CONFORMATIONAL CHANGE OF RUBELLA VIRUS SPIKE PROTEINS INDUCED BY 2-MERCAPTOETHANOL

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SUMMARY: Hemagglutinating (HA) activity of rubella virus was inactivated with 2-mercaptoethanol (2ME) in a dose-dependent manner. But even low concentrations of 2ME, which had little effect on HA activity by themselves, greatly increased the sensitivity of spike polypeptides to the subsequent trypsin treatment. Increased trypsin sensitivity was shown by an enhanced reduction of HA activity and an enhanced proteolytic removal of both E1 and E2 polypeptides from the surface of the virion. The findings indicate that 2ME causes an extensive disruption in the conformation of spikes composed of E1 and E2 polypeptides.

Rubella virus has been shown to be composed of three polypeptides, two spike glycoproteins E1 and E2, and one capsid (1,2) and its structure closely resembles that of alphaviruses, another group of Togaviridae, which include Semliki Forest virus (SFV) (3) and Sindbis virus (4).

Treatment of SFV with 2-mercaptoethanol (2ME) was reported to cause a conformational change of spike proteins as shown by the uncovering of hidden epitopes in the mature forms of spikes (5). At the same time, there was a noticeable change in the electrophoretic mobility of E1 and E2 polypeptides of SFV and also Sindbis virus (4,5). In rubella virus as in alphaviruses, the structural
integrity of spikes is thought to depend on intramolecular and intermolecular disulfide bonds as the treatment with 2ME led to the dissociation of heterodimers and homodimers of E1 and E2 polypeptides and altered electrophoretic mobility of these polypeptides (2). The hemagglutinating (HA) activity of rubella virus spikes released from virions by Tween-ether treatment was abolished by 2ME (6). However, which spike polypeptide(s) are affected by sulfhydryl-reducing agents has not been studied yet.

In this report, we describe the effect of 2ME on rubella virus and its polypeptide components more in detail. By 2ME treatment, HA activity of the virus was reduced and the sedimentation velocity of the virion was slightly reduced. More significantly, both E1 and E2 polypeptides were rendered very susceptible to trypsin digestion.

The M33 strain of rubella virus grown in a baby hamster kidney cell line, BHK-21, was used. Propagation of cells and growth and purification of the virus were described elsewhere (7). Purified virus was suspended in TES buffer (10 mM Tris: HCl, pH 7.5, 1 mM EDTA, 100 mM NaCl). A 0.1 volume of 2ME of an appropriate concentration was added to an aliquot of purified virus sample. The virus-2ME mixture was kept at 37 C for 30 min, unless otherwise mentioned, and then chilled at 0 C. After dilution in an appropriate buffer, the 2ME-treated virus was tested for HA activity or subjected to trypsin treatment. HA activity of the virus was assayed by the microtiter method using goose red blood cells at 4 C (8).

Treatment of rubella virus with 2ME resulted in a linear decline of HA activity as a function of time (Fig. 1). Infectivity was reduced in parallel (data not shown). The rate of decline was proportional to the final concentration of 2ME. When the virus was treated with 1% 2ME, HA activity was lost completely within 20 min. To see the additional effect of trypsin treatment, rubella virus with partially inactivated HA activity by treatment with 0.06 or 0.12% 2ME was used. The treatment with 0.12% 2ME for 30 min at 37 C caused 65% reduction in HA activity (Fig. 1) and 73% reduction in infectivity. The sedimentation of 125I-labeled virus to be described below was studied by centrifugation through a linear 10-40% (w/w) sucrose gradient at 38,000 rpm for 4 hr in a Beckman SW41 rotor. Virus treated with 2ME (0.12%) sedimented by two fractions (out of 31) more slowly than untreated virus. As 2ME-treated virus sedimented as a single peak as did untreated virus, it was unlikely that reduced infectivity and HA activity resulted from aggregation of the treated viruses. 2ME-treated virus was then subjected to trypsin digestion as described elsewhere (7). Briefly, a 0.25 volume of trypsin of an appropriate concentration was added to a 2ME-treated virus sample.
Fig. 1. Effect of 2-mercaptoethanol (2ME) on HA activity of rubella virus. Rubella virus was treated with the indicated final concentrations of 2ME at 37 C, and aliquots were taken at the indicated times.

The virus-trypsin mixture was kept at 37 C for 30 min and the reaction was stopped by the addition of a 0.25 volume of a trypsin inhibitor mixture. Although 2ME was not removed and its effect continued during the trypsin treatment, the effect of 2ME at the concentrations employed in Fig. 2 was negligible when compared with that of trypsin. It will be seen that the HA activity was very sensitive to trypsin after 2ME treatment and the trypsin sensitivity increased in proportion to the concentration of 2ME used for pretreatment (Fig. 2). The combined effect of 2ME and trypsin on radiolabeled rubella virus glycoproteins was then studied. Virus metabolically labeled with 1,6-3H(N)-glucosamine hydrochloride (42.5 Ci/m mol, DuPont/NEN Research Products, Boston, MA) was purified from infected culture fluid by centrifugation through sucrose gradient (9) and mixed with approximately 512 HA units of unlabeled virus so as to achieve trypsin treatment at a similar virus-to-trypsin ratio as in the above-described experiment for the effect of trypsin on the HA activity of rubella virus. To prevent the non-specific adsorption of labeled viral materials, all plastic tubes, pipette tips and ultracentrifuge tubes were precoated with silicon and bovine serum albumin.
Fig. 2. Trypsin sensitivity of HA activity of 2ME-exposed rubella virus. Rubella virus either unexposed or exposed to 2ME at a concentration of 0.06 or 0.12% at 37 C for 30 min, was further treated with the indicated final concentrations of trypsin at 37 C for 30 min. HA titer before 2ME treatment was taken as the original titer.

