In Situ Fluorescence Imaging of Myelination

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SUMMARY We describe a novel fluorescent dye, 3-(4-aminophenyl)-2H-chromen-2-one (termed case myelin compound or CMC), that can be used for in situ fluorescent imaging of myelin in the vertebrate nervous system. When administered via intravenous injection into the tail vein, CMC selectively stained large bundles of myelinated fibers in both the central nervous system (CNS) and the peripheral nervous system (PNS). In the CNS, CMC readily entered the brain and selectively localized in myelinated regions such as the corpus callosum and cerebellum. CMC also selectively stained myelinated nerves in the PNS. The staining patterns of CMC in a hypermyelinated mouse model were consistent with immunohistochemical staining. Similar to immunohistochemical staining, CMC selectively bound to myelin sheaths present in the white matter tracts. Unlike CMC, conventional antibody staining for myelin basic protein also stained oligodendrocyte cytoplasm in the striatum as well as granule layers in the cerebellum. In vivo application of CMC was also demonstrated by fluorescence imaging of myelinated nerves in the PNS. (J Histochem Cytochem 58:611–621, 2010)

KEY WORDS myelin staining demyelination remyelination coumarin imaging multiple sclerosis PNS nerves 3-(4-aminophenyl)-2H-chromen-2-one

MYELINATION IS ONE OF THE MOST fundamental biological processes during development of the vertebrate nervous system. Myelin sheaths are unique structures in the nervous system that foster rapid and efficient conduction of impulses along the length of axons. Destruction of or changes in myelin occur in many acquired or inherited neurodegenerative diseases, such as multiple sclerosis (MS). For therapeutic intervention in MS and other myelin-related neurological disorders, significant efforts have been made to develop novel drugs that are aimed at preventing demyelination and/or promoting remyelination. To facilitate these studies, it is imperative to develop an imaging tool that allows direct visualization of myelin sheaths in vivo.

Currently, visualization of myelin sheaths relies on the use of conventional myelin stains such as luxol fast blue (LFB) (Kluver and Barrera 1953; Presnell and Schreibman 1997; Kiernan 1999; Bancroft and Gamble 2002), fluoromyelin (Kanaan et al. 2005), Black-Gold (Schmued and Slikker 1999), Black-Gold II (Schmued et al. 2008), and antibodies for myelin basic protein (MBP) and proteolipid protein (Horton and Hocking 1997). These histochemical methods are limited to staining of myelin sheaths after the animal has been sacrificed and the tissue has been fixed, owing to the lack of permeability across tissue barriers, including the blood–brain barrier. Thus, tissues must be sectioned prior to examination of any myelin changes. Selection of tissues is often made on an empirical basis, and only small samples of tissue sections are stained. Although myelin staining of postfixed tissue may suffice in preclinical studies in many cases, it cannot be applied directly to clinical studies in human subjects. This is because myelinated nerves perform critical functions and complex signal transduction; even biopsies cannot be conducted to examine myelin changes. In addition,
myelin changes often occur at random along myelinated nerves across different regions; consequently, local examination with myelin staining in limited regions may not represent the global changes in myelin throughout the patient. Therefore, it is necessary to develop novel agents that can be used to identify myelinated nerve fibers in situ. Such agents have the potential to be used for intra-operative fluorescent imaging, which has become an indispensable tool for nerve mapping in surgical procedures (Figueiredo et al. 2010; Nguyen et al. 2010).

Thus, it is necessary to develop novel myelin-imaging agents that can be intravenously administered to stain myelinated nerves in vivo. Such a procedure will allow immediate, dynamic characterization of myelinated nerves. Over the past several years, we have developed analogs of LFB that are considerably more lipophilic while maintaining myelin-binding affinity and specificity. Neutral derivatives mimic the backbone of LFB by eliminating the acidic sulfonic acid groups and carcinogenic diazo bonds. These considerations led us to develop a wide array of lipophilic distyryl benzene and stilbene agents (Stankoff et al. 2006; Wu et al. 2006; Wang et al. 2009). To enhance the potential for routine studies, we have also developed a series of stilbene derivatives for use as novel myelin-imaging agents (Wu et al. 2008). To date, our studies with these agents have focused on imaging central nervous system (CNS) myelin.

