Association of Myxoviruses with an Adenosine Diphosphatase / Adenosine Triphosphatase as Revealed by Chromatography on DEAE-Cellulose and by Density Gradient Centrifugation

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It has been found that haemolysis by Sendai virus is partially inhibited by adenosine 5'-diphosphate (ADP) or adenosine 5'-triphosphate (ATP) and that a relationship exists between haemolytic and neuraminidase activities of the virus. The finding that influenza viruses are associated with an adenosine diphosphatase (ADP-ase) rose the question of whether this enzyme would also play a role in the manifestation of the haemolytic activity of the virus. We therefore studied the ADP-ase activities of haemolytic and nonhaemolytic myxoviruses. A part of the results, demonstrating a close association between the enzyme, which can also split ATP, and the virus particles, is reported in the present paper.

Materials and methods

The following viruses were used in the form of preparations purified from infected allantoic fluids by two cycles of alternate low (3,000 x g, 20 mins.) and high speed centrifugations (conditions are given in parentheses): Inhibitor resistant variant of A 2 (Singapore) 57 influenza virus (48,000 x g, 40 mins.), previously purified by adsorption onto and elution from rooster red blood cells, the Blackstock strain of Newcastle disease virus (NDV) (40,000 x g, 30 mins.), and Sendai virus (58,000 x g, 60 mins.). The sedimentable component from uninfected allantoic fluids (NC) of 12 day old chick embryos (75,000 x g, 120 mins.) was used in control experiments. The conditions of virus multiplication in chick embryos and the method of determining haemagglutination (HA) titres were those reported previously.

The ADP-ase activities were determined as follows: 0.4 ml. portions of samples in 0.1 M Tris(hydroxymethyl)aminomethane (Tris)-HCl, 0.05 M NaCl pH 7.8 (TS) were mixed with 0.1 ml. of a solution containing 5 μ moles of magnesium salt of ADP (Sigma) and 0.5 μ moles of ethylenediaminetetraacetate (disodium salt) (EDTA) in one ml. of TS, and incubated for 45 mins. at 37 °C. The samples were then immediately chilled in ice and the inorganic phosphorus split off (P) was extracted and determined according to the method of Martin and Doty, adapted to small volumes. Samples containing ADP only served as controls and the results were corrected for traces of P occasionally found in the suspensions tested.

Adenosine tri-phosphatase (ATP-ase) activities were determined under similar conditions, using ATP instead of ADP as substrate. After incubation, 0.1 ml. of a 6% solution of HClO₄ was added to the samples and the decrease of ATP concentration determined using the kit for ATP determination of Boehringer & Soehne. DEAE-cellulose chromatography and sucrose density gradient centrifugation of the viruses listed above and of the NC were performed under conditions which will be published in detail elsewhere. About 150,000 HA units of each virus preparation (the yield from 35 embryos) and NC from 57 embryos were used for chromatography. Chromatographic columns (1 x 20 cm) were prepared, using DEAE-cellulose (Serva, Heidelberg) which had been previously washed with the starting buffer (SB) (0.02 M Tris-HCl pH 7.8). Elution occurred by stepwise addition of 10 ml. quantities of SB containing raising amounts of NaCl (0.05, 0.10 M, etc., concentrations increment = 0.05 M NaCl). 3 ml. portions were collected at a flow rate of 30 ml./hour. Individual fractions were appropriately pooled, their HA titres estimated, and then centrifuged at 60,000 x g for 20 mins.

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60 mins. (75,000 x g for 120 mins. with NC). The pellets were resuspended in TS and the enzymatic activities determined. 10,000 HA units of NDV and A 2 influenza virus, 4,000 HA units of Sendai virus (yields from 3 embryos), each in 0.25 ml. of TS and the NC from 20 embryos were used for sucrose density gradient centrifugation. The NDV preparation employed was previously purified by chromatography on DEAE-cellulose. The suspensions were layered over a continuous sucrose gradient (60—5% w/v) and the tubes were centrifuged in the Spino swinging bucket rotor SW 39 L at 20,000 rev./min. for 15 minutes. Fractions of 0.25 ml. each were collected by puncturing the bottom of the tube. The volume of each fraction was adjusted by addition of TS to one ml., and the HA and enzymatic activities determined.

