Haemagglutinin of Measles Virus: Purification and Storage with Preservation of Biological and Immunological Properties

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SUMMARY

Measles virus envelope haemagglutinin (H) was purified rapidly with Triton X-100-solubilized virions by a two-step anion-exchange chromatography using fast protein liquid chromatography. The purity of the glycoprotein in its dimeric form was demonstrated by SDS–PAGE followed by silver staining or autoradiography. The purified H glycoprotein was further freed from contaminating detergent by dialysis of octylglucoside detergent. This purification procedure, together with subsequent lyophilization and storage at −70 °C of the H glycoprotein which was incorporated into phospholipid vesicles allowed the full preservation of its haemagglutinating activity, its reactivity with a monoclonal anti-H antibody that recognized a conformational epitope and its capacity to elicit anti-H antibodies with haemagglutination-inhibiting and neutralizing activities.

INTRODUCTION

Measles virus, which belongs to the Paramyxoviridae family, is involved in the induction of a highly infectious acute respiratory disease and may give rise to major cerebral complications such as acute encephalitis and subacute sclerosing panencephalitis. In vitro studies have shown that antibodies to the paramyxovirus H (haemagglutinin) and F (fusion) haemagglutinating and fusion-inhibiting surface glycoproteins prevent viral infection (Norrby, 1985). It is difficult to draw analogies between the in vivo and in vitro properties of these antibodies. For example, despite the ability of formalin-inactivated measles virus to induce neutralizing and haemagglutination-inhibiting (HI) antibodies, it has been found to be poorly efficient as a vaccine in vivo (Norrby et al., 1975). It has been suggested that important antigenic sites on the glycoproteins are lost during virus inactivation (Norrby et al., 1975). More recently, a monoclonal antibody recognizing a specific epitope of the H glycoprotein while preventing acute infection was found to induce a delayed encephalitis when administered simultaneously with the intracerebral inoculation of a neuro-adapted measles virus strain in mice (Giraudon & Wild, 1985). The use of purified viral surface components with well-defined biological properties as subunit vaccines should further help in clarifying the role of antigenic epitopes of the purified protein in virus–host interactions. The aim of the present study was to purify biochemically the measles virus H glycoprotein and to determine storage conditions allowing the preservation of its biological and immunological properties for subsequent cellular and immunological studies.

METHODS

Cells and virus. African green monkey kidney mycoplasma-free (Vero) cells maintained in RPMI 1629 culture medium supplemented with 5% foetal calf serum were infected at subconfluence with 0.01 p.f.u./cell of the LEC-ki strain of measles virus (Norrby et al., 1982) in 150 cm² tissue culture flasks. The virus was collected from 25 tissue culture flasks on days 4, 5 and 6 in serum-free medium, centrifuged at 2800 r.p.m. for 20 min to eliminate cell debris and kept on ice until use. The supernatants (4.5 l) were pooled and concentrated to 90 ml by ultrafiltration at...
of 1251 was usually very low, only slightly above the gamma counter background. After 24 h of incubation, 1% aprotinin (Sigma) was added, the cells were frozen and thawed once and then disrupted for 20 min at room temperature by adding lysis buffer (LB: 2% Triton X-100, 0.15 M-NaCl, 0.6 M-KCl, 5 mM-EDTA, 1% aprotinin in 0.01 M-Tris –HCl pH 7.8). After ultracentrifugation at 100000 g the supernatant was used as crude soluble radiolabelled viral antigen.

