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The SU Glycoprotein 120 from HIV-1 Penetrates into Lipid Monolayers Mimicking Plasma Membranes

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Abstract. Increasing evidence suggests that the HIV envelope binds through its surface (SU) gp120 not only to receptors and coreceptors, but also to other components of the cellular membrane where the glycolipids appear to be good candidates. To assess the ability of HIV-1 SU gp120 to penetrate into phospholipid membranes, we carried out a study of the interactions between a recombinant SU gp120 from HIV-1/HXB2 and artificial lipid monolayers mimicking the composition of the outer leaflet of the lymphocytes and which were spread at the air-water interface. We show that the protein, in its aggregated form, has amphipathic properties and that the insertion of this amphipathic species into lipids is favored by the presence of sphingomyelin. Furthermore, cholesterol enhances the penetration into mixed phosphatidylcholine-sphingomyelin monolayers. Coexistence of different physical states of the lipids and thus of domains appears to play a major role for protein penetration independently of the presence of receptors and coreceptors.

Key words: Lipid monolayers — Protein insertion — gp120 — Amphipathic properties — Lipid domains

Introduction

It is generally admitted that HIV-1, HIV-2 and SIV strains interact with cells through binding of the viral envelope glycoprotein gp120 to membrane receptors of target cells, this binding promoting the fusion process. However, some questions remain open due to the fact that the expression of CD4 and CXCR4 in some cell types did not confer susceptibility to infection by all HIV-2 isolates. This suggested that another factor may influence the ability of HIV-2 to penetrate human cell types that express the appropriate receptor(s) (McKnight et al., 1998). This point was also addressed on the basis of experiments dealing with heat and protease-insensitive components of red blood cells (Dragic, Picard & Alizon, 1995; Puri et al., 1996). All these observations suggest that another nonproteic membrane component could be involved in gp120 cell binding.

Among the nonproteic membrane components, glycosphingolipids are known to interact with a large number of proteins to mediate biological functions such as cell adhesion and can also act as a type of receptors for viruses, bacteria or other parasites (Nieva et al., 1994). Galactosylceramide (GalCer) and/or its sulfated derivative (SgalCer), and glycosphingolipids, which are mainly expressed in intestinal and neural tissues, have been shown to mediate HIV gp120 binding to some $CD4^-$ cells (Harouse et al., 1991; Fantini et al., 1993; Gadella et al., 1998; Hammache et al., 1998*a*). It has also been reported that GalCer is involved in the transcytosis of infectious HIV-1 across epithelial cells (Bomsel, 1997) and that the binding of gp120 to the neutral glycosphingolipid Gb3 is required for CD4/CXCR4 dependent fusion (Puri et al., 1998) while GM3 is directly associated to the CD4 for promoting the fusion process (Sorice et al., 1997; Hammache et al., 1999). The role of sphingolipids in the fusion process was recently confirmed by examination of the influence of L-cycloserine. Indeed, this compound is a potent inhibitor of serine palmytoyl transferase, which initiates the biosynthesis of sphingolipids (Williams et al., 1987) and was shown to inhibit HIV-1 cytopathic effects, replication and infectivity

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(Mizrachi et al., 1996). However, these observations showed that glycosphingolipids played an important role in the inhibition process of the viral infection but did not address the question of a possible direct interaction between gp120 and membrane lipids. To assess the ability of HIV-1 gp120 subunit to bind directly phospholipid membranes, we undertook a study using model membrane systems at the air-water interface. Such an approach is appropriate since the thermodynamic relationship between monolayer and bilayer membranes is direct and the monomolecular films at the air-water interface overcomes limitations such as regulation of lipid lateral-packing density and lipid composition which occur in bilayers (Brockman, 1999). One of the major outcomes of this study is that gp120 can increase the surface pressure of monolayers with a lipid composition close to that of lymphocyte plasma membranes (Aloia, Tian & Jensen, 1993) and that gp120 inserts into these monolayers.

Materials and Methods

Chemicals

All lipids, namely natural erythrocytes bovine sphingomyelin (SM), monosialoganglioside (GM_3), and synthetic Lα-phosphatidylcholine β-palmitoyl-γ-oleoyl (POPC) and cholesterol were purchased from Sigma (St. Louis, MO). Solvents: chloroform, methanol and phosphate buffer products were from Merck (Darmstadt, Germany). Sodium chloride salt was baked before use and water was tridistilled (once on MnO_4K).

