Reduced Temperature Can Block Different Glycoproteins at Different Steps during Transport to the Plasma Membrane

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SUMMARY

Reduced temperature has been shown to block the cell surface expression of Sendai virus haemagglutinin–neuraminidase (HN) and fusion (F₀) glycoproteins at different steps of their intracellular transport. At 20 °C, HN was confined to the rough endoplasmic reticulum or cis Golgi compartment, while F₀ acquired complete resistance to digestion by endo-β-N-acetylglucosaminidase-H and therefore was blocked at a more distal location in the pathway of cell surface expression. The significance of these results for different pathways of transport to the cell surface is discussed.

Membrane glycoproteins are synthesized by rough endoplasmic reticulum (RER) membrane-bound ribosomes and are subsequently transported via the Golgi apparatus to the cell surface (Sabatini et al., 1982). During this transport, the glycoproteins undergo various modifications. The high mannose glycans, added cotranslationally in the RER, are trimmed and converted to complex sugars (Hubbard & Ivatt, 1981). In addition, proteins can be modified by acylation or proteolysis (Schmidt, 1982; Homma & Ohuchi, 1973; Scheid & Choppin, 1974).

To establish precisely the sites at which these various modifications take place, one would ideally like to block the processing of the glycoproteins at definite biochemical steps and at defined intracellular sites, to be able to correlate directly the biochemical protein composition and intracellular location. To achieve this goal different approaches are currently used.

Drugs preventing high mannose sugar addition, like tunicamycin (Takatsuki et al., 1975), or blocking the transport of the glycoprotein from the Golgi onwards, like monensin (Tartakoff, 1983), are widely used. Tunicamycin which blocks high mannose sugar addition is useful in defining the role of glycosylation in glycoprotein transport, and drugs like monensin allow the correlation between an intracellular location and a particular step in glycoprotein processing. Drugs, however, have the major drawback of their possible direct effect on the cellular machinery involved in transport.

Another approach is to generate mutant cell lines deficient in enzymes involved in the glycosylation processing, and to follow the transport and properties of the glycoproteins in such cell lines (Vischer & Hughes, 1981).

The availability of a series of exo- and endoglycosidases with defined substrate requirements also facilitates the analysis of glycoproteins isolated at different stages of their transport and are useful in defining, for instance, the rate of transport through the Golgi apparatus (Kobata, 1979; Koide & Marumatsu, 1974; Tarentino & Maley, 1974; Elder & Alexander, 1982).

As well as the methods cited above, a very interesting and simple one has been reported by Matlin & Simons (1983). They observed that influenza virus haemagglutinin was not detected at the surface of infected cells within 2 h of its synthesis when the cells were incubated at 20 °C. This contrasted with the surface appearance of the glycoprotein within 15 min during incubation at 37 °C. At 20 °C, however, terminal glycosylation of the protein was taking place,
suggesting that the block in cell surface expression occurred distal to the Golgi apparatus, presumably between the Golgi complex and the plasma membrane. Supporting this interpretation was the quick externalization of the haemagglutinin when the incubation temperature was raised from 20 to 37 °C. Incubation at 20 °C thus appears to represent a way of blocking glycoprotein at a specific step of its transport.

To analyse whether the results obtained with the influenza virus haemagglutinin would apply more generally, we decided to investigate the effect of 20 °C incubation on the cell surface expression of the Sendai virus haemagglutinin-neuraminidase (HN) and fusion (F₀) glycoproteins. HN and F₀ are viral surface glycoproteins with mol. wt. of 67,000 (67K) and 65K respectively. They both contain N-linked glycans which constitute 9% and 15% of their respective final protein mass (Choppin & Compans, 1975; Kohama et al., 1978; Yoshima et al., 1981). While F₀ is anchored in the membrane by its C-terminus (Blumberg et al., 1985a), HN has its N-terminus spanning the membrane (Blumberg et al., 1985b; Hsu & Choppin, 1984). The rate of transport (at 37 °C) of the two proteins to the cell plasma membrane differs: F₀ appears at the surface with a half-life of about 15 min, and HN with a half-life of 45 min (Blumberg et al., 1985b). The native mature structure of the two proteins as estimated by their ability to react with antibodies is generated at different rates also. F₀ reacts fully with antibodies raised against its mature native form soon after its synthesis and addition of high mannose glycans. HN immunoreactivity, on the other hand, matures with a half-life of about 30 min after completion of its synthesis and addition of high mannose sugars. This maturation step is presumably taking place in the RER and/or the cis Golgi (Mottet et al., 1986). In consequence, apart from extending the results of Matlin & Simons (1983) to other glycoproteins, this study also compares the effect of 20 °C incubation on the transport of membrane glycoproteins exhibiting different characteristics.

