Activity of herpes simplex virus type 1-specified glycoprotein C antigenic site II epitopes reversibly modulated by peripheral fucose or galactose units of glycoprotein oligosaccharides

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We have previously shown that the herpes simplex virus type 1 (HSV-1)-specified glycoprotein C (gC-1) produced in epitheloid cells contains epitopes of peptide nature, which are dependent on galactose of oligosaccharides for their expression. In the present communication we report that these epitopes are expressed in a mouse neuroblastoma cell line (C1300) with low levels of galactosyl transferases. However, in place of galactose the glycoprotein from C1300 cells was found to contain oligosaccharides with additional fucose units. Fucosidase treatment, but not galactosidase treatment, abolished the antigenic activity of the carbohydrate-dependent epitopes. Altogether the results indicated that the carbohydrate-dependent epitopes of gC-1 from C1300 cells were stabilized by peripheral sugars of N-linked oligosaccharides rather than O-linked ones and that fucose could substitute for terminal galactose in promoting the activity of the carbohydrate-dependent epitopes. This is the first demonstration of the involvement of fucose in the establishment of a carbohydrate-dependent epitope of peptide nature. The results also demonstrated that reversible carbohydrate–peptide interactions were responsible for the activity of the carbohydrate-dependent epitopes.

Introduction

The glycoproteins of enveloped viruses, being exposed both on the virion and on the surface of infected cells, constitute important targets for humoral and cellular immune responses (for a review, see Datema et al., 1987). As far as is known the carbohydrate composition even of genetically large viruses such as herpes simplex virus (HSV) is specified entirely by the host cell genome (Campadelli-Fiume & Serafini-Cessi, 1985; Lundström et al., 1987b). An immediate consequence of this host dependence is the expression of multiple host structures on the surface of the viral glycoprotein, which may act to diminish antiviral immune responses in an infected individual (Datema et al., 1987). For example, Skehel et al. (1984) have shown that the selection pressure on influenza virus might result in the creation of new glycosylation sites whose oligosaccharides are engaged in masking of previously available epitopes. Such data suggest that controlled deglycosylation could be an alternative strategy both for prophylactic and therapeutic strategies.

However, the feasibility of such strategies is restricted by the fact that the interplay between viral glycoproteins and their carbohydrate complements are complex processes (Rademacher et al., 1988), involving both positive and negative modulation of antigenic properties (Sjöblom et al., 1987; Huso et al., 1988). Accordingly, several reports indicate that complete enzymic deglycosylation (Alexander & Elder, 1984) or prevention of N-glycosylation by tunicamycin treatment of infected cells could result in loss of antigenic reactivity of glycoproteins specified by several enveloped viruses (Kaluza et al., 1980; Merz et al., 1981; Long et al., 1986). Moreover, recent data indicate that even changes in the peripheral composition of the large complex-type N-linked oligosaccharides have a significant potential to modulate the immunological properties of viral glycoproteins (Sjöblom et al., 1987; Huso et al., 1988). All these data emphasize the need for information about the mechanisms by which carbohydrates interact with the protein component to modulate its conformation and antigenic properties.

To this end we have developed an experimental system to show the influence of peripheral carbohydrate determinants on antigenic properties of viral glycoproteins and to identify specific carbohydrate determinants, acting as positive or negative modulators of antigenicity of a model protein (Sjöblom et al., 1987). Our system includes sequential degradation of oligosaccharides of
the HSV-1-specified glycoprotein C (gC-1) coated onto microplates and an ELISA-based detection system to demonstrate changes in the reactivity of specific antibodies (Sjöblom et al., 1987). This glycoprotein contains a number of small O-linked oligosaccharides, clustered in a pronase-resistant array in the amino-terminal region of the protein, and a maximum of nine N-linked oligosaccharides, as outlined in Fig. 1. By comparing the antibody reactivity to that of a panel of lectins in an enzyme-linked assay we were able to correlate transient changes in antigenicity with discrete steps in deglycosylation (Sjöblom et al., 1987). Two different classes of epitopes, tentatively designated as carbohydrate-dependent [antigenic site II, nomenclature according to Marlin et al., (1985), as indicated in Fig. 1] or carbohydrate-independent epitopes (antigenic site I) could be recognized. The carbohydrate dependence of site II epitopes was correlated with the presence of terminal galactose of the oligosaccharides in gC-1. Accordingly, degalactosylation resulted in complete loss of reactivity of these epitopes which, although they were mapped to a very carbohydrate-rich portion of gC-1 (Fig. 1), were of peptide and not of carbohydrate nature (Sjöblom et al., 1987). The site I epitopes, on the other hand, were expressed even in the absence of peripheral carbohydrates, although they were inactivated if gC-1 was produced in cells in which N-glycosylation had been prevented by tunicamycin.