Protein Production

Recombinant monomeric gp120 protein was produced in baculovirus system as previously described (Missé et al., 1998). Briefly, the recombinant soluble gp120 was subcloned in p119P baculovirus transfer vector. Insect SF9 cells were cotransfected by a modified baculovirus expression vector AcSLP10 and by the recombinant p119P vector. Recombinant baculovirus were plaque purified. SF9 cells were infected at a density of 5×10^5 cells/mL and at a multiplicity of infection of 5 PFU/cell. Cell supernatant was collected 6 days postinfection and gp120 was concentrated and immmunopurified by chromatography with the anti-gp120D,7324Ab (Aalto, Dublin, Ireland) linked to bromoacetyl-sepharose. The purity of the produced gp120 was verified by separation on sodium dodecyl sulfate-Phast Gel gradient (Pharmacia, Upsalla).

Methods

Surface pressures were measured by the Wilhelmy plate method using a platinum plate and a Prolabo tensiometer (Paris, France) and a Kipp and Zonen (Delft, The Netherlands) X-Y recorder model BD 91 as previously reported (Vidal et al., 1998). The lipid monolayer was obtained by spreading a chloroform solution of the lipid at the air-water interface. At a selected initial surface tension, aliquots of an aqueous solution of gp120 were injected in the subphase. Measurements were made at equilibrium which was obtained after 2 to 3 hr of gentle stirring with a magnetic stirrer. The subphase was a solution of 0.154 M NaCl buffered at pH = 7 with 0.1 M phosphate buffer.

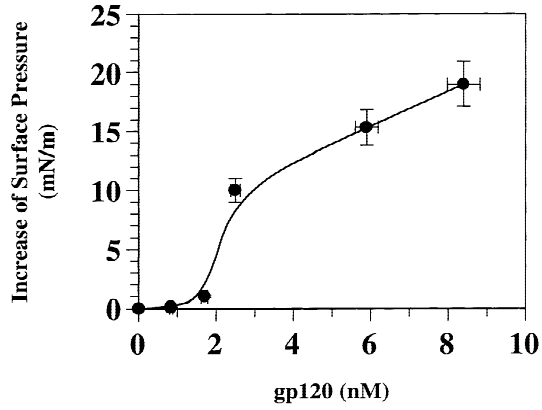

Fig. 1. Variation of the surface pressure of the air-aqueous buffer interface upon increasing the concentration of gp120 in the subphase.

Results

Adsorption at the Air-Aqueous Buffer Interface

Without Lipid

To identify whether the gp120 has amphipathic properties, the protein was allowed to adsorb at the surface of an aqueous buffer. The surface pressure of the adsorbed monolayer, defined as the surface tension of the subphase, was measured for increasing concentrations of protein in the subphase. Figure 1 shows that the surface pressure increases when increasing the gp120 concentration in the bulk and reveals an abrupt increase when the gp120 concentration is between 1.6 nM and 3 nM.

In the Presence of Lipids

The ability for the protein to insert into lipids was determined by measuring the increase of the surface pressure of a lipid monolayer spread at the air-aqueous buffer interface at a selected initial surface tension (Rafalski, Lear & DeGrado, 1990) when injecting aliquots of an aqueous solution of the protein in the subphase. Owing to lipidic composition of lymphocyte plasma membranes (Gottfried, 1967) we have selected phosphatidylcholine and sphingomyelin.

Pure Lipids

The first part of the study was devoted to the identification of the interactions occurring between gp120 and monolayers made of pure phosphatidylcholine or sphingomyelin. To be as close as possible to natural mem-

Table. Critical pressures of insertion of gp120 into various lipid media in a monolayer situation

Pur POPC	Pur SM	SM/POPC 1/3	SM/POPC 3/1	SM/POPC/Chol (1/3)/0.6	GM3/POPC 1/3
19.5 mN/m	38 mN/m	25 mN/m	34 mN/m	35 mN/m	29 mN/m

brane conditions, palmitoyl-oleoylphosphatidylcholine (POPC) (with an unsaturated hydrocarbon chain) and sphingomyelin (SM) of beef erythrocytes were selected.

