Virocidal Cleavage of Disulphide Bonds within Structural Proteins of Sendai Virus

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SUMMARY

Dithiothreitol (DTT), a reagent which cleaves disulphide bonds, destroyed the haemagglutinin, neuraminidase and ‘haemolysin’ of Sendai virus. The treated virus particles appeared morphologically intact. They failed to bind haemagglutination-inhibiting (HI) and neuraminidase-inhibiting (NI) antibodies. Antisera against DTT-treated virus inhibited haemolysis induced by intact virus, although they did not contain detectable HI, NI or virus-neutralizing antibodies. Treated and untreated virus particles were labelled with $[^{14}C]$-iodoacetamide or with $[^{14}C]$-$N$-ethylmaleimide, disrupted with SDS and subsequently electrophoresed in polyacrylamide gels. Thus, polypeptide species with accessible cysteinyl residues, either originally present in intact virus or resulting from reduction of disulphide bonds, were distinguished. The significance of these findings is discussed.

INTRODUCTION

During attempts to elucidate the role, if any, of neuraminidase in haemolysis induced by Sendai virus, we tried to inhibit selectively the enzyme with reagents containing thiol groups (Rafelson, Schneir & Wilson, 1966). Surprisingly, two such reagents, 2-mercaptoethanol (ME) and dithiothreitol (DTT), destroyed the neuraminidase and also the haemagglutinin (HA) and the haemolytic activity of Sendai virus. The HA and neuraminidase were not inactivated when intact influenza viruses were treated with DTT under the same conditions, although interchain disulphide bonds seem to occur in both influenza HA and neuraminidase (Laver, 1971; Stanley & Haslam, 1971; Lazdins, Haslam & White, 1972). Our findings suggested that (a) envelope components of Sendai virus contained disulphide bonds, (b) at least some of these bonds were essential for the biological activity of HA, neuraminidase and ‘haemolysin’ and (c) all or part of these bonds were accessible to DTT or ME when the virus components were assembled within the envelopes of intact particles. An analysis of the interaction between DTT (ME) and Sendai virus is the subject of this report.

METHODS

Propagation and purification of viruses. The influenza viruses $A_2$/Aichi/68, $B$/Mass/66, $A_6$/Taiwan/64, Newcastle disease (NDV) and Sendai viruses were all propagated in the allantoic sacs of chick embryos. The viruses were partially purified by centrifuging at 17,500

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Treatment of viruses by reagents reducing disulphide bonds. One-tenth ml samples of virus suspensions in 0.13 M-NaCl, 0.01 M-tris-acetate, pH 7.0 (TS) (corresponding to a 50-fold concentrate of allantoic fluid), were mixed with an equal volume of solutions containing various dosage levels of either DTT, ME or cysteine hydrochloride (all from Calbiochem, La Jolla, California) in TS. The mixtures were incubated for 20 min at 37 °C and centrifuged at 22,000 rev/min for 5 min in the rotor SW 39 of an L 4 preparative ultracentrifuge (Spinco Division, Beckman Instruments, Palo Alto, California). The virus pellets were either resuspended in 1.0 ml of TS and analysed for haemagglutinin (HA), neuraminidase and haemolytic activity or radioactively labelled as described below.

Radioactive labelling. Sendai virus was labelled in vivo by injecting chick embryos at the time of infection with 10 μCi/embryo of a mixture of [14C]-amino acids (Algal hydrolysate; 1 mCi/mg; International Chemical and Nuclear Corporation, Irvine, California). For in vitro labelling, pellets of intact virus or of virus treated by DTT (final concentration 0.032 M) were suspended either in 0.2 ml of TS containing 10 μCi of iodoacetamide-1-[14C] (71.5 μCi/mg) or in 0.5 ml of TS containing 2 μCi of N-ethyl-[1-14C]-maleimide (9.8 mCi/m-mol). The virus was stored with the reagents (both obtained from New England Nuclear, Boston, Massachusetts) overnight at 4 °C in a dark room. The suspensions were then adjusted to 1.0 ml by addition of TS and centrifuged at 20,000 rev/min for 20 min in the rotor SW 65. The pellets were resuspended in 1 ml of TS, and recentrifuged. The final pellets were used for electrophoresis. The approximate number of reagent molecules incorporated per haemagglutinating unit (H.A.U.) of the virus was calculated on the basis of the specific radioactivity given by the manufacturer and the radioactivity determined from a sample of the treated viruses.