In the present paper we have addressed three questions, which may be summarized as follows. Is the galactose-dependent modulation of antigenic activity a reversible property? Are carbohydrate determinants other than galactose engaged in the formation of carbohydrate-dependent epitopes? Do the carbohydrate determinants promoting carbohydrate-dependent antigenicity belong to the N-glycosidic or O-glycosidic class of oligosaccharides? We now report that the carbohydrate-induced modulation, as described above, is a reversible process, that fucose or galactose of N-linked oligosaccharides are the active carbohydrate determinants and that O-linked oligosaccharides are at most, of indirect importance for the expression of site II epitopes. This is the first demonstration of fucose in the establishment of a carbohydrate-dependent epitope of peptide nature.

Methods

Virus and cells. The HSV-1 strain F was used throughout the study. Mouse neuroblastoma C1300 cells (ATCC no. CCL 147) and baby hamster kidney (BHK-21) cells (ATCC no. CCL 10) were used for production of viral glycoproteins. All cells were cultivated in Eagle's minimal essential medium supplemented with 10% calf serum. For maintenance the same medium was used, without serum but with antibiotics (penicillin (100 units/ml) and streptomycin (100 μg/ml)).

Preparation of immunosorbent-purified gC. The details for production of immunosorbent-purified gC have been reported previously (Olofsson et al., 1983). Briefly, a membrane fraction from HSV-infected cells was produced by the method of Springer et al. (1977). This fraction was solubilized in 0.1 M-glycine buffer pH 8.8, containing 1.0% (v/v) Triton X-100 and centrifuged at 100000 g for 1 h. The solubilized glycoprotein was subjected to SDS-PAGE and was found to migrate as single bands corresponding to an apparent Mr of 130K for gC-1 from Sepharose, as previously outlined (Olofsson et al., 1983). The purified glycoprotein was subjected to SDS-PAGE and was found to migrate as single bands corresponding to an apparent Mr of 130K for gC-1 from BHK cells and 105K to 115K for gC-1 from C1300 cells, as previously described by Lundström et al. (1987b). In some cases gC-1 from C1300 cells was prepared by means of affinity chromatography on Helix pomatia lectin, as previously described (Olofsson et al., 1983). In this case the gC-1 fraction was eluted with 5 mM-N-acetylglactosamine (GalNAc).
Antisera and monoclonal antibodies. A characterization of the gC-specific monosaccharide antibody B1C1 has been published previously (Olofsson et al., 1983). In addition, gC-specific monosaccharide antibodies, designated C1, C3, C8, C11 and C13, as characterized by Marlin et al. (1985), were used for control purposes.

Periodate treatment and enzyme-linked assays. Sequential removal of carbohydrates from gC-1, coated on microplates, was carried out as previously described (Sjöblom et al., 1987). Polystyrene microplates (Nunc) were coated with purified preparations of gC-1 (in 0-1 M-sodium carbonate buffer pH 9-6) by incubation for 2 h at 37 °C at 100 µl per well. In most experiments the coating concentration of gC-1 was 0.25 µg/ml. The protein content was measured by the method of Lowry et al. (1951), modified for use in Triton-containing solutions (Dulley & Grieve, 1975). The plates were subsequently treated with 50 µl of sialidase (Behringwerke; 100 mU/ml) at 37 °C for 2 h and thereafter with serial dilutions of periodate, according to Woodward et al. (1985). The plates were subsequently incubated with mouse monoclonal antibodies against gC-1. The reactions were visualized as described below. All serum dilutions used in this assay were optimized by checkerboard titrations where the antigen coating concentration and the antibody dilution were varied (Sjöblom et al., 1987). The reactivity against antibody B1C1 per µg glycoprotein coated onto the microplate of gC-1 from C1300 and BHK cells (prepared and analysed during the standard conditions of the present study) did not differ significantly at coating concentrations ranging from 0.2 to 1.5 µg/ml.

