THE CONFORMATION OF INFLUENZA VIRUS HAEMAGGLUTININ

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Received 1 June 1977

1. Introduction

The haemagglutinin of influenza virus is the major glycoprotein of the virus membrane [1,2]. It is responsible for the attachment of the virus to the plasma membrane of the cell to be infected [3] and in an additional as yet undefined manner in initiating infection [4,5]. In infectious virus particles the glycoprotein appears to be a trimer of molecular weight about 210 000 in which each monomer consists of two disulphide linked polypeptides HA_1 and HA_2 [6–8]. During replication these are formed as the result of proteolytic cleavage of a precursor polypeptide (HA_0) of molecular weight about 70 000 which is the translation product of a single virus gene. Recently this cleavage has been shown to be essential for the formation of infectious virus particles even though in its absence particles which can bind to cells can be assembled. In addition these non-infectious particles containing HA_0 can be activated in vitro by digestion with trypsin and concomitant generation of HA_1 and HA_2 [4,5].

The protein may be isolated following dissociation of virus particles with detergents [9] or by digesting them with the protease bromelain [10]. The bromelain released protein (BHA) is soluble in the absence of detergent and lacks the hydrophobic region of the intact molecule which is associated with the lipid bilayer of the virus particle. As a result it is more amenable to physical studies aimed at elucidating its conformation than the detergent solubilized intact molecule (HA). Wiley and Skehel [11] have recently reported the initial results of an X-ray diffraction study of BHA crystals. In this communication the circular dichroism (CD) spectra of HA_0, HA and BHA are presented. Since such spectra are sensitive to conformational changes in the protein molecules the effects on the structure of HA_0 of the proteolytic cleavage which results in the production of HA can be examined and in addition a comparison can be made between the structures of HA and BHA.

2. Materials and methods

Virus particles containing HA or HA_0 were purified from infected egg allantoic fluid or infected CEF culture medium respectively as described previously [6].

Detergent solubilized haemagglutinins (HA_0 and HA) were prepared by treating purified virus particles with a 1% solution of polyoxyethylene 10-lauryl ether (Lubrol PX or Brij 36T) in PBS, pH 7.2. The extracted membrane glycoproteins were separated from the virus cores by spinning down the latter at 100 000 × *g* for 30 min. Bromelain-released haemagglutinin (BHA) was prepared as described previously [10].

The in vitro cleavage of HA_0 by trypsin was achieved by incubating virus particles containing HA_0 in PBS, pH 7.2, at 37°C for 1 h, with 1/10 their weight of diphenyl carbamyl chloride treated bovine pancreatic trypsin. The reaction was stopped by the addition of a molar equivalent of soya bean trypsin inhibitor and the suspension centrifuged at 100 000 × *g* for 30 min. The virus pellet was resuspended in PBS and the haemagglutinin solubilized in Brij 36T as described above.

Circular dichroism (CD) spectra were measured with a Roussel-Jouan CD 185 model II dichrograph. Spectra between 190 and 250 nm were analysed in terms of contributing helical, beta and aperiodic conformations using the reference data of Chen, Yang and Martinez [12]. Absorption spectra were measured with a Cary 118 spectrophotometer. Tyrosine/tryptophar

ratios were obtained by the method of Beaven and Holiday [13].

3. Results and discussion

3.1. *The polypeptide composition of the isolated glycoproteins*

The use of the non-denaturing polyoxyethylene acyl ether detergent to isolate HA rather than harsher acyl sulphates [9,14] avoids contamination of the glycoprotein by the internal core components of the virus particles but also results in solubilization of the other membrane glycoprotein, the neuraminidase. This can be seen when the solubilized proteins are examined by SDS-polyacrylamide gel electrophoresis (fig.1A) However, since the neuraminidase was always less than 5% of the total protein in such preparations no further purification of the detergent solubilized haemagglutinin, HA, was attempted.