Electrophoresis in polyacrylamide gels. Radioactively labelled virus suspended in 0.025 M-phosphate buffer, pH 7.35, was dissociated in the presence or absence of ME (2.5 %, v/v) by addition of SDS (2.5 %, w/v) and boiling for 90 s. Sucrose and tracking dye (Canalco, Inc., Rockville, Maryland) were added to the samples, which were electrophoresed in 8 % gels containing 0.5 M-urea under conditions described previously (Neurath et al. 1972a).

Density-gradient sedimentation. Intact and DTT- or ME-treated [14C]-amino acid-labelled virus (0.2 ml of each) were layered on top of a 5 to 50 % sucrose gradient in TS (4 ml) and centrifuged for 45 min at 18,000 rev/min in the rotor SW 65.

Preparation of subvirus HA. A suspension of [14C]-amino acid-labelled Sendai virus in TS-0.1 % Tween 80 (0.7 ml) was mixed with an equal volume of diethyl ether for 1 h at 4 °C. The water phase freed from ether was cooled in ice and mixed with 0.15 ml of packed chick red blood cells (RBC). After 20 min the agglutinated RBC were sedimented, mixed with 0.5 ml of TS and incubated 2 h at 37 °C. The eluted HA was used for further studies.

Preparation of antisera. Rabbits were immunized with 0.25 ml of a suspension of intact Sendai virus (7500 H.A.U./ml) mixed with the same volume of Freund's adjuvant, or with an equivalent amount of DTT-treated virus. One week later the rabbits received an additional dose of virus twice as large as the first one. After another week the animals were exsanguinated by cardiac puncture. The γ-globulins were isolated from the sera using the procedure of Perper et al. (1967).

Treatment with trypsin. One-tenth of a ml of DTT-treated or intact Sendai virus (7500 H.A.U.) was mixed with 0.1 ml of trypsin (2 mg/ml in TS; 1 × crystallized; Worthington Biochemical Corporation, Freehold, New Jersey) and incubated 4 min at 37 °C. Soybean
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Trypsin inhibitor (300 μg in 0.7 ml of TS) was added and the sample was centrifuged at 22,000 rev/min for 20 min in the rotor SW 65. The pellet was resuspended in an appropriate buffer and further analysed.

Electron microscopy. Specimens were deposited by capillary tubes on carbon-coated copper grids and negatively stained with 1.0% sodium phosphotungstate (PTA), pH 6.9, or 0.5% uranyl acetate (UAc), unbuffered. The grids were examined in an RCA-EMU-3H electron microscope, equipped with double condenser illumination, at a magnification of x39,000 at 50 kV.

Other methods. Procedures for measurement of radioactivity, titration of HA, determination of neuraminidase, titration of haemagglutination-inhibiting (HI) and neuraminidase-inhibiting (NI) antibodies and measurement of haemolysis were as described before (Neurath et al. 1971, 1972a, b).

In HI-blocking tests 0.5 ml of serial twofold dilutions of DTT-treated virus in TS were mixed each with 0.1 ml of antiserum (against intact virus) diluted so that it would just inhibit haemagglutination by 10 H.A.U. of virus. After the mixtures were kept 30 min at room temperature, 10 H.A.U. (in 0.1 ml) of intact virus were added. Following further incubation for 30 min at room temperature, 0.2 ml of 1% RBC were added to the tubes. The haemagglutination pattern was read after the tubes had been standing for 1 h at 4 °C.

NI-blocking tests were performed in the same way as NI-tests, except that a constant dose of DTT-treated virus was added to each serum dilution in addition to the constant amount of intact virus.

For haemolysis-inhibition (HbI) tests, 0.1 ml samples of appropriately diluted hydroxyethylendiaminetriacetate (HEDTA)-treated virus (Neurath et al. 1972b) were added to serial twofold dilutions of antiserum (0.5 to 1.0 ml). The mixtures were kept 30 min at room temperature. One ml of a 10% suspension of RBC was added to all tubes and haemolysis was determined after 2 h incubation at 37 °C. HbI-blocking tests were carried out in the same way, except that the serum dilutions were preincubated for 30 min at room temperature with a constant dose of DTT-treated virus before the addition of HEDTA-treated virus.

