Polysialylation Increases Lateral Diffusion of Neural Cell Adhesion Molecule in the Cell Membrane*§

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Polysialic acid (PSA) is a polymer of N-acetyleneuraminic acid residues added post-translationally to the membrane-bound neural cell adhesion molecule (NCAM). The large excluded volume created by PSA polymer is thought to facilitate cell migration by decreasing cell adhesion. Here we used live cell imaging (spot fluorescence recovery after photobleaching and fluorescence correlation spectroscopy) combined with biochemical approaches in an attempt to uncover a link between cell motility and the impact of polysialylation on NCAM dynamics. We show that PSA regulates specifically NCAM lateral diffusion and this is dependent on the integrity of the cytoskeleton. However, whereas the glial-derivative neurotrophic factor chemotactic effect is dependent on PSA, the molecular dynamics of PSA-NCAM is not directly affected by glial-derivative neurotrophic factor. These findings reveal a new intrinsic mechanism by which polysialylation regulates NCAM dynamics and thereby a biological function like cell migration.

The spatial and temporal expression pattern of cell adhesion molecules is a prerequisite for the correct development and functioning of the nervous system. These molecules fulfill their role by regulating cell-cell and cell-substrate adhesion as well as intracellular signaling pathways (1, 2).

NCAM (neural cell adhesion molecule), the prototype member of the immunoglobulin superfamily proteins, is widely expressed in the nervous system. NCAM mediates a large number of biological functions both through homophilic interactions and heterophilic interactions with other membrane receptors such as fibroblast growth factor and glial-derivative neurotrophic factor (GDNF) receptors (3, 4). For the latter, Paratcha et al. (4) and Iwase et al. (5) showed that the NCAM140 isoform can function as a co-receptor of the GDNF family receptor α1 (GFRα1) independently of Ret tyrosine kinase, a known GDNF-signaling receptor. The functional outcome of this cross-talk is an increase of axonal growth in neurons and cell migration in Schwann cells.

In vertebrates, NCAM is the only carrier of polysialic acid (PSA), a long carbohydrate composed of α2,8-linked N-acetyleneuraminic acid (Neu5Ac) residues (6). The polysialylated form of NCAM (PSA-NCAM) plays a critical and unique role during brain development and in some brain tumors, modulating adhesion between cells, stimulating cell migration and neurite outgrowth (7, 8). Observations based upon enzymatic digestion of PSA by endoneuraminidase (EndoN) (9), knock out of the polysialyltransferase coding genes responsible for addition of PSA to NCAM (10), or the use of mimotope peptide of PSA (11) indicate that the carbohydrate, more than the core protein, is critical to account for PSA-NCAM biological functions. At the molecular level, previous studies have demonstrated that PSA doubles the hydrodynamic radius of the extracellular domain of NCAM (12, 13) and thereby increases the range and magnitude of intermembrane repulsion (14). In this vein, it is accepted that the repulsion conferred by PSA is a mechanism by which cell-cell interactions are decreased. However, there is no direct evidence that PSA volume affects NCAM dynamics at the cell membrane.

Therefore, exploring whether PSA would regulate NCAM spatial distribution, endocytosis, mobile fraction, lateral diffusion, or NCAM confinement in a living cell could help in understanding PSA-NCAM biological functions. In this study, to investigate whether these parameters were affected by PSA removal we used a cellular model in which PSA potentiates cell migration and conducts a chemotactic effect. Biochemical and molecular imaging techniques performed on living cells allowed us to show that polysialylation conveys to NCAM an intrinsic capacity to modify its lateral diffusion at the cell surface even when molecules are engaged in mediating cell-cell contact or activated by an extracellular factor such as GDNF. In
addition, polysialylation of NCAM influenced ERK phosphorylation and increased actin stress fiber formation. Thus, our data place the addition of a carbohydrate by post-translational modification to a cell surface receptor as an efficient way to control its lateral diffusion and its relationship with the cytoskeleton.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—EndoN was produced by the Institut de Biologie du Développement Marseille Luminy according to a procedure described by Wang et al. (15) and used at 0.8 units/ml. Rat GDNF protein was used at 200 ng/ml (R&D Systems, Minneapolis, MN). Latrunculin B was used at 1 μM (Calbiochem). Rabbit polyclonal antibodies against NCAM (1:5000; Chemicon-Millipore, Temecula, CA), actin (1:2500; Chemicon), phospho-p44/42 ERKs (1:500; Cell Signaling Technology, Danvers, MA), Fyn (1:1000; Upstate-Millipore, Charlottesville, VA), Rab5 (1:1000; provided by P. Chavrier, Institut Curie, Danvers, MA), and mouse anti-human transferrin (1:5000; Invitrogen), and mouse anti-human transferrin receptor (1:500) were used for immunoblotting experiments. Rabbit IgG anti-NCAM (1:1000; Zymed Laboratories Inc., San Francisco, CA) were used for immunocytochemical experiments. Rabbit IgG anti-NCAM (0.7 μg/ml; Chemicon) or mouse IgM anti-PSA (1:400) (16) antibodies were used for immunocytochemical experiments.