Biochemical purification procedure. The purified viral preparation (1 or 2 ml) was thawed quickly, and after addition of 0.3 mg/ml of phenylmethysulphonyl fluoride (from a 200 mg/ml stock solution in dimethylsulphoxide) ultracentrifuged for 5 min at 160000 g in an Airfuge (Beckman). The virus pellet was solubilized in 1% Triton X-100, 20 mM-piperazine pH 9.5 (1 mg of protein/ml) for 20 min at room temperature, and the insoluble material was removed by a second ultracentrifugation. After filtration through a 0.45 μm filter the soluble viral antigen (SVA) was applied to a Mono Q anion-exchange column (1 ml/min) in 20 mM-piperazine buffer containing 0.03% Triton X-100 followed by elution with a linear 0 to 1 M-NaCl gradient in 30 ml using the fast protein liquid chromatography device from Pharmacia. The three 0.5 ml fractions containing H glycoprotein as determined in a dot immunobinding assay (DIA) (see below) were diluted 1:20 in piperazine buffer containing 30 mM-n-octyl-β-D-glucopyranoside (ODG) and applied for a second run on the Mono Q column which had been re-equilibrated with 30 mM-ODG in 20 mM-piperazine buffer pH 9.5. The elution was performed in stages using NaCl as counter ion in 3 ml of 0 to 0.1 M gradient, then 1 ml of 0.1 M to 0.3 M gradient and 8 ml of 0.3 to 1 M gradient. The three 0.5 ml fractions containing H protein were pooled and immediately treated for subsequent storage. The biochemical purity of the H preparation was assessed by SDS-PAGE using freshly prepared slab gels (Sheshberadaran et al., 1983) or ready-to-use minigels run on PhastSystem (Pharmacia). The protein bands were revealed by ultrasensitive silver staining (Ansorge, 1985).

Elimination of the detergent and storage procedure. To eliminate the ODG detergent, the procedure described by Sechey et al. (1986) was used. Briefly, the purified H glycoprotein solution was dialysed for 24 h against several batches of PBS, initially at 37 °C for 1 h, then at 20 °C. The dialysis tubing was then immersed in SM-2 beads (Bio-Rad) and suspended in a minimal volume of PBS. After 24 h, the SM-2 beads were replaced with fresh ones. The H protein was recovered after 24 h, split into small aliquots, frozen immediately in liquid nitrogen, lyophilized for 4 h and then stored at −70 °C. For incorporation of H glycoprotein into liposomes, an equal volume of 10 mg/ml phospholipids (distearoylphosphatidylcholine, dicetylphosphate and cholesterol in 7:2:1 molar ratio (Gerlier et al., 1983) or ready-to-use minigels run on PhastSystem (Pharmacia). The protein bands were revealed by ultrasensitive silver staining (Ansorge, 1985).

DIA. To detect viral proteins, 8 μl of each fraction collected from the ion-exchange chromatography were spotted on 1 cm² of nitrocellulose (BA85/20; Schleicher & Schüll). After cold drying the nitrocellulose sheets were immersed in PBS containing 10% skimmed milk powder for 20 min and then washed quickly 10 times with PBS. They were incubated with mouse monoclonal anti-measles virus ascitic fluid diluted 1:1000 in milk-PBS for 20 min. After 10 washes with PBS, the nitrocellulose sheets were further incubated for 20 min with 5 to 10 ml of goat Fab anti-mouse Ig (Biosys, Compiègne, France) and radiolabelled with 125I (Gerlier & Avice, 1984). The sheets were washed until no radioactivity could be detected in the supernatant, dried with a hair-dryer and the radioactivity was determined using a gamma counter. In the absence of H glycoprotein, the non-specific binding of 125I was usually very low, only slightly above the gamma counter background.

Haemagglutination and HI assays. These assays were performed as previously described (Norrby & Gollmar, 1975). Briefly, for the haemagglutination assay, 50 μl of 1% fresh vervet monkey red blood cells suspended in PBS were incubated with 50 μl of serial dilutions of measles virus or soluble H glycoprotein in round-bottomed 96-microwell plates for 1 h at 37 °C then 1 h at 4 °C. The last dilution inducing red blood cell agglutination was scored as 1 haemagglutination unit. To test the HI activity of mouse antiserum, the haemagglutination test was modified by preincubating serial dilutions of serum with 4 haemagglutination units of SVA for 30 min at 37 °C before the addition of vervet red blood cells. The highest serum dilution that inhibited SVA-induced haemagglutination was used to define the HI antibody titre.