In order to study the effect of reduced temperature on HN and F₀ cell surface expression, Sendai virus-infected BHK cells were pulse-labelled with [35S]methionine for 15 min at 37 °C and then chased for increasing periods of time at 37 or 20 °C. At the end of the chase times, the extent of cell surface expression of HN and F₀ was estimated as described in the legend to Fig. 1. At 37 °C, a significant amount of F₀ (about 40%) was already seen at the cell surface at the end of the pulse (Fig. 1a, b). This amount increased during subsequent incubation at 37 °C to reach a maximum level by 20 to 30 min of chase. HN, however, was expressed at the cell surface much more slowly; not detected after the 15 min pulse, HN gradually reached the surface with a half-life of about 40 to 45 min. These results have been presented more extensively before (Blumberg et al., 1985b). At 20 °C, on the other hand, the degree of both F₀ and HN surface expression seen during the chase did not significantly exceed that observed at the end of the 15 min pulse. This demonstrates that incubation at 20 °C efficiently limits the cell surface expression of F₀ and HN. In fact, incubation at 20 °C drastically slows down the rate of HN and F₀ transport to the cell surface rather than blocks it. After 2 h of chase at 20 °C, about 55 to 60% of F₀ and about 30% of HN was at the surface and by 16 h most of the HN was eventually seen at the surface (data not shown).

To define at which intracellular location the proteins are restricted in their transport to the surface, pulse-labelled HN and F₀ were chased at 37 or 20 °C as in Fig. 1, isolated by immunoprecipitation after cell disruption (total cell immunoprecipitation) and analysed for their sensitivity to endo-β-N-acetylglucosaminidase-H (endo-H). Endo-H cleaves the high mannose sugars [(gluc)₃-(man)₉-(GlucNAc)₂] from the protein backbone before they are processed to [(man)₅-(GlucNAc)₂], at which point the sugars become resistant to cleavage (Tarentino & Maley, 1974). As this trimming takes place in the RER up to the cis Golgi compartment, sensitivity to endo-H reflects RER or cis Golgi localization of the glycoprotein and resistance reflects its transport from the RER to more distal compartments of the Golgi (Hubbard & Ivatt, 1981; Tarentino & Maley, 1974). As shown in Fig. 2, HN as well as F₀ were totally sensitive to endo-H at the end of the 15 min pulse at 37 °C (0 h, Fig. 2a, b). After 1 h of chase at 37 °C about 50% of HN and 100% of F₀ had acquired resistance, and after 2 h of chase about 80% of HN was resistant. These different rates of acquisition of endo-H resistance at 37 °C which correlate with the different rates of cell surface expression have been noticed.
Fig. 1. Cell surface expression of HN (●, ■) and F₀ (○, □) at 20 °C. BHK cell samples grown in 9 cm diam. Petri dishes were infected with Sendai virus (m.o.i. of 40). Eighteen h post-infection, the infected cells were pulse-labelled for 15 min at 37 °C with [35S]methionine, chased at 37 °C (○, ●) or 20 °C (□, ■) for the times (min) indicated with cold methionine (10 mM) and then reacted in situ with Sendai virus antiserum. The cells were then washed with phosphate-buffered saline to remove excess antibody and solubilized in RIPA buffer and the immune complexes were recovered with the aid of Staphylococcus aureus beads as previously described (Blumberg et al., 1985a, b). Identical samples of each cell sample were then separated by SDS-PAGE. (a) Autoradiograph of the gel; lane V, viral protein markers. (b) Scanning of the autoradiograph to show the amount of protein recovered at the cell surface expressed as a function of chase time and temperature.

previously (Mottet et al., 1986). At 20 °C, even if the rate of acquisition of endo-H resistance for F₀ was decreased (only about 70% resistant after 1 h of chase), F₀ nevertheless reached complete resistance within 2 h of chase. This contrasts with HN which remained totally sensitive to endo-H at 20 °C during the same period of observation. These results suggest that, at 20 °C, HN remains completely in the RER or the cis Golgi compartment while F₀ is efficiently transported from the RER to the Golgi. As the apparent molecular weight of F₀ analysed after 2 h of chase at 20 °C corresponds to that of the mature protein, F₀ presumably undergoes terminal sugar processing.