**Reverse Transcription-Polymerase Chain Reaction**—Total RNA was extracted from TRizol reagent (Invitrogen) according to the manufacturer’s protocols. cDNA was produced using the Superscript III first-strand reverse transcription PCR system kit (Invitrogen). Briefly, 4 μg of RNA was used in reaction with 50 ng of random hexamer primers. The GoTaq DNA polymerase (Promega) was used to amplify cDNA in mix with 500 nm of each primer. The cycle used for PCR was as follows: 94 °C for 120 s 1 time; 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 30 s 40 times; and 72 °C for 600 s 1 time. Primers used were as followed: NCAM, 5’-GAC-ATCACCTGTACTC-3’ and 5’-TCATGCTTTGCTCTCA-TTCTC-3’; GFRα1, 5’-TCAGCAAGTGGACATC-3’ and 5’-AGCATTCCGTAGCTGTGCTT-3’. Primers for NCAM were designed to discriminate between the 180- and 140-kDa NCAM isoforms. PCR products expected were 361 and 1120 bp for NCAM140 and -180, respectively, and 210 bp for GFRα1.

**Transwell Migration Assay**—The migration ability of rhabdomyosarcoma TE671 cells was assessed in a transwell migration assay. Cells were seeded (5 × 10⁵ cells/well) on an 8-μm-pore top culture insert (Corning Inc., Corning, NY) of a 6-well culture plate containing in upper and lower reservoirs Dulbecco’s modified Eagle’s medium and incubated in a humidified atmosphere of 5% CO₂ at 37 °C. After 24 h, cells were stained with 4’,6-diamidino-2-phenylindole, washed twice with phosphate-buffered saline, and fixed with 3.7% formaldehyde for 20 min. Migrated cells and non-migrated cells were manually counted using a light microscope (Axiophoto 2; Zeiss, Oberkochen, Germany). Cells in five random fields of three independent experiments were counted, and data were reported as the ratio between migrated and non-migrated cells.

**Isolation of Detergent-resistant Membrane Fractions and Immunoblot**—Protein samples and flotation on sucrose density gradients were prepared as previously described (3). For total cell lysates, cells were grown in mass culture, pretreated or not with EndoN for 24 h and GDNF for 30 min. Cells were lysed with a radioimmune precipitation buffer containing 1% Nonidet P-40 and protease inhibitors. Protein lysates were clarified and analyzed by Western blotting.

**Phalloidin Staining**—Cells were fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100. Rhodamine-phalloidin (Sigma-Aldrich) was added for 30 min at a final concentration of 2 μg/ml. Slides were rinsed and coverslipped, and images were collected as described above.

**Biotinylation of Cell Surface Proteins for Endocytosis Assay**—A biotinylation assay was used to monitor internalization of NCAM upon different conditions. Briefly, TE671 cells were grown in mass cultures and cell surface proteins biotinylated with the reversible membrane-impermeable derivative of N-hydroxysulfosuccinimide esters (Pierce) (1.5 mg/ml for 30 min at 4 °C). Cells were then incubated at 37 °C, a permissive temperature for internalization, in HBSS (Invitrogen) containing no added factor, EndoN, GDNF, or EndoN + GDNF. Then the remaining cell surface biotin was cleaved by reducing its disulfide linkage with a 50 mM l-glutathione cleavage buffer, and cells were lysed with radioimmune precipitation buffer. Biotinylated proteins were precipitated using UltraLink-immobilized NeutrAvidin beads (Pierce), eluted from the beads with boiling Laemmli buffer, resolved by SDS-PAGE, and immunoblotted with an antibody directed against NCAM. Non-biotinylated proteins (supernatants) were immunoblotted in parallel with a rabbit anti-actin antibody.