During the course of our studies, we have found that coumarin derivatives that possess the same pharmacophore as stilbene also selectively bind to myelin. Coumarin is a naturally occurring compound in plants with many important biological activities (Lee et al. 2006 and literature cited therein), and coumarin derivatives have been widely applied for clinical use (Marshall et al. 1991). However, their myelin-binding properties have not been reported. Here, we report 3-(4-aminophenyl)-2H-chromen-2-one (termed casein aldehyde at 4C.

To remove and incubated for 24 hr in 4% paraformaldehyde in PBS followed by 4% paraformaldehyde in PBS. Brains were removed and incubated for 24 hr in 4% paraformaldehyde at 4C.

Animal Preparation
All animal experiments were performed in accordance with guidelines approved by the Institutional Animal Care and Use Committee of Case Western Reserve University (Protocol 2006-0176). The animals were subjected to minimal stress during tail vein injections.

Animals. Two-month-old Swiss-Webster R/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME, and used as the control. The plp-Akt-DD transgenic mice were generated as described previously (Flores et al. 2008; Narayanan et al. 2009) and used at 2 months of age. Briefly, the transgenic mice expressing constitutively active Akt (HAAkt308D473D, Akt-DD) driven by the plp promoter (Wight et al. 1993) were generated and used as a hypermyelinated animal model. The Akt cDNA was inserted into the AsciI/PaciI sites of the modified plp promoter cassette, and hypermyelination was induced after the plp promoter/Akt-DD insert was injected into SJL/SWR F1 mice. Positive founders were identified by PCR amplification of tail DNA using IntronSV40F (5′-GCAGTGGACACGGGTCACT-3′) and Akt lower (5′-CTGGCAACTAGAACGCACAG-3′) primer pairs. Analyses were done from wild-type littermate mice in all developmental experiments and, when possible, with older animals. In all three lines, plp-Akt-DD was used for the studies.

Euthanasia. Mice were deeply anesthetized with isoflurane and perfused via the ascending aorta with PBS followed by 4% paraformaldehyde in PBS. Brains were removed and incubated for 24 hr in 4% paraformaldehyde at 4C.

Tissue Processing. Brain tissue was rinsed in PBS. After treatment with 15% sucrose for 1 hr, the tissues were incubated in 30% sucrose until submerged. Free-floating or frozen sections were used for tissue staining. For preparation of free-floating sections, the cryoprotected tissues...
were first frozen in dry ice followed by axial sectioning (30 µm) using a sliding microtome. For preparation of fresh-frozen sections, the cryoprotected tissues were first frozen in OCT on dry ice before axial sectioning (20 µm) with a cryostat at −20C. For both types of sections, tissue from the midline of the brain containing the whole corpus callosum was selected for staining. Stained sections were covered with fluorescence mounting medium (Vectorshild; Vector Laboratories, Burlingame, CA) and stored at 4C for future analysis.

**Immunohistochemistry**

Free-floating sections were rinsed in 1× PBS (2 × 5 min), incubated in 1% H2O2/10% Triton X-100, and then washed with 1× PBS (3 × 5 min). After incubation in 3% normal goat serum for 1 hr, the free-floating sections were then immunostained overnight at 4C with rabbit anti-MBP antibody (Chemicon-Millipore, Bedford, MA; 1:2000) in 3% normal goat serum in 1× PBS. Sections were incubated for 1 hr at room temperature in IRDye 800CW goat anti-rabbit (LI-COR Biosciences, Lincoln, NE; 1:5000), and then rinsed in 1× PBS (2 × 5 min). Images of the stained mouse brain sections were acquired on a LI-COR Odyssey infrared imaging system (LI-COR Biosciences). MBP immunoreactivity of the stained sections was then quantified on the LI-COR Odyssey infrared imaging system using 21-µm resolution, 1.2-mm offset with highest quality, and 3.0 channel sensitivity. The integrated densities of the midline corpus callosum were obtained using the associated Odyssey software. Statistical analysis was performed using a non-paired Student’s t-test (GraphPad Prism; GraphPad Software, La Jolla, CA).

**CMC Tissue Staining**

Free-floating sections (30 µm thick) were incubated in 1% H2O2/10% Triton X-100 in 1× PBS for 10 min, and then in CMC (100 µM) in 1% DMSO/1× PBS for 30 min at room temperature. The sections were then washed three times for 5 min each with 1× PBS. Images of the stained mouse brain sections were acquired on a Leica DMI6000 inverted microscope (2.5× objective, BP530/30-nm filter; Leica Microsystems, Inc., Bannockburn, IL) with a Hamamatsu Orca-ER digital camera (Bridgewater, NJ) operated with Improvision’s Volocity software (PerkinElmer; Waltham, MA). Image J software (http://rsb.info.nih.gov/ij/) was used to quantify pixel intensity values. The corpus callosum between the midline and below the apex of the cingulum was defined as the region of interest. The density of myelin in the corpus callosum of wild-type mice was given the arbitrary value of 100, and the density of myelin in plp-Akt-DD mice was determined as a percentage of that in wild-type mice.