The effect of the combined sialidase-periodate treatment on the oligosaccharide side-chains was determined by the use of biotinylated Ricinus communis lectin (RCA) (Sjöblom et al., 1987). The reactions were visualized by addition of alkaline phosphatase-conjugated avidin (lectin assays) or goat antibodies against mouse IgG (antibody assay) and the plates were read at A405.

Galactosyl transferase assay. The galactosyl transferase assay [UDP-galactose: N-acetylgalcosaminyl-glycoprotein galactosyl transferase (EC 2.4.1.22), obtained from Sigma (code G5507)] was carried out by the method of Grimes (1970), as modified by Olofsson et al. (1980), directly on gC-1 coated on microplates. Acceptor structures were prepared by treatment with sialidase and β-galactosidase (see below). The donor solution (0·1 M-Tris–HCl, 0·3% Triton X-100 and 0·03 M-MnCl₂) contained UDP-galactose. The reaction mixture consisted, except for acceptor gC-1 coated onto the walls of each cavity, of 40 µl donor solution (2-5 mM final concentration), 40 µl enzyme dilution (final concentration of 2-0 µg protein/ml) and 20 µl water. The reaction was carried out at 37 °C for 1 h and stopped by repeated washes with TBS (0·15 M-NaCl, 0·02 M-Tris–HCl pH 7·5). Addition of galactose was detected by the increase in RCA activity, as described above.

Treatment with glycosidases. Digestion with β-galactosidase (Sigma, grade IX) was carried out on purified gC-1, coated on microplates, as described above. Prior to galactosidase treatment terminal sialic acid was removed as described above. The wells were incubated with 100 µl 0·1 M-phosphate buffer pH 7·4, supplemented with 1 mM-MgCl₂, containing 1, 10 or 100 units/ml of β-galactosidase for 4 h at 37 °C. Thereafter the plates were washed and lectin or antibody affinities were assayed as described above.

Treatment with α-fucosidase (Boeringer; from beef kidney) was carried out in a similar manner, except that 80 units (in 0·2 M-buffer pH 4·5) was added per well.

Measurement of fucose abundance. Petri dishes (50 mm) containing C1300 or BHK cells were infected with HSV-1 F and radiolabelled at 4·5 h.p. with 4 µCi of [³H]fucose or [³H]glucosamine (GlCN). At 18 h.p. the cells were harvested by scraping and solubilized in TBS containing 10 mg/ml of Triton X-100. Insoluble material was pelleted by centrifugation at 10000 × g for 1 h and gC-1 was purified by an immunoabsorbent technique (Olofsson et al., 1983), using a gC-1-specific rabbit antiserum coupled to Protein A-Sepharose. Antibody-gC-1 complexes were eluted with 1 M-acetic acid and quantified by liquid scintillation counting. The cell extracts were treated with sialidase (0·1 units/ml at 37 °C overnight) to rule out the possibility that differences in the sialylation capacities between C1300 and BHK cells could influence the incorporation of [³H]GlCN into gC-1. Two independent experimental series were performed.

Results

We have previously shown that galactose supports expression of carbohydrate-dependent epitopes (Sjöblom et al., 1987). In the present paper we determined whether these epitopes were expressed in gC-1 produced from a galactosyl transferase-deficient mouse neuroblastoma cell line that completely lacks the ability to add galactose to O-linked oligosaccharides and has a restricted capacity to add galactose to N-linked oligosaccharides (Lundström et al., 1987b). To demonstrate the possible existence of carbohydrate-dependent epitopes purified gC-1 from C1300 cells was coated onto microplates and the peripheral carbohydrates were removed in a sequential series by sialidase treatment and increasing concentrations of periodate, as previously described (Sjöblom et al., 1987). The disappearance of sialic acid and galactose was monitored by the use of biotinylated RCA, with main specificity for terminal βl-4-linked galactose (Goldstein & Poretz, 1986). Detection of trace amounts of galactose in gC-1 from C1300 cells was possible due to the high sensitivity of the enzyme-linked lectin assay (Sjöblom et al., 1987). Thus after sialidase treatment the RCA-binding ability was increased (Fig. 2), due to removal of sialic acid from penultimate galactose residues (Sjöblom et al., 1987). This activity decreased on treatment with increasing concentrations of periodate, confirming removal of the galactose residues. The reactivity of gC-1 towards antibody B1C1, which binds to one of the carbohydrate-dependent epitopes described in Fig. 1 (Sjöblom et al., 1987) was decreased by the addition of periodate. Similar results were recorded for other antibodies recognizing carbohydrate-dependent epitopes, whereas the reactivity of antibodies C1 and C11, reacting with carbohydrate-independent epitopes (Sjöblom et al., 1987), was not affected by the periodate treatment of gC-1 from C1300 cells (data not shown). This is in essence similar to the situation for gC-1 produced in BHK cells (Sjöblom et al., 1987).