**Time-lapse Confocal Imaging**—A cDNA encoding the NCAM140 isoform was cloned into pEGFP-N1 vector (BD Biosciences Clontech) between Xhol and Sall restriction sites. Briefly, primers containing restriction sites were designed to amplify the open reading frame without stop codon. DNA was amplified from a plasmid containing NCAM140 with Accuprime Pfx DNA polymerase (Invitrogen). NCAM-GFP expression was obtained by electroporating cell culture using a Nucleofector I apparatus (Amaza, Cologne, Germany). To electroporate TE671 cells, we used cell line Nucleofector kit V, 24 h after transfection, cell-loaded coverslips were transferred into a humidified recording chamber (37 °C, 5% CO₂) containing Dulbecco’s modified Eagle’s medium-buffered salt medium. Confocal time-lapse images were collected using a spinning disc confocal head (PerkinElmer) run by the Metamorph software on an inverted microscope with a ×63 lens (model Axiovert 200 M; Zeiss). Time-lapse measurements were performed from four different experiments.
Spot Fluorescence Recovery after Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS) Analysis—Spot FRAP and FCS measurements were performed on a Zeiss C-Apochromat custom apparatus as previously described (17). Experiments were performed at 37 °C in Dulbecco’s modified Eagle’s medium-buffered salt solution with 10 mM HEPES, pH 7.4, by illuminating the sample at the back aperture of a 40×/0.75 NA objective lens with an excitation power of 3 milliwatts for photobleaching and 3 microwatts for pre-bleach and post-bleach measurements (for FRAP) and 3 microwatts (for FCS). All measurements were obtained in three to five different areas on a minimum of five different cells.

For FRAP experiments, the signal recovery is described by Tsuji and Ohnishi (18) as shown in Equation 1

\[
F(t) = \frac{\alpha}{F_0} \sum_{n=0}^{\infty} \left( \frac{-K^n}{n!} \right) \frac{1}{1 + n(1 + 2t/T_0)} + (1 - \alpha)F_0
\]  

(Eq. 1)

where \( \alpha \) represents the fraction of the mobile species \( M_f \), \( K \) a parameter related to the degree of bleaching, \( T_0 \), the characteristic time of recovery, and \( F_0 \) and \( F_0 \) the fluorescence intensities prior to and immediately after the bleach event, respectively.

For FCS experiments, each measurement was obtained from 20 runs. Autocorrelations were processed by a hardware correlator (ALV-GmbH, Langen, Germany), and data were analyzed with built-in functions of IgorPro (Wavemetrics, Lake Oswego, OR). For the disruption of F-actin, cells were incubated at 37 °C for 5 min with 1 \( \mu \)M latrunculin B before the FRAP and FCS experiments.

Statistical Analysis—One-way analysis of variance test followed by Bonferroni post-test was run for statistical significance.

RESULTS

PSA Influences ERK Phosphorylation—Rhabdomyosarcoma TE671 cell line (19), which endogenously expresses the polysialylated 140-kDa NCAM (NCAM140) isoform and the glycosylphosphatidylinositol-linked GFRα1 (Fig. 1A), was chosen as
a cellular model to investigate the biological effect of PSA on NCAM, and GDNF signaling via NCAM. We verified that under EndoN treatment it was possible to remove more than 97% of PSA from the cell surface (Fig. 1B). We also combined this enzymatic treatment with the addition of GDNF, a chemotactic factor described as signaling through NCAM in complex with GFRC1 (4). We first observed that removal of PSA by EndoN resulted in an increase in the basal level of ERK phosphorylation (pERK). We found that this effect was maintained upon GDNF treatment (Fig. 1B). NCAM modification by PSA thus interferes with the pERK intracellular signaling pathway threshold as previously reported (20), and this effect was independent of GDNF treatment.

PSA Promotes Cell Migration—To address the physiological significance of PSA in cell migration, we examined the requirement of polysialylation using transfilter migration assays. We observed that removal of PSA decreased by half the ratio between migrating and non-migrating cells (0.49 ± 0.07) (Fig. 2A). Furthermore, removal of PSA dramatically reduced the enhancing effect of GDNF on migration (0.73 ± 0.01), which was clearly observed in the GDNF-treated condition (1.58 ± 0.08). Thus, polysialylation of NCAM plays a role in promoting cell migration but also in efficiently conducting the chemotactic effect of GDNF in TE671 cells.