To assess the RER localization of HN during the 20 °C incubation, the degree of maturation
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Fig. 2. Endo-H sensitivity of pulse-labelled and chased HN and F₀. Sendai virus-infected BHK cell samples were pulse-labelled with [35S]methionine and chased (0, 1 or 2 h) as in the experiment shown in Fig. 1. The HN and F₀ proteins, recovered by total cell immunoprecipitation using monoclonal antibodies (Roux et al., 1984), were resuspended in 1% SDS, 50 mM-Tris-HCl pH 6.8, 0.5% 2-mercaptoethanol, 2 mM-phenylmethylsulphonyl fluoride and boiled for 5 min. After a 10-fold dilution with 125 mM-sodium citrate pH 5.0, aliquots of the proteins were digested overnight at 37 °C with 80 mU/ml endo-H (Nenzymes) (+) or mock-treated (−). The proteins were then concentrated by acetone precipitation (8:1, v/v) and analysed by PAGE. (a) HN; (b) F₀. Lane V, viral protein markers.

of HN native immunoreactivity was controlled. HN immunoreactivity has been shown to mature during the first hour following the synthesis of the protein (Mottet et al., 1986). This maturation step takes place in the RER or at the most in the cis Golgi compartment. It was thus of interest to analyse whether HN immunoreactivity would mature at 20 °C. The immunoreactivity of HN for a monoclonal antibody raised against the native mature form of the molecule was therefore estimated as described in detail elsewhere (Mottet et al., 1986) and the results of these estimations are shown in Fig. 3. In this experiment, the infected cells are pulse-labelled and chased as in Fig. 1 and the HN is recovered by total immunoprecipitation as in Fig. 2. At 37 °C, HN immunoreactivity matured during the first hour following its synthesis as evidenced by the increased efficiency at which the protein was recovered by immunoprecipitation. This maturation did not take place at 20 °C. This experiment thus confirms that HN is blocked in the RER at low temperature. As expected, F₀ which shows no immunoreactivity maturation (Mottet et al., 1986) is not affected in its immunoreactivity by incubation at low temperature.

Therefore, if incubation at 20 °C efficiently prevents cell surface expression of Sendai virus
HN and F₀ glycoproteins, it appears to affect the intracellular transport of the two proteins in different ways. HN transport is efficiently blocked in the RER or the cis Golgi compartment. F₀ transport, on the other hand, can still proceed until the protein becomes totally resistant to endo-H, but is arrested before cell surface expression. In conclusion, this study confirms and extends the observation of Matlin & Simons (1983). Incubation at 20 °C appears to be a reliable method to block cell surface expression of membrane glycoproteins. The step at which the proteins are blocked, however, may differ from protein to protein.

The differential effect of reduced temperature in restricting HN and F₀ is yet another factor which can be added to the list of differences already reported, namely in the oligosaccharide patterns, in the rate of cell surface expression, in the orientation of anchorage in the plasma membrane and finally a difference in the maturation process of their native mature immunoreactivity (Yoshima et al., 1981; Blumberg et al., 1985 a, b; Mottet et al., 1986). This list
of different properties exhibited by HN and F₀ questions the processing pathway(s) that the two glycoproteins follow to mature and to reach the cell surface. Classically, HN and F₀ would follow one unique pathway, and the differences observed between the two proteins would only reflect intrinsic properties linked to each protein. Thus, the different rate in the cell surface expression could result from the difference in the orientation of anchorage in the membrane which basically depends on the primary structure of the protein. The different effect of reduced temperature in restricting HN and F₀ cellular transport could in this case only result from the difference in the rate of transport. If transport from RER or cis Golgi to the more distal compartments of the Golgi is more sensitive to low temperature than transport from the middle Golgi onwards, then the transport of the fast moving F₀ protein could be less affected since F₀ could possibly reach the middle Golgi before the effect of low temperature is present. This interpretation does not satisfactorily account for the different patterns of glycosylation as already mentioned by Yoshima et al. (1981) and for the evolution of F₀ endo-H resistance at 20 °C observed here. Alternatively, HN and F₀ could follow different pathways with different glycosylation machinery, different rates of transport and different sensitivities to reduced temperature. Although the different characteristics of HN and F₀ do not constitute a formal proof of the existence of such different pathways, they certainly indicate that this possibility has to be seriously considered.

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REFERENCES


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