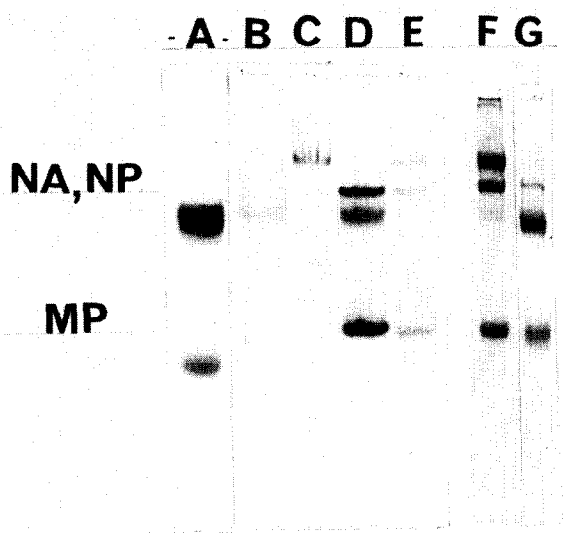

Fig.1. SDS-polyacrylamide gel electrophoresis of A2/Japan/57 (H_2) influenza virus and its glycoprotein components. (A) BHA, (B) HA, (C) HA_0, (D) infectious virus, (E) and (F) non-infectious virus, (G) Brij 36 T solubilized glycoproteins from non-infectious virus following trypsinization. Electrophoresis in phosphate buffered SDS solutions pH 7.2 was as described previously [6]. Lanes A–E were electrophoresed at 50 V for 18 h. Lanes F and G for 12 h. The abbreviations NA, NP and MP refer to the neuraminidase, nucleoprotein and matrix protein respectively [6].

HA_0 was also released from virus particles following Brij 36T treatment (fig.1C). However, following trypsinization the preparation of cleaved HA_0 contained in addition to the two haemagglutinin glycopolypeptides an amount of the nucleocapsid polypeptide which amounted to about 5% of the protein (fig.1G). Again no attempt was made to purify the haemagglutinin further.

The polypeptide components of BHA (BHA_1 and BHA_2) are shown in fig.1B.

3.2. *Circular dichroism spectra of the glycoproteins*

Figure 2 shows the far UV CD spectrum of BHA and assuming only helical, beta and aperiodic contributing peptide conformations the composition of the protein is shown in table 1. Though these values may be misleading as accurate predictions of structure (e. g. the data for the basic set of globular proteins may not be applicable to membrane proteins) the analysis is useful for comparative purposes. No difference within the experimental error exists between the different spectra for all three protein preparations HA_0, HA and BHA.

In the near UV BHA was found to possess a characteristic CD spectrum. This is shown in fig.3 for

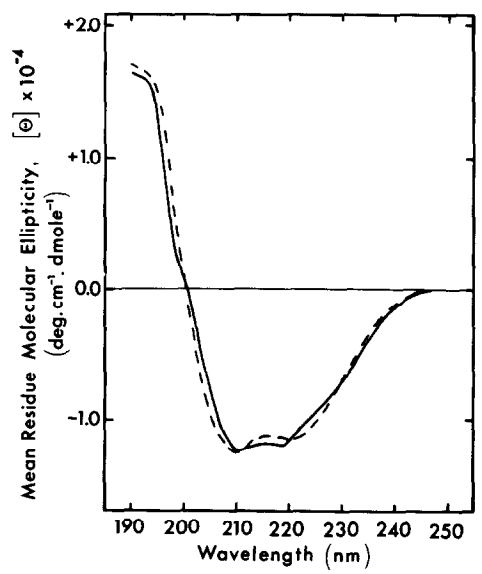

Fig.2. The far UV-CD spectrum of HA isolated from A_2/Japan/57 (H_2). (———) Experimental curve. (- - - - -) Best fit to the data of Chen, Yang and Martinez [12].

Table 1
Analysis of the CD spectra (190–250 nm) of haemagglutinin isolated from the strain, A_2/Japan/57 (H_2), using the reference data of Chen, Yang and Martinez [12].