Titrations of infectious virus were performed in 10-day-old chick embryos. The presence of HA in allantoic fluids was considered as an indicator that the virus had multiplied. To determine the virus-neutralizing (VN) titres of antisera, 1500 H.A.U. (in 0.02 ml) of intact virus were added to each of serial twofold dilutions of serum in TS (1.0 ml). The mixtures were incubated at 37 °C for 30 min, at 4 °C overnight, and then injected into chick embryos. The VN-titres of the sera were expressed as the highest dilution at which the infectious titre of the virus was reduced tenfold.

RESULTS

Inactivation of Sendai virus by DTT or ME

Treatment of Sendai virus with solutions containing appropriate amounts of DTT in TS for 20 min at 37 °C resulted in partial or complete loss of HA, neuraminidase, and 'haemolysin'. The extent of inactivation was about the same for all the three activities associated with the virus (Fig. 1). Total inactivation was achieved at concentrations of DTT exceeding 0.01 M (not shown in the figure). Treatment with 0.032 M-DTT resulted in a decrease of infectious titre from 10⁶ to 10⁵ ID₅₀/ml; other concentrations of the reagent were not tested. ME had a similar effect except that about 10 times higher concentrations (in mol/l) of this reagent were required, as compared with DTT, to achieve the same degree of inactivation. Cysteine was even less effective; approximately tenfold reductions of HA-titre and of
neuraminidase activity were observed when the virus was incubated in 0.057 M-cysteine hydrochloride at pH 7.0. Only treatment with 0.032 M-DTT was used for further investigations concerning Sendai virus to ensure the decrease of HA titre and of neuraminidase and haemolytic activities to undetectable levels.

The HA-titre and neuraminidase of three influenza viruses, A~/A~/A~cm/68, B/MASS/66 and A~/TAIWAN/64, remained unaltered after incubation in 0.032 M-DTT or 0.064 M-ME for 20 min at 37 °C. Under the same conditions, the neuraminidase activity and the HA titre of NDV was reduced about fivefold, while 0.002 M-DTT did not affect that virus (compare with Fig. 1).

To establish whether or not DTT or ME affected the physical integrity of the virus particles, treated and untreated preparations of radioactive Sendai virus were submitted to rate zonal sedimentation in sucrose gradients. Intact and treated virus particles were distributed across the gradient equally (Fig. 2). The broad distribution of radioactive particles

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**Fig. 1.** Effect of DTT on the haemagglutinin (●), neuraminidase (○) and haemolytic activity (■) of Sendai virus.

**Fig. 2.** Rate zonal sedimentation (18000 rev/min for 45 min, rotor SW 65) in 5 to 50 % sucrose gradients in TS of[^14C]-amino acid-labelled untreated Sendai virus (bottom) and of virus treated for 20 min at 37 °C by 0.064 M-ME (middle) or 0.032 M-DTT in TS (top). Fraction 1 = bottom of gradient.
Fig. 3. Inhibition of Sendai virus neuraminidase by antiserum to intact virus in the presence of DTT virus, added in 50-fold excess over intact virus (O--O) and in the absence of DTT virus (●●●●); ×···×, inhibition by serum from a non-immunized rabbit.

Fig. 4. Inhibition of Sendai virus-induced haemolysis by (1) antiserum against intact virus in the presence of DTT virus added in twofold excess over intact virus (□···□) and in the absence of DTT virus (■■■); (2) antiserum against DTT virus (●●●); and (3) normal rabbit serum (○○○).

was due to the pleomorphism of Sendai virus (Hosaka, Kitano & Ikekuchi, 1966). No evidence for the release of slowly sedimenting radioactive components from the virus particles was found. Electron microscopy revealed that treatment with DTT failed to disrupt the virus particles but apparently increased the loss of spikes during specimen preparation; untreated virus particles lacked some peripheral spikes to a lesser degree. It was concluded that the destruction of virus-associated biological activities must be due to structural changes which are not reflected in alterations of gross morphology.

Since DTT inactivated HA, it was expected that treated particles would fail to attach to RBC. Indeed, treated radioactive particles were not adsorbed by RBC.