The gp120 protein was injected into the subphase of POPC and SM monolayers at an initial pressure of 14 mN/m, a pressure that is close to the maximum obtained for the protein at the air-water interface. The rise of the surface pressure reflects the interactions of the protein with the lipids (Rafalsky et al., 1990). Figure 2*A* shows the variation of the surface pressure induced by the presence of gp120 for monolayers made of POPC or SM. For both lipids which are in the same physical state (liquid expanded) at this initial pressure (Maggio, Cumar & Caputto, 1978), the general trend is almost the same. After a marked increase for protein concentrations below 3 and 2 nM for SM and POPC, respectively, an inflexion occurs which is reminiscent of that found for compounds characterized by a CMC.

Since the penetration of gp120 into lipid monolayers depends on the surface density of lipids, and in order to determine the critical pressure of insertion above which the protein no longer inserts into the monolayer (Maget-Dana et al., 1995), the surface pressure increase was measured at different initial pressures of lipids using a constant concentration of gp120 in the subphase. The results are shown in Fig. 2*B* and indicate that for a concentration of gp120 of 4 nM, the extrapolated maximum pressure of insertion observed within the pure POPC and SM monolayers is 19.5 mN/m and 38 mN/m, respectively.

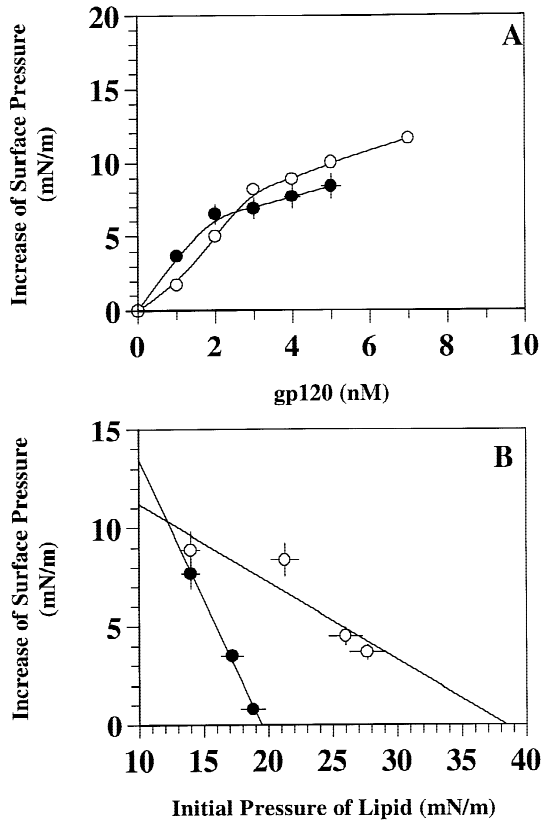

Fig. 2. (*A*) Variations of the surface pressure of lipid monolayers at initial pressures of 14 mN/m upon increasing the concentration of gp120 in the subphase ● POPC lipid monolayer. ○ SM lipid monolayer. (*B*) Determination of the critical pressure of insertion in lipid monolayers at a gp120 concentration of 4 nM. ● POPC lipid monolayer. ○ SM lipid monolayer.

Mixtures of Lipids

To assess the penetration of gp120 into mixed monolayers mimicking the outer leaflet of lymphocyte membranes, mixed POPC/SM monolayers were used at two different molar ratios: 3/1 and 1/3. Figure 3*A* shows that when POPC is the major compound, (POPC/SM 3/1) adsorption is favored for gp120 concentrations lower than 3 nM. In contrast, when SM is the major compound (POPC/SM 3/1) adsorption is favored for concentrations of gp120 higher than 3 nM. Figure 3*B* shows the extrapolations for the determinations of the critical surface pressures of insertion into the mixed monolayers using a protein concentration of 4 nM close to that used by other authors (Hammache et al., 1998*a*,*b*). These values are 24.5 mN/m and 34 mN/m for POPC/SM at the 3/1 and 1/3 molar ratios, respectively.