The virus was treated with 2ME followed by trypsin. Trypsin-treated virus was then layered on the top of the discontinuous sucrose gradient as described elsewhere (7). After centrifugation, viruses either intact or trimmed with trypsin recovered in the bottom and the released components in the top fractions were collected separately and subjected to sodium dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) as previously described (9). The exposure of the virus to 2ME markedly changed its trypsin susceptibility. The E1 and E2 polypeptides of control virus withstood up to 0.8 µg/ml of trypsin (Fig. 3A), while the E1 polypeptide of 2ME-exposed virus disappeared completely and so did the E2 nearly completely (Fig. 3B). The E2 polypeptide of 2ME-exposed virus was slightly more resistant to trypsin than the E1 (Fig. 3B) as found in control virus (Fig. 3A). A
Fig. 3. SDS-PAGE of trypsin-treated rubella virus. $^3$H-glucosamine-labeled rubella virus either unexposed (A) or exposed to 0.12% 2ME at 37 C for 30 min (B) was further treated with various concentrations of trypsin. Trypsin-treated virus was separated into pellet and supernatant fractions by centrifugation through a sucrose cushion as described in the text. The pellet and supernatant were analyzed by SDS-PAGE. The number above each lane denotes the final concentration of trypsin in µg/ml and that on right denotes molecular weight (in kd). The 87 kd polypeptide band is indicated with an arrow.

homodimer of E1 (2 × E1 in Fig. 3) and a heterodimer of E1 and E2 (E1 + E2 in Fig. 3) were also digested with trypsin (Fig. 3B). The 87 k dalton (kd) polypeptide associated with the virion was probably derived from one of these dimers (Fig. 3B, pellet). The relatively higher susceptibility of E1 polypeptide in 2ME-exposed virus to trypsin than E2 may be explained by the higher content of cysteine residues in the former (10,11). At least three species of polypeptides with the
Fig. 4. SDS-PAGE of $^{125}$I-labeled rubella virus. Purified rubella virus radiolabeled with $^{125}$I was exposed to 0.12% 2ME at 37 C for 30 min and further treated with trypsin at a final concentration of 0.8 µg/ml at 37 C for 30 min. Trypsin-treated virus was separated into pellet and supernatant fractions as described in the text. The pellet and the supernatant were subjected to immuno-precipitation with a human convalescent serum in the presence of detergent. Lane 1, $^{125}$I-labeled virus; lane 2, immunoprecipitated materials from pellet; and lane 3, immunoprecipitated materials from the supernatant.

The molecular weight of 42 to 50 kd, 34 kd, and 22 kd were released from 2ME-exposed trypsin-treated virus (Fig. 3B, sup).

To determine which polypeptide released the polypeptide products, purified rubella virus was labeled with $^{125}$I with Iodogen (7) and treated with 2ME and trypsin as described above. Trypsin released two cleavage products, polypeptides of 34 kd and 22 kd, from 2ME-treated $^{125}$I-labeled virus (Fig. 4, lane 3). Because the E1 polypeptide is preferentially labeled with $^{125}$I (Fig. 4, lane 1) (7), the above two polypeptides are thought to have been derived from the E1 polypeptide. On the
other hand, the third polypeptide of 42-50 kd released from 3H-glucosamine labeled virus (Fig. 3B, sup) was not seen in the supernatant of 125I-labeled virus. This polypeptide was possibly derived from the E2 polypeptide. Two E1-derived cleavage products in the supernatant were precipitated with a convalescent human serum (Fig. 4, lane 3). Virus-associated polypeptides E1 and of 87 kd were also precipitated with the convalescent human serum (Fig. 4, lane 2).

In contrast to HA activity, the antigenicity of E1 or E2 polypeptide was not affected by 2ME treatment as far as immunoprecipitation with five anti-E1 and one anti-E2 monoclonal antibodies, a rabbit anti-E1 serum and a convalescent human serum is concerned (data not shown).

We have reported that the exposure of rubella virus to pH 5 causes a conformational change shown by the changed trypsin sensitivity. Major cleavage products derived from the E1 polypeptide were virion-associated 41 kd polypeptide and released 50 kd polypeptide (7). Completely different species of cleavage products were generated from 2ME-treated rubella virus by trypsin treatment, indicating that the conformational change caused by 2ME is different from that induced in the acidic environment.

REFERENCES