. 1).

Isolation of X-7F1 neuraminidase

Particles of the recombinant virus, X-7F1, which contained A0/NWS haemagglutinin subunits and A0/RI/5+ neuraminidase subunits, were disrupted with SDS and the neuraminidase subunits were isolated by electrophoresis on cellulose acetate strips as described previously (Webster, Laver & Kilbourne, 1968; Laver & Valentine, 1969). Neuraminidase subunits, isolated from X-7F1 virus in this way, completely retained their enzyme activity.

SDS-polyacrylamide gel electrophoresis showed that the neuraminidase subunits of X-7F1 virus contained two polypeptides with slightly different mol. wt. This finding was in agreement with those of others (Skehel & Schild, 1971; Webster, 1970).

Amino acid analyses

The individual polypeptides isolated from A0/BEL and B/LEE virus particles and the A9 neuraminidase subunits (consisting of two slightly different size polypeptides) isolated from X-7F1 virus were hydrolysed with acid and the hydrolysates were analysed for amino acids. The amino acid composition of the polypeptides is given in Table 1. No corrections have been made for losses of labile amino acids, e.g. serine and threonine, during hydrolysis.

DISCUSSION

This paper reports the amino acid composition of polypeptides isolated from particles of influenza viruses. Several of the virus proteins (the neuraminidase of LEE and X-7F1 viruses and the haemagglutinin of BEL virus) were positively identified by their biological activity. The other proteins, which were denatured during the isolation procedure, were identified by their mobilities during SDS-polyacrylamide gel electrophoresis (Compans et al. 1970; Schulze, 1970).

The neuraminidase subunits of LEE and the haemagglutinin subunits of BEL and LEE viruses were cleanly separated from the other virus proteins during electrophoresis on cellulose
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Expressed as mol of amino acid per 100 mol recovered.
Figures in parentheses refer to number of analyses done.
Data for BEL HA1 and BEL HA2 were taken from Laver (1971).
ND = not determined.
acetate strips (Fig. 1). Pure neuraminidase subunits from X-7F1 virus were also obtained in this way (Webster et al. 1968). The polypeptides of these proteins were obtained pure, but the other polypeptides, which did not separate so cleanly during electrophoresis on cellulose acetate (BEL neuraminidase and the membrane protein (M) and ribonucleoprotein (NP) of BEL and LEE viruses), were not completely pure. However, the amounts of contaminating proteins in the preparations of these polypeptides were small (Fig. 1) and probably did not greatly affect the amino acid analyses reported in Table 1.

None of the polypeptides had an unusually large or small quantity of any amino acid, but some noticeable differences between the polypeptides are worth commenting on.

The heavy polypeptide (HA 1) from the haemagglutinin subunits of LEE and BEL viruses contained much more proline than the light polypeptide (HA 2). The external proteins of the virus particles, namely the heavy and light polypeptides of the haemagglutinin subunits and the polypeptides of the neuraminidase subunits, contained more $\frac{1}{2}$-cystine than the internal proteins (NP and M). These internal polypeptides, on the other hand, were somewhat richer in arginine than the external polypeptides but the differences between the two groups were small and none of the polypeptides contained sufficient arginine to justify its description as an 'arginine-rich protein'.

The nucleocapsid (NP) and membrane (M) proteins from LEE (type B) influenza virus had a different amino acid composition from the nucleocapsid and membrane proteins of BEL (type A) influenza virus. This is not an unexpected finding since the nucleocapsid proteins from these two influenza virus types are completely different immunologically, and maps of the tryptic peptides from the membrane proteins of BEL and LEE viruses have been found to differ greatly.

The number of polypeptides in the neuraminidase subunits of influenza virus is not known. The neuraminidase of the $A_0-A_2$ recombinant virus, X-7F1, appeared to consist of two species of polypeptides which migrated with slightly different mobilities during polyacrylamide gel electrophoresis. This confirmed the findings of others (Webster, 1970; Skehel & Schild, 1971) but it was not known whether the neuraminidase subunits of X-7F1 virus contained two different species of polypeptides or whether there was a single species, some molecules of which carried a different carbohydrate component from the others. The neuraminidase of BEL (type A) influenza virus appeared to consist of a single species of polypeptide of about 60,000 mol. wt. (Fig. 1) but the enzyme of this virus was inactivated during its isolation and possibly one of the polypeptides had been lost. The neuraminidase of LEE (type B) influenza virus, on the other hand, was isolated in pure form without any loss of enzymic activity and, when examined by polyacrylamide gel electrophoresis, appeared to consist of only one species of polypeptide (Fig. 1).

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REFERENCES


Amino acids of influenza polypeptides


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