Influence of Cholesterol on the Penetration of gp120 into Mixed Lipid Monolayers

Cholesterol is present in all plasma membranes and its presence modifies their fluidity (Raffy & Teissié, 1999). It was therefore of major importance to check its influence on the gp120-lipid interactions. For this purpose a lipid/cholesterol mixture was used at a molar ratio of 1/0.6 where the lipid component was the POPC/SM mixture at the 3/1 molar ratio. Figures 3*A* shows the variation of the surface pressure when increasing the concentration of gp120 and reveals that cholesterol favor the penetration for concentrations of gp120 higher than 3 nM. Figure 3*B* which allows the determination of the critical

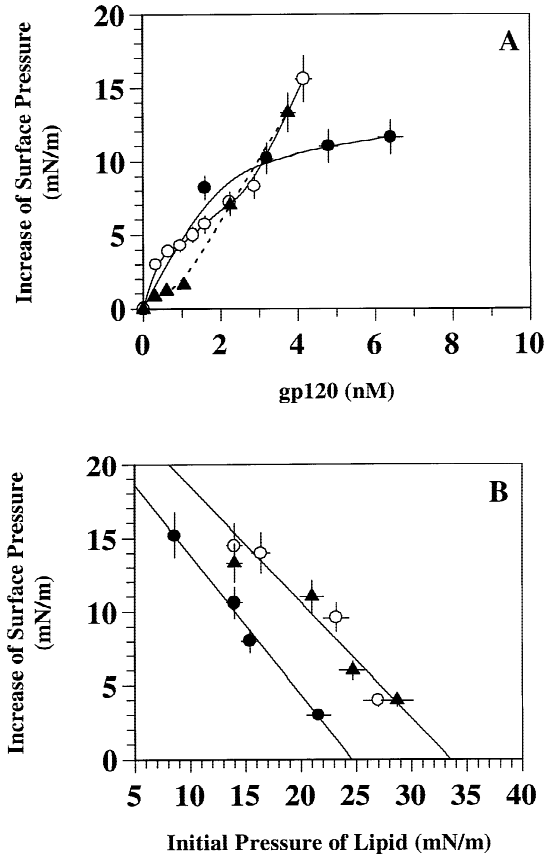

Fig. 3. (*A*) Variations of the surface pressure of mixed lipid monolayers at initial pressures of 14 mN/m upon increasing the concentration of gp120 in the subphase ● POPC/SM 3/1. ○ POC/SM 1/3 and ▲ (POPC/SM)/cholesterol (3/1)/0.6. (*B*) Determination of the critical pressure of insertion in lipid monolayers at a gp120 concentration of 4 nM of the corresponding mixed monolayers.

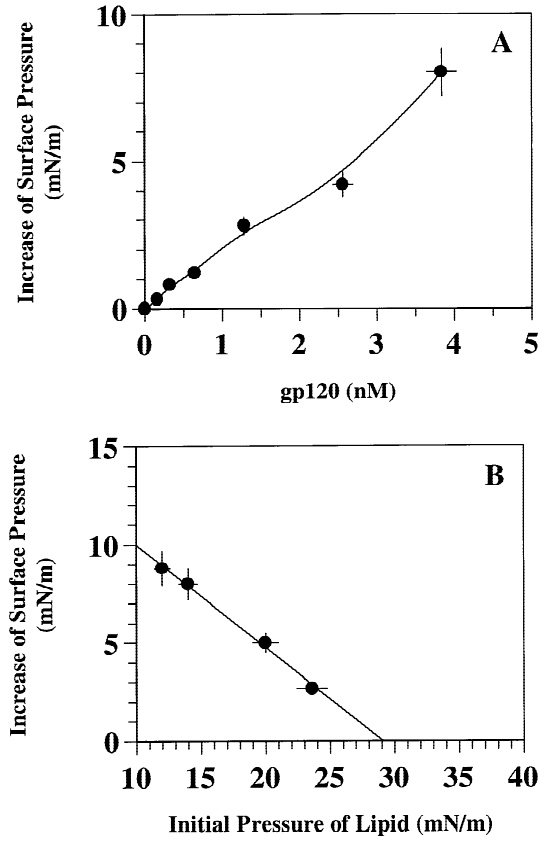

Fig. 4. (*A*) Variations of the surface pressure of a mixed lipid POPC/GM3 3/1 monolayer at an initial pressure of 14 mN/m upon increasing the concentration of gp120 in the subphase. (*B*) Determination of the critical pressure of insertion in lipid monolayers at a gp120 concentration of 4 nM of the corresponding mixed monolayer.

pressure of insertion indicates that upon addition of cholesterol to the mixed lipid monolayer, this pressure of insertion is increased from 24.5 to 34 mN/m.