Altered immunological specificity of DTT-treated virus

It was of interest to determine whether or not the DTT-induced functional losses of HA and neuraminidase would also affect their interaction with HI and NI antibodies, respectively.

HI-blocking tests revealed that DTT-treated virus added in 6000-fold excess over intact virus (10 H.A.U.) failed to interfere with the inhibition of haemagglutination by a minimal dose of antiserum against untreated virus. DTT-treated virus also failed to block the inhibition of neuraminidase by the same serum (Fig. 3). On the other hand, the treated virus partially interfered with inhibition of virus-induced haemolysis (Fig. 4, the two curves on the right side). This result suggested that in addition to HI-antibodies (which would prevent the adsorption of the virus to RBC), antibodies to sites on the virus other than HA or neuraminidase may play a role in inhibition of haemolysis. In order to investigate this possibility further an antiserum against DTT-treated virus was prepared. This antiserum inhibited haemolysis (Fig. 4, the two left curves) although it failed to inhibit haemagglutination and had a NI and VN titre of less than 4. Since every batch of serum from unimmunized rabbits inhibited haemolysis to a lesser degree, the γG-fraction was isolated. The γG fraction from the serum against DTT-treated virus inhibited haemolysis while normal rabbit γG did not.
Evidence that treatment by DTT resulted in cleavage of disulphide bonds within structural proteins of the virus

DTT reduces disulphides quantitatively and is capable of maintaining thiols in the reduced state (Cleland, 1964). Therefore, the changes induced in Sendai virus by this reagent were expected to be caused by the reduction of disulphide bonds within virus proteins. If such cleavage of disulphides had occurred, the treated virus should incorporate larger quantities of $[^1C]$-iodoacetamide or $[^1C]$-N-ethylmaleimide (reagents reacting with thiol groups) than intact virus. An enhanced incorporation of these reagents into polypeptides of DTT-treated virus was, in fact, observed (Figs. 5, 6). A single polypeptide species (no. 5), presumably rich in thiol groups, became predominantly labelled when untreated virus was reacted with the radioactive reagents. Treatment with DTT resulted in increased labelling of several additional polypeptide species, which must have contained half-cystine residues. The polypeptide patterns obtained with $[^1C]$-iodoacetamide and $[^1C]$-N-ethylmaleimide-derivatized DTT-treated virus differed from each other. The calculated number of iodoacetamide and N-ethylmaleimide molecules incorporated into the virus as the result of treatment with DTT was $7 \times 10^{10}$ and $3.4 \times 10^{13}$/H.A.U., respectively. Since these two values differed from each other, the number of thiol groups, which became available after virus had been treated with DTT, could not be calculated. Similar problems were encountered when thiol groups of ribosomal proteins were labelled with the two reagents (Moore, 1971). The estimated mol. wts. of the polypeptide species 1 to 6 were 83,000, 71,000, 60,000, 55,000, 40,000 and 19,000.

Fig. 5. Separation of $[^1C]$-iodoacetamide-treated polypeptides from intact (bottom) and DTT-treated (top) Sendai virus by electrophoresis in polyacrylamide gel. ME was present during dissociation of the viruses by SDS.
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The disulphide bonds cleaved with DTT may have been either interchain or intrachain polypeptide linkages. In order to distinguish between these two types of bonds, virus labelled *in vivo* with \[^{14}C\]-amino acids (either unreduced or reduced with DTT) was submitted to electrophoresis in the presence of SDS. Identical mobilities of polypeptide species from DTT-treated and untreated virus particles were considered as an indication that these polypeptides did not participate in interchain linkages.

Only two major polypeptides (nos. 3 and 5) were discerned by electrophoresis of unreduced virus (Fig. 7, bottom). Therefore these polypeptides could not have been involved in covalent interchain disulphide links. A relatively small amount of radioactivity occurred in the gel at the position corresponding to polypeptide no. 1. Radioactive material, probably representing aggregates of polypeptides, remained near the top of the gel. Additional radioactive material failed to penetrate the gel since the total recovery of radioactivity was only 75 % of that obtained with DTT-treated virus. Additional major polypeptides nos. 1 and 4 and a minor component (6) were detected by electrophoresis of reduced virus (Fig. 7, middle). These three polypeptides were thus involved in interchain disulphide links within intact virus particles. Polypeptide 2, which is a glycoprotein (own data to be published), was not separated from the non-glycosylated polypeptide 3 when we used virus labelled *in vivo*. 