To determine whether the low level of galactosyl residues present in gC-1 from C1300 cells was responsible for the maintenance of epitope expression, we monitored the effect of de-galactosylation and subsequent re-galactosylation on the antigenic activity (Fig. 3). After digestion with sialidase gC-1 was treated with Escherichia coli β-galactosidase, specific for β1-4 linkages (Kobata, 1979; Lundström et al., 1987b), and re-
Fig. 2. Effects of the combined sialidase-periodate treatment on peripheral carbohydrates and the epitope recognized by antibody B1C1. Open squares denote the antibody reaction and filled squares denote RCA binding. Bars indicate S.E.M. (n = 3).

Fig. 3. Reversible galactose dependence of the B1C1-binding epitope of gC-1 produced in BHK cells. Microplates coated with gC-1, were subjected to treatment with, in order, sialidase (S), β-galactosidase (G) and galactosyl transferase (T), as indicated. The reactivity against RCA or antibody B1C1 (Ab) was determined as described in Methods. Purified gC-1 from C1300 cells and BHK cells were analysed. Bars indicate S.E.M. (n = 6).

Fig. 4. Influence of combined sialidase–fucosidase treatment on the B1C1-binding epitope of gC-1, produced in C1300 cells. Purified gC-1 was coated onto microplates and the wells were treated with sialidase (Si). Subsequently the plates were treated with α-fucosidase (fuc) [600 mU/ml from beef kidney (Boehringer) in 0.2 M-acetate buffer, pH 4.5] and/or β-galactosidase (Gal) (100 U/ml) as indicated. The S.E.M. values (n = 4) are indicated.

galactosylated to the same glycosidic linkage with UDP-galactose N-acetylglucosaminyl-glycoprotein galactosyl transferase (Strous, 1986). Based on the reported structures of O-linked oligosaccharides of gC-1 produced in BHK cells and C1300 cells (Lundström et al., 1987a, b), these enzymes should only be active on N-linked oligosaccharides. By using biotinylated RCA we could confirm that exposure and removal of galactose, as well as re-galactosylation, was achieved as expected by these treatments. We found that β-galactosidase treatment of gC-1 from BHK cells, in contrast to gC-1 from C1300 cells, resulted in a prominent decrease in the activity of the epitope reacting with antibody B1C1. When galactosyl transferase and UDP-galactose were added an increase in antigenicity was noted for gC-1 from BHK cells, but not for gC-1 from C1300 cells. The finding that it was possible to regenerate the epitopes by the action of galactosyl transferase clearly demonstrated that the contribution of carbohydrates to antigenicity is based on reversible interactions between the polypeptide and the peripheral carbohydrates.

However, neither removal of galactose residues nor exogenous addition of galactose residues altered the antigenic activity of the carbohydrate-dependent epitopes of gC-1 produced in C1300 cells. This finding indicated that RCA-binding β1-4-linked galactose residues of N-linked oligosaccharides were not essential for
Table 1. Fucose abundance in gC-1 from C1300 and BHK cells

<table>
<thead>
<tr>
<th>Cellular origin of gC</th>
<th>Radiolabel in gC</th>
<th>Ratio fucose/GlcN</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1300 Expt. I</td>
<td>[3H]Fucose 1491</td>
<td>[3H]GlcN 7284</td>
</tr>
<tr>
<td>Expt. II</td>
<td>1355</td>
<td>6007</td>
</tr>
<tr>
<td>BHK Expt. I</td>
<td>1971</td>
<td>27408</td>
</tr>
<tr>
<td>Expt. II</td>
<td>1823</td>
<td>28171</td>
</tr>
</tbody>
</table>

the antigenic activity of carbohydrate-dependent epitopes of gC-1 produced in these cells. Furthermore, as gC-1 from C1300 cells lacks O-linked galactose-containing oligosaccharides (Lundström et al., 1987b), the data suggest that a periodate-sensitive carbohydrate determinant other than galactose is engaged in the expression of carbohydrate-dependent epitopes.