Neutralization (N) assay. The assay was performed in flat-bottom 96-well plates, by incubating 50 μl of serial dilutions of serum with 20 p.f.u. of measles virus in 100 μl final volume of RPMI 1629 containing 2% foetal calf serum for 1 h at 37 °C. After addition of 104 Vero cells to each well, the plates were incubated at 37 °C in a 5% CO2-supplemented humidified atmosphere for 5 days. The cell monolayer was then stained with methylene blue and the plaques of lysis were scored. The last serum dilution preventing the c.p.e. was used to define the N antibody titre.
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Immunoprecipitation assay. To 50 μl of crude soluble [35S]methionine-radiolabelled viral antigen, 3 μl of mouse serum or monoclonal antibodies directed against H, the nucleoprotein (NP), the membrane protein (M) (kindly provided by P. Giraudon) (Giraudon & Wild, 1985) and F (provided through the courtesy of E. Norrby) were added and incubated in a final volume of 500 μl of LB on ice for 2 h. The immune complexes were precipitated by adding Protein A-Sepharose CL-4B (Pharmacia) according to the procedure described by Sheshberadaran et al. (1983). After dissociation, the radioactive precipitated was analysed by SDS–PAGE, followed by gel soaking in Enlightning (New England Nuclear) and autoradiography using Agfa Gevaert Curix RP2 100AFW films with a Dupont Cronex lightning plus screen. The autoradiographs were scanned using an automatic densitometer.

Mouse immunization. Inbred BALB/cByJlco mice (IFFA-CREDO, St Germain sur l’Arbresle, France) were injected twice subcutaneously with 1 μg purified H material emulsified in complete Freund’s adjuvant with a 3 week interval. The serum was collected from individual mice just before the booster injection and 3 weeks thereafter.

RESULTS

Purification of measles virus H glycoprotein

To determine the best separating conditions for use in ion-exchange chromatography, 0-1 mg of viral proteins solubilized with Triton X-100 and dialysed against the starting buffer were applied to an anion-exchange Mono Q column or cation-exchange Mono S column using different buffers containing 0.03% Triton X-100, followed by elution with a 0 to 1 M-NaCl linear gradient in 30 ml. After analysis of each 0.5 ml fraction in 10% SDS–PAGE, H glycoprotein was found to bind to the cation-exchange column only when running in buffer at or below pH 5.5. From the anion-exchange column, H glycoprotein was eluted at approx. 0.2 M-, 0.35 M- and 0.2 M- NaCl in bis-Tris pH 6.5, Tris pH 8.0 and piperazine pH 9.5 buffers, respectively. These ion-binding properties are overall within the data range expected from the pI 5-2 for H glycoprotein determined by isoelectric focusing analysis (Christie et al., 1981), since the protein should be negatively charged above its pI and positively charged below. However, only elution in piperazine buffer from the anion-exchange column resulted in a relatively sharp peak of H glycoprotein reactivity only slightly contaminated by other proteins as revealed by silver staining (data not shown). The anion-exchange chromatography in 20 mM-piperazine pH 9-5 buffer was further used for rapid (less than 8 h) purification of H glycoprotein. Purified virus (1 to 2 mg) was pelleted by ultracentrifugation, and solubilized for 20 min with 1% Triton X-100 in piperazine buffer. The soluble material was then applied for a first run on a Mono Q column and eluted with 0 to 1 M-NaCl in the presence of 0.03% Triton X-100. The fractions were tested by DIA, the three H glycoprotein-rich fractions were pooled, diluted in 20 ml of piperazine buffer and applied for a second run on the re-equilibrated Mono Q column. The gradient profile used for the elution was modified as described in Methods. Most of the H glycoprotein reactivity was again recovered within three or four 0.5 ml fractions. The elution profile of H glycoprotein and that of the other main viral proteins were compared after the first chromatographic run of the SVA. As illustrated in Fig. 1 (a) NP and M measles virus proteins were eluted in fractions later than those highly enriched in H glycoprotein. F glycoprotein was eluted slightly ahead of H glycoprotein. When the H-rich pooled fractions were applied to the column for a second run using a different shape of NaCl gradient, F and H glycoproteins were eluted in different fractions corresponding to the two major protein peaks. The H glycoprotein seemed to exhibit some microheterogeneity in its elution profile since several sharp H peaks could be observed within the major H peak (Fig. 1b). From DIA data, it could be estimated that the F/H ratio in the H-rich fractions after the second run was reduced to below 0.026 whereas the F/H ratio of the initial SVA was around 0.25 and that of H-rich fractions after the first run around 0.12. The H-rich fractions after the second run were also found to be unreactive with monoclonal anti-NP, or -M antibodies (data not shown) and to migrate in 10 to 15% gradient SDS–PAGE under non-reducing conditions as an almost unique band with an apparent M_0 of 160K (Fig. 2a) in accordance with the reported dimeric form of the H molecule (Christie et al., 1981; Lund & Salmi, 1981). Under reducing conditions, one major band with the expected M_0 of 78K corresponding to the monomeric form of the H glycoprotein and two minor bands were observed (Fig. 2a). These two bands were also seen when using sample buffer containing...
Fig. 1. Elution profile of Triton X-100-solubilized measles virus proteins after anion-exchange chromatography in 20 mM-piperazine buffer pH 9.5. Data obtained from two separate experiments are illustrated: (a) first run, (b) second run of pooled H-rich fractions from the first run. The material was eluted with a 0 to 1 M-NaCl gradient (---) and the optical density (○) was recorded. Fractions were tested for their reactivity with anti-HA (●), anti-F (▼), anti-M (■) or anti-NP (▲) monoclonal antibodies in DIA. The DIA scale was arbitrary. In these experiments, fractions 9 to 11 from the first run and fractions 13 to 16 from the second run were selected as H-rich fractions.