PSA Expression Is Associated with an Increased Number of Actin Stress Fibers—We performed a phalloidin staining to visualize the filaments of polymerized actin. When these filaments are anchored to the extracellular substrate at focal adhesion sites, they form extensive actin stress fibers. TE671 cells expressing PSA-NCAM displayed more actin stress fibers than cells expressing NCAM. These stress fibers were particularly dense and not limited at the peripheral zone of the cytoplasm (Fig. 2B). This was true for

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**FIGURE 3.** A, sucrose gradient fractionation and immunoblot analysis of TE671 cell lysates in untreated, pretreated by EndoN, and/or GDNF conditions. NCAM immunoblotting was performed to detect the distribution of PSA-NCAM and NCAM in the fractions (lanes 1–8). Lane 8 corresponds to the high density sucrose fraction. Rab5 and horseradish peroxidase-conjugated cholera toxin (GM1 immunoblot) were used as markers of the high density sucrose fraction and lipid raft domains, respectively. IB, immunoblot for the molecules indicated. B, immunocytochemistry of NCAM, PSA-NCAM, and GM1 in TE671 cells. Confocal images of cells stained for NCAM or PSA-NCAM (green) and GM1 (red) under the different conditions cited above. Scale bar, 10 μm. C, bar graph shows quantification of fluorescence signal of GM1 with either PSA or NCAM staining under the different conditions cited above.

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isolated cells as well as for cells in contact with neighboring cells. These data indicate that PSA participates in the organization of actin stress fibers and its presence may affect the relationship of NCAM with the cytoskeleton.

PSA Does Not Modify Localization of NCAM in Cell Subcompartments—To facilitate efficient signal transduction upon stimulation, membrane subcompartments are postulated to serve as platforms to recruit components involved in the signaling complex (21). We asked whether the effect of PSA on the basal level of pERK and cell migration could result from a change in localization of NCAM in membrane subcompartments. We first used well established criteria involving detergent membrane preparations and flotation on sucrose density gradients. We determined that both PSA-NCAM and NCAM were predominantly present in the Triton X-100-soluble fraction corresponding to the high density sucrose fraction (Fig. 3A). Second, we used fluorescent-conjugated cholera toxin that binds to ganglioside GM1 for lipid raft detection (22), followed by immunostaining with either anti-NCAM or anti-PSA antibodies. As shown in Fig. 3, B and C, and in agreement with the density gradient analysis, the majority of NCAM or PSA-NCAM and GM1 stainings were distinct because no more than 35% of colocalization of NCAM or PSA-NCAM with the lipid raft marker was observed. These results are consistent with data published for other cell lines (3).

As GDNF signaling via GFRα1 is thought to occur in lipid rafts (23), we also investigated whether GDNF induced a redistribution of PSA-NCAM or NCAM in subcompartments of the plasma membrane. Addition of GDNF had no significant effect on the partitioning of PSA-NCAM or NCAM (Fig. 3A). Furthermore, colocalization of PSA-NCAM or NCAM with GM1 did not increase upon GDNF treatment (Fig. 3, B and C). These data show that while polysialylation of NCAM influences GDNF-induced cell migration, it is not by inducing long-lasting redistribution of PSA-NCAM in lipid raft.

PSA Does Not Modify NCAM Endocytosis—Given that endocytosis of cell surface molecules can be a mechanism involved in signal transduction responsible for many biological processes, such as cell motility (24), we examined whether PSA would promote internalization of NCAM. We employed a live cell surface biotinylation assay that provides a measure of internalization of endogenous NCAM bearing or not (EndoN pre-treated) PSA in cultured cells (Fig. 4). The efficiency of cell surface biotinylation of both PSA-NCAM and NCAM on surface of living cells was assayed and is represented in lanes 1 and 4. Incubation at 37 °C allowed internalization to occur; then biotin remaining at the cell surface was cleaved such that only endocytosed molecules remained biotinylated. These biotinylated molecules were purified and analyzed for their content in NCAM and PSA-NCAM. Neither EndoN (Fig. 4, lanes 2 and 5) nor GDNF treatment (Fig. 4, lanes 3 and 6) resulted in NCAM endocytosis over this 30-min period of time. Using transferrin we verified that endocytosis could take place in these cells (Fig. 4).