As fucose is a prominent constituent of oligosaccharides in glycoproteins derived from neural cells it was essential to determine whether this monosaccharide could be involved in the expression of carbohydrate-dependent epitopes of gC-1. In the first series of experiments we wanted to determine whether fucose was more abundant in gC-1 from C1300 cells than in gC-1 from BHK cells. This was accomplished by comparing the ratio of [3H]fucose/[3H]GlcN label incorporated in gC-1 produced in C1300 cells and in gC-1 produced in BHK cells, respectively (Table 1). We have previously shown that gC-1 from C1300 cells contains considerably less sialic acid than gC-1 from epitheloid cells (Lundström et al., 1987b) and to rule out the possibility that the differences in ratio reflected GlcN label metabolized into sialic acid, we subjected the gC-1 preparations to sialidase treatment before purification. However, despite the sialidase treatment, it was found that the ratio of fucose to GlcN in oligosaccharides for gC-1 from infected C1300 cells was threefold higher than was found for gC-1 from BHK cells, indicating that fucose is more abundant in gC-1 from C1300 than in gC-1 from BHK cells.

To determine whether such peripheral fucose residues could be responsible for the maintenance of carbohydrate-dependent epitopes we examined the effect of fucosidase treatment on the carbohydrate-dependent epitope recognized by monoclonal antibody B1C1 (Fig. 4). We found that treatment of gC-1 with α-fucosidase after sialidase treatment caused a significant decrease in the binding of antibody B1C1. As expected, the control experiment including combined sialidase and β-galactosidase treatment did not significantly decrease the ability of gC-1 to react with B1C1. Moreover, β-galactosidase treatment in combination with fucosidase treatment did not further decrease the binding of antibody B1C1. These data demonstrated that peripheral fucose residues, such as in structure (ii) of Fig. 1, but not galactose, significantly contributed to the expression of carbohydrate-dependent epitopes in gC-1 produced in C1300 cells.

**Discussion**

The antigenic activity of the epitopes associated with antigenic site II, as defined by Homa et al. (1986) and Marlin et al. (1985), is extensively affected by relatively small changes in the peripheral structures of the glycoprotein oligosaccharides (Sjöblom et al., 1987). We have previously concluded that the actual binding site of these epitopes, despite their carbohydrate dependence, is of peptide nature and does not involve carbohydrates as direct binding determinants. This conclusion is further strengthened by the observation of the present paper that antibody B1C1 defined an epitope that was dependent on either galactose or fucose on adjacent oligosaccharides. If either of these monosaccharides were a physical part of the binding determinant it has to be assumed that a methyl-containing sugar with the L-configuration (fucose) and an unsubstituted hexose with the D configuration (galactose) are interchangeable in forming an active binding site. This possibility must be regarded as highly unlikely. Since the expression of antigenic activity was abolished by β-galactosidase treatment, but was partly regenerated by enzymic re-galactosylation, the carbohydrate–protein interactions responsible for expression of the carbohydrate-dependent epitopes are reversible. It is therefore reasonable to assume that the peptide surface harbouring antigenic site II has a pronounced plasticity, permitting carbohydrate-dependent reversible changes between different conformational stages.