![Fig. 1](image1.png)

![Fig. 2](image2.png)

**Fig. 1.** HA titres (empty columns) and ADP-ase activities (shaded columns; absolute amount of inorganic phosphorus split off under experimental conditions given in the text) of fractions eluted from DEAE-cellulose columns. Note: In the case of NDV and A 2 influenza virus in some of the fractions (empty columns without shaded parts) ADP-ase activities could not be determined due to great losses of virus during the sedimentation of pooled fractions.

**Fig. 2.** HA titres (dotted lines) and ADP-ase activities (solid lines; absolute amount of inorganic phosphorus split off under experimental conditions given in the text) of fractions from sucrose density gradient centrifugation. Fraction 1 — bottom, Fraction 19 top.

**Results**

The results of DEAE-cellulose chromatography of the virus preparations and NC are summarized in Fig. 1. It is evident that there is a coincidence of peaks corresponding to HA and ADP-ase activities, although each of the three viruses was eluted at a different ionic strength. In no case could an indication of the separation of both activities be observed. The NC seems to be considerably heterogeneous.

The results of sucrose density gradient centrifugation are also in accordance with the concept of a close association between the enzyme and the virus particle. In the case of Sendai and influenza A 2 viruses the peaks of HA and ADP-ase activities did not coincide perfectly, suggesting a heterogeneity of
virus particles in respect to their ADP-ase activities. The density gradient centrifugation of both Sendai and ND viruses revealed two peaks of HA and ADP-ase activities, respectively, the lighter component of NDV corresponding to the noninfectious haemagglutinin described by Rott et alias.\(^6\)\(^7\) and that of Sendai virus corresponding to an analogous component.\(^8\)

ATP-ase activities have been determined in the fractions after DEAE-cellulose chromatography of NC and of A2 influenza and Sendai viruses. The peaks of both ATP- and ADP-ase activities coincided for all preparations, ATP-ase activities in fractions corresponding to the peaks of ADP-ase activities after density gradient centrifugation have also been determined. The average ratios of ATP-ase/ADP-ase activities (decrease of molar concentration of ATP using ATP as substrate per decrease of ADP concentration using ADP as substrate) were 3.6; 4.7 and 1.9 for NC, A2 influenza and Sendai viruses respectively. Since variations of this ratio during a more detailed study of the enzymatic activity of Sendai virus have been observed, no significance can be ascribed to the differences mentioned.

Discussion

An association of virus particles with an ATP-ase has been first observed with avian myeloblastosis virus\(^10\) and with some bacteriophages.\(^11\) The “ATP-ase” of these viruses possesses some features similar to the analogous enzyme of myxoviruses: inhibition by EDTA\(^9\)\(^11\)\(^12\)\(^13\), splitting of both ATP and ADP\(^9\)\(^10\)\(^14\) and competitive inhibition of ATP splitting by ADP\(^9\)\(^15\). Though a correlation between ATP-ase activity and the injection by bacteriophages of the DNA into the host cell could be clearly demonstrated\(^13\)\(^16\)\(^17\)\(^18\), the possible function of this enzyme in the process of cell penetration by myxoviruses, which differs from that of bacteriophages, morphologically intact virus particles being capable of entering a cell\(^19\)\(^20\), remains obscure.

It has been proposed\(^14\) that the viral enzyme represents a “reminiscence” of those parts in the host cell which have participated in the virus synthesis. In this respect it would be of great interest to compare the properties of the viral enzyme with those of subcellular particles coming into question. The activity of the enzyme of myxoviruses has been shown\(^9\) to be insensitive to cardiac glycosides and organic mercurials, indicating that it is probably derived from the microsomes\(^21\)\(^22\).

Whether the ATP-(ADP-)ase activity of Sendai and ND viruses after density gradient centrifugation found in the top fraction is associated with the small haemagglutinin or is derived from NC, must be elucidated by further experiments.

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