Influence of the Presence of GM3 on the Penetration of gp120 into Mixed Lipid Monolayers

To assess the influence of the nature of the polar headgroups of sphingolipids on the penetration of the SU gp120 into mixed lipid monolayers, two mixed monolayers differing only by the nature of the sphingolipid have been compared. The two lipid mixtures (POPC/GM3 and POPC/SM) were at a molar ratio of 1/3 and as above the initial pressure was 14 mN/m. The variation of the surface pressure by increasing the gp120 concentration is reported in Figs. 3*A* and 4*A*. Comparison of these figures indicates that the presence of GM3 lowers the pressure increase compared to SM (9 mN/m compared to 14 mN/m at a gp120 concentration of 4 nM). However, the presence of GM3 increases the critical pressure of insertion (*see* Fig. 4*B*): 29 mN/m which has to be compared with 24.5 mN/m for POPC/SM.

Discussion and Conclusion

This work shows that the SU gp120 exhibits amphipathic properties that are required for an adsorption at a lipid containing air-water interface. Taking into account the globular shape of gp120 (Rizzuto et al., 1998) the strong increase of the surface pressure can be attributed to the formation of amphipathic oligomer species which are probably formed after a partial unfolding of the protein facilitating thus hydrophobic interactions. This unfolding is reminiscent of that occurring for a protein when engaged in an anisotropic interface such as the air-water interface, a phenomenon sometimes called surface denaturation (Andrade, 1985; Green, Hopkinson & Jones, 1999).

Among the lipids composing the lymphocyte plasma membrane, phosphatidylcholine (PC) is the major

compound (43.6%) and is mainly (80%) located in the outer leaflet. SM which represents 10% of the lipids is also essentially located in this leaflet (Gottfried, 1967). There is a balance between the distribution of these two lipids (Hildebrand, Marique & Vanhouche, 1975) and in a comparative study, Aloia et al. (1993) have described an increase of PC and a decrease of SM for cells infected by either HIV-1_{RF} or HIV-2_{LAI}. Using a single component monolayer approach (Fig. 2*A*) we have shown that the interactions between SU gp120 from HIV-1/HXB2 and pure POPC and pure SM are almost identical for a lipid initial pressure of 14 mN/m (liquid expanded state). From the critical pressure of insertion (Fig. 2*B*) it can be stated that the protein could insert spontaneously (Demel et al., 1975) into membranes made of pure SM (critical pressure of insertion 38 mN/m) and not in POPC membranes (19.5 mN/m). Since POPC remains in the liquid expanded state whatever the surface pressure while a liquid expanded to liquid condensed phase transition for SM begins at 17 mN/m (Maggio et al., 1978; Koynova & Caffrey, 1995; Smaby et al., 1996*a*) the differences between the critical pressure of insertion reflect the higher affinity of gp120 for lipids in phase separation (Dumas et al., 1997).

When considering mixed monolayers, examination of the variations of the surface pressures for different POPC/SM molar ratios reveals that the insertion is strongly dependent on this molar ratio. It can be stated that for gp120 concentrations higher than 3 nM, the insertion of the protein is favored when SM is the major compound (for example 1/3 in the present case). In addition, the critical pressure of insertion suggests that gp120 could insert spontaneously for membranes where SM is the major compound. Therefore, the existence of domains in phase separation will strongly favor the protein insertion.