Fig. 2. SDS–PAGE analysis of H glycoprotein purified by anion-exchange chromatography. (a) Silver staining of SVA (lanes 2, 5), H glycoprotein purified after two chromatographic runs (lanes 1, 4) and Mr markers (94K, 67K, 43K, 30K, 20K and 14K) (lanes 3, 6) run on a 10 to 15% gradient gel under non-reducing (lanes 1 to 3) and reducing (lanes 4 to 6) conditions: (b) Autoradiogram of purified [35S]methionine-radiolabelled H glycoprotein (lane 7) and initial crude soluble radiolabelled viral antigen (lane 8) run on a 10% gel under reducing conditions.
mercaptoethanol and so were probably due to interference by mercaptoethanol (or chemical impurities) with the silver staining. The same procedure was used to purify $^{35}$S-methionine-radiolabelled H glycoprotein from a crude soluble radiolabelled viral antigen extract. As illustrated in Fig. 2(b), after autoradiography one major band migrating with an apparent $M_r$ of 78K under reducing conditions was observed when the pooled H-rich fractions from the second chromatographic run were analysed. In addition two minor bands migrating with higher $M_r$ (approx. 120K to 130K and 210K) were observed. These two additional bands, not seen after silver staining, were also observed after immunoprecipitation with a specific anti-H monoclonal antibody and Protein A-Sepharose (see Fig. 3) and after affinity immunopurification of H glycoprotein (B. Mougin, B. Cristau, C. Bottex & D. Gerlier, unpublished data). They may correspond to unreduced dimeric or multimeric forms of H glycoprotein. The biochemical purification process of H glycoprotein was highly reproducible (Table 1). The purified H material was still able to agglutinate vervet monkey red blood cells and this haemagglutination activity was fully inhibited in the presence of either polyclonal anti-measles virus antibodies or monoclonal CL55 anti-H antibody. From data obtained in haemagglutination assay or DIA, the yield was estimated to be 34 to 37% of H reactivity and 9.4 to 14.7% of the initial SVA protein content (Table 1). The specific activity of the purified H glycoprotein fractions could be
Table 1. Elution of H glycoprotein by anion-exchange chromatography on Mono Q in 20 mM-piperazine buffer pH 9.5

<table>
<thead>
<tr>
<th></th>
<th>First run</th>
<th>Second run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial input (mg protein)</td>
<td>0.150</td>
<td>0.300</td>
</tr>
<tr>
<td>Retention volume (ml)</td>
<td>6.610</td>
<td>5.840</td>
</tr>
<tr>
<td>NaCl molarity</td>
<td>0.194</td>
<td>0.168</td>
</tr>
<tr>
<td>Protein recovery (%)</td>
<td>ND*</td>
<td>20.4</td>
</tr>
<tr>
<td>H recovery (%)†</td>
<td>70</td>
<td>54</td>
</tr>
</tbody>
</table>

* ND, Not determined.
† As determined in DIA, the H reactivity of the crude extract being taken as 100%.