NCAM Clusters Are Highly Mobile—We examined to what extent polysialylation might interfere with NCAM dynamics at the plasma membrane at steady state or after stimulation through GNDF. We performed fluorescent time-lapse confocal microscopy on live TE671 cells transfected with a construct of NCAM140 C-terminal-tagged with GFP. This live cell imaging technique has been previously used for studying the dynamic behavior of other transmembrane proteins fused with GFP (25, 26). As for the endogenous NCAM or recombinant untagged NCAM proteins, identical clusters were observed with the recombinant PSA-NCAM-GFP fusion protein (see supplemental Fig. S1), suggesting that the GFP did not affect the formation of NCAM cell surface clusters. We noted that during 50 min of recording PSA-NCAM-GFP clusters were highly and uniformly mobile (Fig. 5A). Removal of PSA or GDNF treatments did not modify this global behavior of NCAM. At a time scale of 5 min with a frequency of two frames/min, some aggregates of NCAM140-GFP clusters were observed to diffuse at the cell surface rather than internalize (Fig. 5B). During this time scale, some aggregates of NCAM140-GFP clusters were also observed to form and scatter randomly (Fig. 5B). Under PSA removal or GDNF cell treatment these behaviors were unchanged. Thus, distribution of NCAM clusters was characterized by rapid mobility at the cell surface that cannot be blocked or switched to internalization by PSA or GDNF.

PSA Enhances NCAM Lateral Diffusion without Affecting Its Mobility—To further explore at the molecular level the role of PSA on NCAM behavior, we performed measurements on NCAM140-GFP-expressing TE671 cells based on a spot FRAP approach. Briefly, the Z-scan for membrane detection allowed us to precisely perform photobleaching at the cell surface (NCAM Mf = 71 ± 2%). Nevertheless, this percentage was

![Figure 4. Endocytosis assay.](image)
PSA Increases Lateral Diffusion of NCAM

Whereas the lateral diffusion of both PSA-NCAM and NCAM in cell-cell contact was significantly lower (×2.5) than the ones observed in isolated cells (Fig. 6C), an equivalent increase in lateral diffusion was also maintained in the contact zones due to the polysialylation of NCAM (PSA-NCAM $D_{\text{FRAP}} = 0.30 \pm 0.01 \mu m^2 s^{-1}$; NCAM $D_{\text{FRAP}} = 0.20 \pm 0.01 \mu m^2 s^{-1}$). However, this effect was independent of the GDNF treatment (Fig. 6B). Thus, this last result provided evidence that PSA effect on NCAM lateral diffusion persists when cells are in contact or when GDNF is present. This overall decrease in lateral diffusion is probably the result of an increase of friction forces due to trans homophilic or heterophilic cell-cell interactions as suggested by Jacobson et al. (27).

**PSA Enhances NCAM Lateral Diffusion without Affecting Its Confinement**—To extend our analysis of polysialylation modulation of NCAM lateral diffusion, we performed measurements of NCAM140-GFP-expressing TE671 cells using a new FCS approach (17, 28, 29). In this method, we determine the apparent diffusion time ($\tau_0$), which represents the time fluorescent molecules stay on average within the confocal volume of radius $\omega$. By measuring this value as a function of the size of the spot of observation (28), we were able to plot what we called the diffusion law. The deviation of the intercept $\tau_0$ from the origin on the time axis and the effective diffusion coefficient ($D_{\text{eff}}$) allowed us to identify potential confinement mechanisms hindering the lateral diffusion from normal behavior (17, 28).

For PSA-NCAM-GFP, we found that $\tau_0$ increased linearly with $\omega^2$ but intercepted the time axis at a positive value ($\tau_0 = 13.11 \pm 0.51$ ms) (Fig. 7A). This suggested that PSA-NCAM-GFP is hindered in its diffusion by confinement within small domains. Upon PSA removal, we found similar confined diffusion behavior ($\tau_0 = 13.50 \pm 0.51$ ms for NCAM-GFP), suggesting that confinement within domains was unrelated to polysialylation. However, the slopes that represent the effective diffusion coefficient ($D_{\text{eff}}$) were significantly different between the two forms of NCAM-GFP (1.16 ± 0.04 and 0.70 ± 0.01 $\mu m^2 s^{-1}$ for PSA-NCAM and NCAM, respectively). Because this value depends on the partition of the molecules within domains and the effective diffusion coefficient outside of the

not significantly different from the polysialylated (PSA-NCAM Mf = 79 ± 2%) or the GDNF condition (NCAM + GDNF Mf = 77 ± 3%; PSA-NCAM + GDNF Mf = 71 ± 3%) (Fig. 6A).