The results of the present paper allow a more detailed definition of the oligosaccharides involved in maintaining the carbohydrate-dependent epitopes. One interesting question is whether it is O-linked or N-linked oligosaccharides that are engaged in the maintenance of carbohydrate-dependent epitopes. The region of gC-1
with the carbohydrate-dependent epitopes contains three N-glycosylation sites (Frink et al., 1983; Marlin et al., 1985), but this region also is situated in close proximity to a peptide stretch containing numerous clustered O-linked oligosaccharides (Lundström et al., 1987a). According to our data the O-linked oligosaccharides may at most be indirect factors of importance for maintenance of the carbohydrate-dependent epitopes. This conclusion is based on the fact that fucose, promoting antigenic activity of these epitopes, is totally absent from O-linked oligosaccharides of gC-1 produced in C1300 cells. Thus gC-1 produced in this cell line contains only O-linked GalNAc residues, not extended beyond the monosaccharide level (Lundström et al., 1987b). Accordingly, the fucose residues essential for expression of carbohydrate-dependent epitopes must be structural determinants of N-linked glycans.

There are reasons to believe that the galactose residues responsible for expression of the carbohydrate-dependent epitopes in BHK cells are also associated with N-linked sugars. This conclusion is based on the following facts. The present results show that these epitopes were regenerated by the action of a galactosyl transferase with the linkage specificity Gal(β1-4)GlcNAc (Strous, 1986). This linkage is obligatory for complex-type oligosaccharides, but relatively scarce in small O-linked oligosaccharides (Kornfeld & Kornfeld, 1985). Previous studies in this laboratory detected Gal(β1-3)GalNAc, but no galactose linked to GlcNAc in O-linked oligosaccharides of gC-1 produced in the cell lines used in this study (Lundström et al., 1987a, b). Altogether these data indicate that structural determinants of N-linked oligosaccharides are directly engaged in modulation of antigenic activity of the carbohydrate-dependent epitopes of antigenic site II, whereas O-linked oligosaccharides play an indirect role.

We have no definite proof regarding the position of these extra fucose residues in gC-1 from C1300 cells. One clue to solving this problem may be linked to the paradox that gC-1 from C1300 cells is able to bind to the galactose-specific lectin RCA despite the low content of suitable galactosyl transferases in these cells. Thus, several cells of neural origin contain highly fucosylated glycoproteins, where fucose units are attached not only to the core GlcNAc residues, but also to the branching GlcNAc, as indicated in Fig. 1 (Krusius & Finne, 1977, 1978). It is well established that difucosylated oligosaccharides, such as the one depicted in Fig. 1, adopt a compact folded conformation of the α(1-6) antenna (Pierce-Crétel et al., 1983; Montreuil, 1987). This rigid conformation, resulting in an extended α(1-3)antenna, has in fact been shown to increase the availability of galactose residues for certain galactose-binding lectins (Pierce-Crétel et al., 1983). It is therefore tempting to speculate that the increased content of fucose in gC-1 produced in C1300 cells is due to these more peripheral fucose residues. If this is the case the low number of galactose residues in gC-1 from C1300 cells could still result in RCA-binding, due to their exposed position on the difucosylated oligosaccharides. There is still one intriguing question regarding the N-glycan conformation responsible for the expression for carbohydrates. If this phenomenon is also a consequence of the folded conformation induced by the multiple fucose residues of the N-linked oligosaccharides synthesized in C1300 cells one would expect that multiple galactose residues of scarcely fucosylated oligosaccharides could result in a similar folding of the (α1-6) branch. Interestingly, solution conformation studies suggest that such oligosaccharides may adopt a similar conformation, at least under certain conditions (Homans et al., 1986; for a review see Montreuil, 1987).

It has been suggested that gC-1 may contribute to the pathogenesis of HSV by acting as a receptor of component C3b of the complement system, thereby acting as an inhibitor of the complement cascade (Cines et al., 1982; Friedman et al., 1984). There are reasons to believe that this is a factor of importance for HSV infections in man (Friedman et al., 1984). The C3b-binding activity is enhanced by sialidase treatment of gC-1 (Smiley & Friedman, 1985) and it was found that this activity is associated with the carbohydrate-dependent conformational site recognized by the antibody B1C1 (Huemer et al., 1989) used in the present study. The carbohydrate composition of gC-1 and other viral proteins differs considerably in a tissue-dependent manner (Datema et al., 1987) and we suggest that the complicated nature of the carbohydrate-dependent conformation variation of gC-1 could be part of a viral strategy to use the variable cellular glycosylation machinery, not only to modulate the antigenic makeup of gC-1, but also for adjustment and optimization of its C3b-binding ability.

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References


Carbohydrate modulation of gC-1 epitopes


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