Preparation	% Helix	% Beta form	% Aperiodic form
HA_0	32.0 ± 1.0	16.8 ± 2.7	49.4 ± 8.5
HA	32.4 ± 1.2	14.0 ± 3.5	53.6 ± 9.5
BHA	29.4 ± 1.2	18.0 ± 3.9	52.6 ± 8.7

the BHA preparations obtained from five strains of influenza which were initially isolated between 1942 and 1968 and were representative of the viruses of the three recorded pandemic eras – 1933 to 1957, 1957 to 1968 and 1968 to the present. Only slight variations occur between any of the strains and no differences were seen between the BHAs of strains closely related immunologically–A_0/Bel/42 (HO subtype) and A_0/Weiss/43 (HO subtype); A_2/Japan/57 (H_2 subtype) and A_2/Korea/68 (H_2 subtype).

The near UV-CD spectrum is dependent on the maintenance of the native conformation of the protein as is shown by its disappearance when the protein is denatured in 4 M guanidinium chloride or at high pH (fig.4). The maximum in the signal strength suggests a tryptophan contribution; the slight red shift on increasing the pH to 10.9, a tyrosine contribution and the fine structure at 261-2 and 267-9 a phenylalanine contribution [15,16]. The minor variation between strains may be due to a slightly different arrangement of the same contributing residues or to the addition or removal of minor contributing species. The aromatic content does vary between strains and tyrosine/tryptophan ratios of 2.10, 1.93 and 1.97 were found for BHA isolated from A_0/Bel/42 (HO), A_2/Japan/57 (H_2) and A_2/Hong Kong/68 (H_3).

Figure 5 shows that the near UV-CD spectra of BHA and HA are very similar. The small differences may be

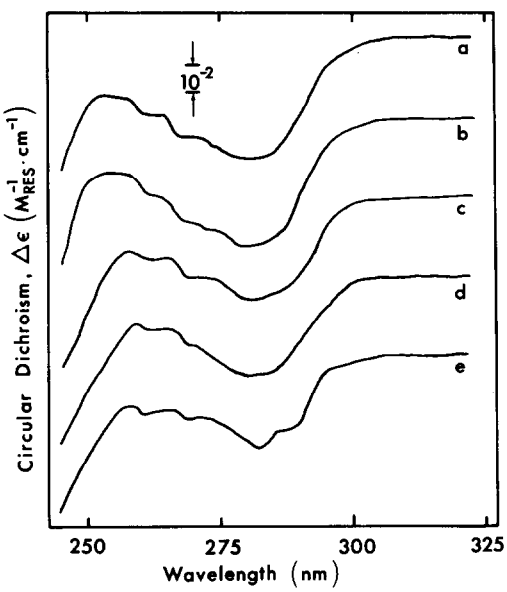

Fig.3. Near UV-CD spectra of BHA isolated from strains: (a) A_0/Bel/42 (H_0), (b) A_0/Weiss/43 (H_0), (c) A_2/Japan/57 (H_2), (d) A_2/Korea/68 (H_2) and (e) A_2/Hong Kong/68 (H_3).

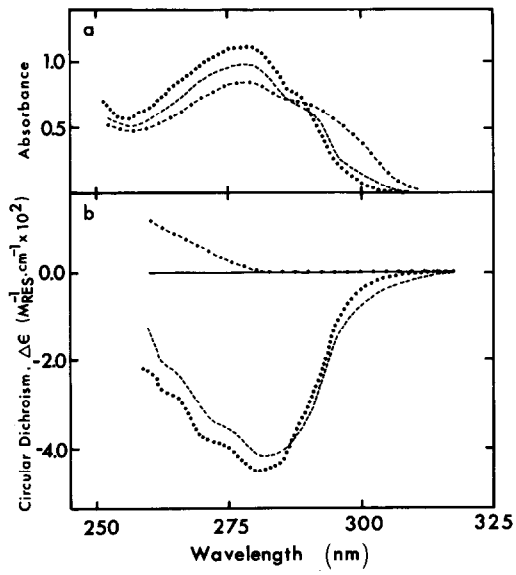

Fig.4. Absorption (a) and near UV-CD (b) spectra of BHA isolated from A_0/Weiss/43 (H_0): (· · · · ·), PBS, pH 7.2; (- - - -), pH 10.9; (-·-·-·), pH 11.2; (———) guanidinium chloride (only in b).