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**Fig. 6.** Separation of \[^{14}C\]-N-ethylmaleimide-derivatized polypeptides from intact (bottom) and DTT-treated (top) Sendai virus by electrophoresis in polyacrylamide gel. ME was present during dissociation of the viruses by SDS.
Fig. 7. Separation of polypeptides from $[^{14}C]$-amino acid-labelled Sendai virus by electrophoresis in polyacrylamide gel. Bottom, intact unreduced virus; middle, virus reduced by DTT; top, virus reduced by DTT subsequently treated with trypsin. ME was absent only during dissociation by SDS of unreduced virus.

For this reason, subvirus HA (SHA) was prepared. The resulting preparation was free of virus nucleocapsids and therefore did not contain polypeptide 3 (see Discussion). It consisted predominantly of elongated particles endowed with spikes. The SHA was associated with neuraminidase. Polyacrylamide gel electrophoresis of $[^{14}C]$-amino acid labelled SHA revealed the presence of major polypeptides 2 and 4 and of smaller quantities of polypeptides 5 and 6 (Fig. 8). Omission of ME during dissociation with SDS resulted only in the disappearance of the peaks of radioactivity corresponding to polypeptides 4 and 6, suggesting that polypeptides 4 and 6, but not polypeptide 2, were involved in interchain links.

**Increased susceptibility of DTT-treated virus to trypsin**

Intact virus seemed to be relatively resistant to short treatment (4 min at 37 °C) with trypsin (1 mg/ml), its HA-titre, neuraminidase activity and appearance under the electron
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Fig. 8. Separation of polypeptides from [14C]-amino acid-labelled subviral HA of Sendai virus by electrophoresis in polyacrylamide gel. ME was present during dissociation of HA by SDS.

microscope remained unchanged, but its haemolytic activity was destroyed (Neurath, 1963). Such treatment also failed to release significant amounts of radioactivity from [14C]-amino acid-labelled virus. However, DTT-treated virus, subsequently exposed to trypsin under conditions described above, contained a reduced amount of polypeptide 4 (Fig. 7, top) and had a surface pattern of spikes distinct from that corresponding to intact virus (Fig. 9).

*Failure to reactivate DTT-treated virus by reagents expected to convert thiol into disulphides*

Exposure of DTT-treated virus to the following oxidizing agents (final concentrations in TS are given in parentheses) causing the formation of disulphide bridges: methyl phenyl-diazene carboxylate (Kosower, Song & Kosower, 1969; saturated solution); I₂ (10⁻⁴ M in 5 × 10⁻³ M-KI) and Cu²⁺ chelated with o-phenantroline (Steck, 1972; 5 × 10⁻³ M-CuSO₄, 10⁻⁴ M-o-phenantroline, pH 8.0) for 20 to 30 min at room temperature failed to reactivate the HA and neuraminidase. The reagents themselves did not inactivate the virus. This result suggested that DTT-induced spatial disarrangements within polypeptides of the virus envelope (including the spikes) were sufficient to prohibit the subsequent proper cross-linking because of a lack of juxtaposition of the thiol groups.

**DISCUSSION**

Treatment of Sendai virus with DTT caused the cleavage of disulphide bonds within virus structural proteins. The resulting structural alterations led not only to the inactivation of virus-associated HA and neuraminidase but also changed their immunological specificity. Alterations of immunological specificities resulting from reduction of disulphide linkages were also observed with immunoglobulins (Litman et al. 1970) and other proteins (Thompson et al. 1972). HA, neuraminidase and the haemolytic activity were equally sensitive to inactivation by DTT. The apparent destruction of 'haemolysin' may have been caused by the failure of DTT-treated particles to adsorb to RBC. In this respect, it seemed important that treated particles elicited the formation of HBI antibodies. This observation suggested that 'haemolysin' was either a distinct entity or that blocking of sites, distinct from HA, by antibodies interfered with the function of 'haemolysin'.