Acquisitions in contact regions between two transfected cells revealed that the mobile fraction remained high (no significant changes between isolated and interacting cells) independently of the treatment (PSA removal or addition of GDNF) (Fig. 6B).

Next, we calculated the coefficients of lateral diffusion ($D_{\text{FRAP}}$) to explore whether the previous conditions tested affected NCAM lateral mobility. We found that, in isolated cells, polysialylation was able to significantly increase by 52% the lateral diffusion of NCAM (PSA-NCAM $D_{\text{FRAP}} = 0.76 \pm 0.03 \mu m^2 s^{-1}$ and NCAM $D_{\text{FRAP}} = 0.50 \pm 0.01 \mu m^2 s^{-1}$). GDNF did not modify the NCAM $D_{\text{FRAP}}$ value, and the effect of polysialylation was preserved upon GDNF treatment (Fig. 6A).
domains, these results confirmed that the lateral diffusion of NCAM significantly increased by 65% when PSA was present.

GDNF stimulation did not modify the PSA-NCAM $D_{\text{eff}}$ value, and the effect of polysialylation on the lateral diffusion was preserved upon GDNF treatment (Fig. 7B). To verify that the EndoN treatment did not induce unspecific modifications of the membrane organization, we analyzed the lateral diffusion of another immunoglobulin superfamily protein, Thy1, which is not polysialylated. By expressing Thy1-GFP construct in TE671 cells, we verified that EndoN was indeed not affecting the lateral diffusion of Thy1-GFP (Fig. 7C). Thus, the differences in NCAM lateral diffusion revealed by EndoN treatment resulted specifically from PSA removal.

Disrupting Actin Organization Suppresses the Confinement of NCAM and the Enhancing Effect of PSA on NCAM Lateral Diffusion—in an attempt to characterize the nature of the confinement in small domains of NCAM and to find a relationship between NCAM lateral diffusion, cellular migration, and actin stress fibers, which are all dependent on the polysialylation
PSA Increases Lateral Diffusion of NCAM

In this study, we have evaluated the influence of PSA on NCAM at the cellular and molecular level. We first characterized a cellular model to investigate the functional consequences of such modification. Our present experiments on the TE671 cell line, which endogenously expresses PSA-NCAM140, combined with the use of EndoN, allowed us to observe that PSA by itself influences pERK. In addition, the chemotaxis assay results showed that PSA contributes to enhanced cell migration, which was correlated to the formation of actin stress fibers. How does PSA exert its effect in mediating biological responses like chemotactic cell migration? Because NCAM is the only carrier of PSA, we addressed this question by delineating PSA-dependent and -independent NCAM dynamics in living TE671 cells. We could exclude that PSA modulates NCAM endocytosis or favors recruitment of NCAM to lipid rafts in the cell membrane. However, if our time-lapse recording data confirmed a homogeneous distribution of PSA-NCAM and NCAM, similar to the situation described for migrating neurons (32), they provide evidence that at the cell surface both PSA-NCAM and NCAM clusters are highly mobile rather than immobile. Considering our time-lapse observations, we investigated precisely the dynamics of NCAM at the molecular level using more sensitive techniques, i.e. spot FRAP and FCS. Measurements obtained by both techniques demonstrated a PSA-dependent increased NCAM diffusivity but a PSA-independent change in the mobile fraction or confinement of NCAM. When PSA-NCAM and NCAM were photobleached in cell-cell contact regions, their lateral diffusion values were decreased but the enhancing effect of PSA on NCAM lateral diffusion was preserved as for isolated cells. It appears then that PSA controls NCAM molecular behavior by regulating specifically its lateral diffusion.