Table 2. Stability and antigenicity of stored H glycoprotein preparation

<table>
<thead>
<tr>
<th>H glycoprotein preparation and storage procedure</th>
<th>CL55 binding activity*</th>
<th>Antibody response† after 14 and 35 days antigen storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mM-piperazine pH 9.5, 0.15 M-NaCl, 30 mM-ODG</td>
<td>Time of storage (days)</td>
<td>Antigen storage</td>
</tr>
<tr>
<td>-70 °C</td>
<td>34.9</td>
<td>33.8</td>
</tr>
<tr>
<td>20 mM-phosphate pH 7.4, 0.15 M-NaCl</td>
<td>-70 °C</td>
<td>12.1</td>
</tr>
<tr>
<td>-70 °C, lyophilized</td>
<td>18.6</td>
<td>31.2</td>
</tr>
<tr>
<td>2560</td>
<td>5120</td>
<td></td>
</tr>
<tr>
<td>Phospholipids, 20 mM-phosphate pH 7.4, 0.15 M-NaCl</td>
<td>-70 °C</td>
<td>21.9</td>
</tr>
<tr>
<td>-70 °C, lyophilized</td>
<td>68.0</td>
<td>96.3</td>
</tr>
<tr>
<td>2560</td>
<td>5120</td>
<td></td>
</tr>
<tr>
<td>14500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results expressed as percentage of initial reactivity.
† Mean of two values.

evaluated and could reach one haemagglutination unit/0.26 ng of protein i.e. a threefold to fourfold increase in the specific activity observed with SVA. Although this H specific activity was found to vary somewhat from one experiment to another and could have reflected different degrees of purity, this more probably reflected variation in the extent of denaturation from one experiment to another since, as pointed out below, determination of H activity was very sensitive to denaturation of the protein.

Storage of H glycoprotein

For subsequent studies of the purified H glycoprotein in vivo and in vitro in the absence of any detrimental detergent, the readily dialysable detergent ODG was used instead of Triton X-100 during the second chromatographic run. The purified H material was immediately and extensively dialysed. This procedure allowed the addition of phospholipids dissolved in a large excess of ODG to the H solution before dialysis to form H-sensitized liposomes. The stability of the purified material freed from any detergent was then explored by analysing the H reactivity in haemagglutination tests and DIA. As shown in Table 2, keeping the H glycoprotein solution or the H-sensitized liposomes frozen at -70 °C resulted in a progressive loss in H glycoprotein reactivity with CL55 anti-H monoclonal antibody. In contrast, lyophilization of the material before storage at -70 °C maintained the CL55 reactivity of H-sensitized liposomes for more than 35 days. In addition, despite an initial approx. 80% loss in CL55 reactivity of the H glycoprotein lyophilized in the absence of phospholipids, this reactivity remained stable thereafter. Studies done using the haemagglutination assay gave similar results.
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Immunogenic properties of purified H glycoprotein

The purified H material, prepared and kept frozen as described above, was tested for its capacity to induce a specific anti-H antibody response. Two mice were each immunized twice 3 weeks apart with either soluble H glycoprotein or H-sensitized liposomes, lyophilized or not, and stored for 14 and 35 days at -70 °C. As shown in Table 2, two immunizations of mice with either H-sensitized liposomes, lyophilized or not, or lyophilized H elicited a strong anti-measles virus antibody response with both haemagglutination and N activities, whereas mice that had received the non-lyophilized H material displayed only a low antibody response. Interestingly, the level of antibody response elicited clearly correlated with the respective biological and immunological integrity of the material injected (Table 2). Sera from mice immunized with lyophilized material were also analysed by immunoprecipitation of crude soluble [35S]methionine-radiolabelled viral antigen and SDS-PAGE migration. The four sera immunoprecipitated one major band with an apparent Mr of 78K corresponding to H glycoprotein (Fig. 3). By densitometric analysis of overexposed autoradiographs, this major band was found to represent 84.4 to 89.4% of the immunoprecipitated radioactive material, a value similar to the 87% 78K material immunoprecipitated by the specific anti-H monoclonal antibody. The pattern of immunoprecipitated material was very similar in both cases; immunoprecipitation of some material migrating with apparent Mr of approx. 210K, 120K to 130K (probably unreduced polymeric forms of H), and 67K to 69K (probably an unglycosylated form of H). However, the sera from purified H-immunized mice also precipitated some material (around 2.5% of the immunoprecipitates) in bands migrating closely with the F1 subunit and other unidentified material (7% of the immunoprecipitates) which migrated with apparent Mr of 51K and 58K (Fig. 3). These two bands might correspond to unglycosylated and glycosylated forms of the uncleaved F0 precursor (Hasel et al., 1987). This material was not immunoprecipitated by preimmune mouse sera, and may be due to the coprecipitation of some F glycoprotein non-covalently linked to H glycoprotein. Alternatively, it may represent either a slight immune response against undetected minor contamination of the purified H preparation by F material or the recognition by polyclonal anti-H antibodies of H degradation products not recognized by the specific anti-H monoclonal antibody.