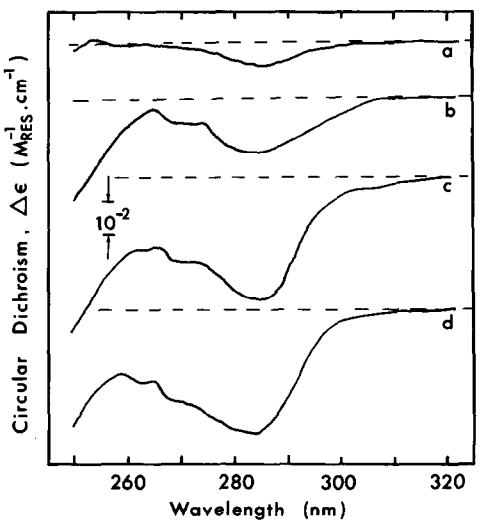

Fig.5. Near UV-CD spectra of (a) HA_0, (b) trypsinized HA, (c) HA and (d) BHA isolated from A_2/Japan/57 (H_2).

due to a contribution from the hydrophobic tail of HA_2 which does contain aromatic residues [14] or from the small amount of contaminating neuraminidase in the preparation. The HA_0 on the other hand possesses hardly any near UV-CD signal but following tryptic digestion a signal similar in shape to that of HA but less than half as strong was recorded.

Since, as judged by the far UV-spectra, no gross change in the structure of the haemagglutinin occurs on cleavage of HA_0 it may be concluded that this change in the near UV-CD spectrum is real. This is quite possible since the range of the interactions giving rise to near UV-CD spectra are usually very short rarely extending to 10 Å (15,16).

The constancy of this near UV spectrum throughout the haemagglutinins of the strains examined which differ in primary structure [1] suggests that it may be generated by a local highly conserved segment of protein possibly essential for function. In this connection a highly conserved sequence adjacent to the precursor haemagglutinin cleavage point has been reported [14] although this could not provide all the contributing residues since the only aromatic residues it contains are phenylalanine. The appearance of the weaker spectrum on cleaving HA_0 in situ with trypsin suggests that a similar but not identical conformational change occurs as in vivo. This is in keeping with the reported infectivity of trypsin treated HA_0 containing virus particles [4,5].

Finally the similarity of both the near and far UV-CD spectra of BHA and HA indicate that no gross structural changes occur during bromelain digestion and that conclusions drawn from the results of studies on BHA will be applicable to the intact membrane glycoprotein.

References

[1] Laver, W. G. (1973) Adv. Virus Res. 18, 57–103.
[2] Schulze, I. T. (1973) Adv. Virus Res. 18, 1–55.
[3] Hirst, G. K. (1942) J. Exp. Med. 75, 47–64.
[4] Klenk, H-D., Rott, R., Orlich, M. and Blodorn, J. (1975) Virology 68, 426–439.
[5] Lazarowitz, S. G. and Choppin, P. W. (1975) Virology 68, 440–454.
[6] Skehel, J. J. and Schild, G. C. (1971) Virology 44, 396–408.
[7] Laver, W. G. (1971) Virology 45, 275–288.
[8] Wiley, D. C., Skehel, J. J. and Waterfield, M. (1977) Virology, in press.
[9] Laver, W. G. (1964) J. Mol. Biol. 9, 109–124.
[10] Brand, C. M. and Skehel, J. J. (1972) Nature New Biol 238, 145–147.
[11] Wiley, D. C. and Skehel, J. J. (1977) J. Mol. Biol. in press.
[12] Chen, Y. H., Yang, J. T. and Martinez, H. M. (1972) Biochemistry 11, 4120–4131.
[13] Beaven, G. H. and Holiday, E. R. (1952) Adv. Protein Chem. 7, 319–386.
[14] Skehel, J. J. and Waterfield, M. D. (1975) Proc. Natl. Acad. Sci. USA 72, 93–97.
[15] Strickland, E. H. (1974) C. R. C. Crit. Rev. Biochem. 2, 113–175.
[16] Strickland, E. H. (1976) in: Handbook of Biochemistry and Molecular Biology (Fasman, G. D. ed.) Vol. 3, 3rd edn, pp. 141–173, CRC Press, Cleveland.