Furthermore, PSA-NCAM confinement and lateral diffusion were dependent on the integrity of the actin cytoskeleton, since latrunculin B-mediated complete disruption of microfilaments led to a change in these parameters. Indeed, the new lateral diffusions observed after this disruption were identical for NCAM and PSA-NCAM. Thus, the cytoskeleton appears as a crucial parameter to reveal the enhancing effect of PSA on NCAM lateral diffusion.
PSA Increases Lateral Diffusion of NCAM

We have also shown that removal of PSA from NCAM led concomitantly to a decrease in actin stress fibers and abolished the chemotactic effect of GDNF on TE671 cells. Therefore, both NCAM lateral diffusion and a directional cell migration are affected by the polysialylation state of NCAM and both involve the cytoskeleton. The actin cytoskeleton therefore appears as a physical link between NCAM lateral diffusion and an associated cellular behavior.

Some NCAM functions are mediated by lateral interactions of NCAM with signal transduction receptors such as GDNF receptors (33). Our data establish that GDNF effect on migration is largely dependent on the polysialylated state of NCAM since removal of PSA reduces drastically the GNF action. Interestingly, PSA has also been reported to be necessary to conduct the chemoattractant effect of several other trophic factors such as platelet-derived growth factor (34) or responses of neurons to brain-derived neuron factor (35) and ciliary neurotrophic factor (36). We failed, however, to detect any change in the molecular dynamics of PSA-NCAM and NCAM upon GDNF stimulation. A possibility could be that PSA, instead of modulating the response of the cell via an interaction with GDNF and/or its cell surface receptors, may in fact be necessary for the stimulated cell to trigger the intracellular signaling cascades and molecular machinery involved in migration and cell polarity. This is also compatible with our observation that PSA by itself influences the ERK phosphorylation level. Further experiments will be necessary to explore this hypothesis and understand the processes by which PSA potentiates the action of growth factors like GDNF.

Altogether, our results showed that in addition to increasing intermembrane repulsion (14) polysialylation conveys to NCAM an intrinsic capacity to modify its lateral diffusion at the cell surface, even when the molecule is engaged in cell-cell contact or triggered by an extracellular factor such as GDNF. So far, changes in the molecular dynamics of proteins have been reported by investigating preferentially the role of the transmembrane and intracellular domains in the molecular mobility and lateral diffusion of cell surface proteins (27, 37) based on the hydrodynamic model developed by Saffman and Delbruck (38). However, a recent model proposed by Gambin et al. (39) postulates a relevant dependence of the diffusion on the radius of the extracellular part of the protein. The carbohydrate PSA is added post-translationally on the extracellular part of NCAM and creates a three-dimensional highly hydrated excluded volume, which appears, according to our results, sufficient to modify the lateral diffusion of NCAM. We suggest that this regulation may be the result of a differential coupling of NCAM to the cytoskeleton. Our data report the first functional characterization obtained in living cells of the role of a post-translational modification to a cell surface receptor as an efficient way to control its lateral diffusion.