DISCUSSION

This report describes the successful rapid biochemical purification and storage of measles virus H glycoprotein in its dimeric form. The method is based on two-step anion-exchange chromatography and as much as 35% of the initial H glycoprotein content could be recovered in a form quite pure biochemically and immunogenically in less than 8 h. However, the preparation could be slightly contaminated by some F glycoprotein, the ion-binding capacities of which are very close to that of H glycoprotein. Greater purity would probably be achieved by adding a non-detrimental step of specific absorption onto anti-F immunoaffinity beads as proposed recently by Varsanyi et al. (1987). The procedure allows the subsequent elimination of any contaminating detergent by using the readily dialysable detergent ODG during the second chromatographic run. It was also shown that the purified H glycoprotein can be stored for several days at -70 °C after lyophilization, and its biological activity (haemagglutination) and recognition by an anti-H monoclonal antibody specific for a conformational determinant can be fully conserved provided that H glycoprotein is associated with artificial membranes. The purification of measles H glycoprotein has been reported previously using lectin affinity in conjunction with gel filtration (Christie et al., 1981; Lund & Salmi, 1981) or immunoaffinity (Bellini et al., 1981; Varsanyi et al., 1984) chromatography. These techniques required the desorption of the material using high salt (3 M-KSCN), 8 M-urea, 1% sodium deoxycholate or low pH (0.2 M-glycine pH 3-0) solutions, i.e. mildly to highly denaturing conditions, and the recovery of the haemagglutination activity of the purified material was either not reported or unquantified. The level of biological and CL55 anti-H binding activity of purified and stored H glycoprotein was found to determine the level of antibodies with biological properties (anti-haemagglutination and virus infection N activity) it was able to elicit in vivo. This indicates that preservation of the native conformational form of a viral glycoprotein can be a critical
parameter to produce the biologically relevant antibodies in vivo. Similarly, the relative inefficiency of an immunopurified Epstein-Barr virus glycoprotein compared to its biochemically purified counterpart has been demonstrated in the induction of in vivo protection of vaccinated monkeys (Epstein et al., 1986).

The role of the H membrane component of paramyxoviruses in the induction of immune protection against virus-induced diseases is still being debated. An apparently normal anti-H antibody response observed after immunization with formalin-inactivated measles virus was found to be unable to protect efficiently against the virus-induced disease (Norrby et al., 1975). By contrast, full passive protection of mice against intracerebrally inoculated measles virus could be observed with several anti-H antibodies that exhibited anti-haemagglutination activity (Giraudon & Wild, 1985). However, one of these anti-H monoclonal antibodies, CL55, although protecting mice against acute measles virus-induced encephalitis, instead induced a delayed encephalitis.

This purification and efficient storage procedure for measles virus H glycoprotein in a biologically active form should allow further insight into the role of the conformational antigen epitope recognized by monoclonal antibody CL55 in the induction of experimental delayed encephalitis, as well as in the relative efficiency of various antigen vectors, such as artificial membranes or immune-stimulating complexes, proposed for the building of subunit vaccines (see Morein & Simons, 1985, for review). In addition, the availability of biosynthetically radiolabelled purified H glycoprotein will enable us to explore further the intracellular events occurring during antigen processing by accessory cells and their relevance in specific recognition by immune T cells.

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REFERENCES


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