A non-random acquisition of disulphide bonds may be an essential step in the assembly of virus components into fully functional particles. It seems possible that the non-haemaglutinating form of Sendai virus, produced in chick embryos inoculated with mouse passage
Fig. 9. (a) Untreated Sendai virus particle. The appearance of the peripheral spikes is "fuzzy". In some areas (small arrow) the spikes appear shorter than on the major part of the virus surface. Occasionally, deposits of negative stain are seen parallel to and about 8 nm from the surface of the virus envelope (wide arrow). (b) Virus treated with DTT and subsequently with trypsin. The surface pattern of spikes is distinct from that of untreated particles; the spikes (about 14 nm long) are clearly defined. Stained with 1 % PTA.
virus (Kato, 1967), lacked some essential disulphide bonds. As shown in this study, the cleavage of disulphide linkages did not result in disruption of the virus, indicating that non-covalent bonds between virus components were sufficient to maintain the morphological integrity of the virus particles.

At least six polypeptides were distinguished by polyacrylamide gel electrophoresis of Sendai virus treated with DTT and subsequently carboxymethylated with [14C]-iodoacetamide (Fig. 5, top). The polypeptide pattern obtained by electrophoresis of [14C]-amino acid-labelled virus was not more complex (Fig. 7, middle). The same number of polypeptide chains, except the minor component 6, was detected by Shimizu, Hosaka & Shimizu (1972) by electrophoresis of unlabelled virus. Others discerned two minor polypeptides, instead of a single one, between the major polypeptides 3 and 5 (Content & Duesberg, 1970; Mountcastle, Compans & Choppin, 1971). These results indicate that all (or all except one) polypeptides of Sendai virus discernible by polyacrylamide gel electrophoresis contain half-cystine residues. If the disulphide bonds within the virus proteins were not reduced by DTT, polypeptide 5 became predominantly labelled as the result of reaction of the virus with either [14C]-iodoacetamide or [14C]-N-ethylmaleimide (Figs. 5 and 6, bottom). Treatment of the virus with DTT led to an even greater incorporation of each of the two radioactive reagents. It was suggested that polypeptide 5 might be the major structural protein of the virus membrane (Mountcastle et al. 1971). This polypeptide was rich in half-cystine residues and was not involved in interchain linkages. This finding appeared to be consistent with the presumed function of this polypeptide, since cysteinyl residues have been implicated in hydrophobic bonds within micelles (Heitmann, 1968). Polypeptide 3, the most abundant protein component of the virus, also did not seem to be involved in interchain linkages. This polypeptide corresponds to the protein subunit of the nucleocapsid (Content & Duesberg, 1970; Mountcastle et al. 1970, 1971). Polypeptides 1, 6 and 4, the last of which is a glycoprotein located in the surface projections of the virus (Mountcastle et al. 1971; Shimizu et al. 1972), contained half-cystine residues involved in interchain linkages. The electrophoretic mobility of polypeptide 2, another virus glycoprotein, seemed to be the same for untreated and DTT-treated SDS-disrupted SHA, suggesting that this polypeptide was not involved in interchain linkages. Since the electrophoretic mobility of glycoproteins in SDS-polyacrylamide gels may depend on factors other than mol. wt., it cannot be excluded that polypeptide 2 may have been involved in an interchain link with another small polypeptide (no. 6?). The actual number of polypeptides in Sendai virus might, perhaps, exceed the number determined by electrophoresis of dissociated virus, since species with similar mol. wt. would not be resolved. If so, the interpretation of experimental results presented here would be partly incorrect.

Treatment of the virus with DTT affected its susceptibility to trypsin. The surface pattern of spikes on untreated and DTT + trypsin-treated particles differed significantly and reproducibly. The ‘fuzzy’ pattern of spikes on intact particles could, perhaps, be explained by the presence of two kinds of spikes differing in length, the shorter spikes being more sensitive to treatment with DTT and trypsin. The occurrence of two distinct populations of spikes could be expected since the virus contains both HA and neuraminidase.

The present study was concerned with the effect of DTT on intact virus. Therefore, only the cleavage of those disulphide bonds, which were accessible to DTT within intact particles, was investigated. The disintegration of the virus into individual components would probably render additional disulphide bonds available to reduction by DTT. Further studies concerning the effect of DTT on virus subunits might, perhaps, contribute to their characterization.
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REFERENCES


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