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REFERENCES
1. Povlsen, G. K., Ditlefsen, D. K., Berezin, V., and Bock, E. (2003) Neurochem. Res. 28, 127–141
2. Rougon, G., and Hobert, O. (2003) Annu. Rev. Neurosci. 26, 207–238
3. Niethammer, P., Delling, M., Sytnyk, V., Dityatev, A., Fukami, K., and Schachner, M. (2002) J. Cell Biol. 157, 521–532
4. Paratcha, G., Ledda, F., and Ibanez, C. F. (2003) Cell 113, 867–879
5. Iwase, T., Jung, C. G., Bae, H., Zhang, M., and Soliven, B. (2005) J. Neurochem. 94, 1488–1499
6. Rutishauser, U. (1998) J. Cell. Biochem. 70, 304–312
7. Hinsby, A. M., Berezin, V., and Bock, E. (2004) Front. Biosci. 9, 2227–2244
8. Kiss, J. Z., and Muller, D. (2001) Rev. Neurosci. 12, 297–310
9. Theodosi, D. T., Bonhomme, R., Vitiello, S., Rougon, G., and Poulin, D. A. (1999) J. Neurosci. 19, 10228–10236
10. Weinhold, B., Seidenfaden, R., Rocke, I., Muhlenhoff, M., Schertzing, F., Konzelmann, S., Marth, J. D., Gerardy-Schahn, R., and Hildebrandt, H. (2005) J. Biol. Chem. 280, 42971–42977
11. Torregrossa, P., Buhl, L., Bancel, M., Durbec, P., Schaefer, C., Schachner, M., and Rougon, G. (2004) J. Biol. Chem. 279, 30707–30714
12. Yang, P., Yin, X., and Rutishauser, U. (1992) J. Cell Biol. 116, 1487–1496
13. Yang, P., Major, D., and Rutishauser, U. (1994) J. Biol. Chem. 269, 23093–23094
14. Johnson, C. P., Fujimoto, I., Rutishauser, U., and Leckband, D. E. (2005) J. Biol. Chem. 280, 137–145
15. Wang, C., Rougon, G., and Kiss, J. Z. (1994) J. Neurosci. 14, 4464–4467
16. Rougon, G., Dubois, C., Buckley, N., Magnani, J. L., and Zollinger, W. (1986) J. Cell Biol. 103, 6 Pt 1, 2429–2437
17. Wawrezinieck, L., Rigneault, H., Marguet, D., and Lenne, P. F. (2005) Biophys. J. 89, 4029–4042
18. Tsuji, A., and Ohnishi, S. (1986) Biochemistry 25, 6133–6139
19. Stratton, M. R., Darling, J., Pilkington, G. J., Lantos, P. L., Reeves, B. R., and Cooper, C. S. (1989) Carcinogenesis 10, 899–905
20. Seidenfaden, R., Krauter, A., Schertzing, F., Gerardy-Schahn, R., and Hildebrandt, H. (2003) Mol. Cell. Biol. 23, 5908–5918
21. Golub, T., Wacha, S., and Caroni, P. (2004) Curr. Opin. Neurobiol. 14, 542–550
22. Lai, E. C. (2003) J. Cell Biol. 162, 365–370
23. Staroma, M. (2001) Trends Neurosci. 24, 427–429
24. Polo, S., and Di Fiore, P. P. (2006) Cell 124, 897–900
25. Okabe, S., Kim, H. D., Miwa, A., Kuriu, T., and Okado, H. (1999) Nat. Neurosci. 2, 804–811
26. Stephens, D. J., and Allan, V. J. (2003) Science 300, 82–86
27. Jacobson, K. A., Moore, S. E., Yang, B., Doherty, P., Gordon, G. W., and Walsh, F. S. (1997) Biochim. Biophys. Acta 1330, 138–144
28. Wawrezinieck, L., Lenne, P. F., Marguet, D., and Rigneault, H. (2004) Proc. SPIE Int. Soc. Opt. Eng. 103, 5462–5492
29. Lenne, P. F., Wawrezinieck, L., Conchonaud, F., Wurtz, O., Bock, E., Guo, X. J., Rigneault, H., He, H. T., and Marguet, D. (2006) EMBO J. 25, 3245–3256
30. Spector, I., Shochet, N. R., Kashman, Y., and Grosseiss, A. (1983) Science 219, 493–495
31. Tang, L., Gao, T., McCollum, C., Jang, W., Vicker, M. G., Ammann, R. R., and Gomez, R. H. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1371–1376
32. Doetsch, F., Garcia-Verdugo, J. M., and Alvarez-Buylla, A. (1997) J. Neurosci. 17, 5046–5061
33. Paratcha, G., Ibanez, C. F., and Ledda, F. (2006) Mol. Cell. Neurosci. 31, 505–514
34. Zhang, H., Vutsikis, L., Calaor, V., Durbec, P., and Kiss, J. Z. (2004) J. Cell. Biol. 117, Pt 1, 93–103
35. Muller, D., Djebara-Hannas, Z., Jourdain, P., Vutsikis, L., Durbec, P., Rougon, G., and Kiss, J. Z. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4315–4320
36. Vutsikis, L., Gascon, E., and Kiss, J. Z. (2003) Eur. J. Neurosci. 17, 2119–2126
37. Thoumine, O., Saint-Michel, E., Dequidt, C., Falk, J., Rudge, R., Galli, T., Faivre-Sarrailh, C., and Choquet, D. (2005) Biophys. J. 89, 140–142
38. Saffman, P. G., and Delbruck, M. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3111–3113
39. Gambin, Y., Lopez-Esparza, R., Reffay, M., Sierieccki, E., Gov, N. S., Genest, M., Hodges, R. S., and Urbach, W. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 2098–2102