This situation is slightly modified when cholesterol is present in the lipid mixture. Examination of the lipidic composition of human lymphocytes reveals that the cholesterol-phospholipid molar ratio is 0.6 (Gottfried, 1967). Cholesterol has a high affinity for phosphatidylcholine (Demel et al., 1977) and induces a condensing effect (Demel et al., 1967; Smaby et al., 1996*b*; Mattjus & Slotte, 1996; Ramstedt & Slotte, 1999) and thus favors the coexistence of lipids in different physical states (liquid condensed or LC and liquid expanded or LE) which can generate a coexistence of domains in phase separation. Moreover, the cholesterol content varies with infection of cells as reported by several authors (Aguilar et al., 1991; Aloia et al., 1993) who have underlined a change of fluidity of lymphocytes infected by HIV associated with a change of the cholesterol/phospholipids ratio from 0.6 to 0.3 for infected *vs.* uninfected cells, respectively. Analysis of Figs. 3*A* and *B* indicates that cholesterol has a comparable effect on gp120 penetration as SM: in the mixture SM/POPC1/3 it induces a decrease of the penetration for concentrations of gp120 lower than 3 nM while an increase of penetration is observed for concentrations higher than 3 nM. Finally the critical pressure of insertion in a POPC/SM 3/1 monolayer with cholesterol is identical to insertion in a POPC/SM 1/3 monolayer without cholesterol. The addition of cholesterol to monounsaturated lipids such as POPC is known to induce condensation (Smaby et al., 1994). We note also, that penetration of gp120 into a SM monolayer at an initial pressure of 14 mN/m induces an increase of pressure of 8 mN/m; the final pressure thus obtained (22 mN/m) is within the LE-LC phase transition pressure of SM. In every case, addition of cholesterol can induce a LE-LC phase transition in monolayers increasing thus the number of domains. Therefore, this increase of number of domains will facilitate the penetration of proteins at the frontiers of the domains (Netz, Andelman & Orland, 1996).

The last point we want to stress is related to the possible influence of the nature of the headgroups of the sphingolipids. Indeed, beside the above-mentioned compounds, the outer leaflet of the plasma membrane also contains a pattern of glycosphingolipids including gangliosides which can act as receptors. The results shown here for a POPC/GM3 mixture at a molar ratio of 3/1 reveal that GM3 enhances slightly the critical pressure of insertion compared to SM.

Several factors can contribute to lipid-protein interactions: electrostatic interactions with the polar headgroups of the lipids, Van der Waals interactions involving the acyl chains and the physical state of the lipids.

(i) Headgroups. Concerning pure lipid monolayers the interactions between gp120 and SM and POPC are similar. Although both have the same phosphocholine polar headgroup, it was shown, on the basis of surface potential experiments, that SM carries a net positive charge (Sha & Schulmann, 1967). This was attributed to ion-dipole interactions arising between the hydroxyl group of sphingosine and the phosphate group. In addition, together with the fact that GM3 (which is a negatively charged) has a high affinity for gp120 (Hammache et al., 1998*b*), it can be stated that the charge carried by the polar headgroups of the lipids does not play any role in the interaction process.

(ii) Physical state. A high penetration level of gp120 into glycosphingolipids and gangliosides has been shown in preceding works with a high affinity for Galcer HFA and GM3 (Hammache et al., 1998*a,b;* Fidelio, Maggio & Cumar, 1986; Maggio, Cumar & Caputto). Recalling that Galcer HFA has two phase transitions and GM3 in the presence of salt has one phase transition (Maggio et al., 1978), it appears that the common mechanism to obtain a high yield of insertion in pure lipid monolayers is the existence of a phase transition at room temperature. On the basis of this last property, i.e., the existence of do-

mains which indicate that when LE and LC phases coexist, the insertion of gp120 very probably at the domain separation is similarly facilitated and can correspond to the situation which is also found for mixed monolayers. Similarly, cholesterol favors domain formation and thus will facilitate the penetration of gp120. The importance of domains is confirmed by several authors (Sato, Serizawa & Okahata, 1994, 1996; Sato et al., 1998) who demonstrated that matrix lipids surrounding GM3 influences the binding of Wheat Germ Agglutinin (WGA) and influenza A virus.

In conclusion, the work reported here shows that the SU gp120 from HIV-1/HXB2 can penetrate and interact with lipids composing the outer leaflet of the plasma membrane when in a monolayer situation independently of the presence of CD4 receptors, coreceptors and sugars and that the existence of a phase transition is one of the major factors that favors the penetration of the protein. Comparison of POPC/SM and POPC/GM3 monolayers indicates that insertion is facilitated for the former mixture and that cholesterol enhances this penetration process. The finding of a critical pressure of insertion of 30 mN/m, a value close to that corresponding to the lipid density of cellular membranes suggests that gp120 could spontaneously insert into biological membranes which have the appropriate lipidic composition.

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