The data were analyzed using GraphPad Prism software with a non-paired Student’s t-test.

**In Situ Staining of CNS Nerves**

A dose of 25 mg/kg of CMC dissolved in DMSO was administered via intravenous injection into the tail vein of 2-month-old wild-type mice and plp-Akt-DD mice. At 10 min, 1 hr, and 24 hr after injection, the mouse brains were perfused and sectioned as described above. Fluorescent images of selected brain sections from the midline were directly acquired on a Zeiss Axiovert 200M inverted microscope (2.5× objective, DAPI filter) with an AxioCam digital camera (Carl Zeiss MicroImaging, Inc.; Thornwood, NY).

To examine the myelin-binding properties of CMC and to correlate CMC staining with immunohistochemistry, we double-stained selected tissue sections with CMC and anti-MBP monoclonal antibody. Briefly, fresh-frozen sections were first stained with CMC as described above. The CMC-stained sections were then rinsed in 3% normal goat serum in 0.1% Triton X-100/1× PBS (2 × 5 min) and washed with 1× PBS (3 × 5 min). The fresh-frozen sections were immunostained overnight at 4C with rabbit anti-MBP antibody (Chemicon-Millipore; 1:1000) in 3% normal goat serum in 1× PBS. Sections were incubated for 1 hr at room temperature in IRDye 800CW goat anti-rabbit (LI-COR Biosciences; 1:1000) and rinsed in 1× PBS (2 × 5 min). Images of the stained mouse brain sections were acquired on a Leica DMI6000 inverted microscope (1.25× and 5× objectives, BP530/30-nm filter).

**In Situ Staining of PNS Sciatic Nerves**

A dose of 25 mg/kg of CMC dissolved in DMSO was administered to 6-week-old C57BL/6 black mice via tail vein injection. One hr after the injection, mice were perfused, and tissues from the upper region of the legs containing sciatic nerves were removed and cryoprotected as described above. Following cryostat sectioning (20 µm), images of the in situ–stained sections were acquired on a Leica DMI6000 inverted microscope (BP530/30-nm filter).

**In Vivo Visualization of PNS Nerves**

Six-week-old C57BL/6 black mice were fasted for 72 hr to minimize autofluorescence inside the body. Then, a dose of 25 mg/kg of CMC was administered via tail vein injection. Five min postinjection, PNS nerves in the mouse ears were imaged. The images of the mouse ears were acquired with a Sony digital camera under normal light and subsequently under ultraviolet (UV) light (365 nm). Then the mice were perfused as described above. The images of the mouse ears were acquired again under the same conditions for comparison. For in vivo visualization of sciatic nerves, the same dose
of CMC was administered via tail vein injection. Ten min postinjection, the mice were deeply anesthetized with isoflurane. Sciatic nerves in the upper leg regions were then surgically exposed and imaged under normal light and subsequently under UV light (365 nm).

**Results**

To date, we have screened several coumarin derivatives that potentially bind to myelin membranes. The structures of these coumarin derivatives are shown in Figure 1. All of these compounds are intrinsically fluorescent. CMC is moderately lipophilic with a calculated LogP value of 2.68, and it shares a similar pharmacophore with the other myelin-imaging agents (Figure 2). The emission and excitation wavelengths of CMC were determined in three different solvents: methylene chloride, DMSO, and methanol in order of increasing polarity. As shown in Figure 3, the emission and excitation bands of CMC ranged from the blue region to the near-infrared region depending on the polarity of the solvent used. The maximum excitation shifted from 348 nm in methylene chloride to 407 nm in DMSO to 655 nm in methanol. Similarly, the maximum emission shifted from 479 nm in methylene chloride to 551 nm in DMSO to 674 nm in methanol.

**In Vitro Tissue Staining**

Fluorescent tissue staining represents a direct approach to evaluating the binding specificity of CMC for myelin sheaths. The fluorescent nature of CMC allows direct comparison of tissue staining with other myelin stains. As shown in Figure 4, CMC stained mouse brain tissue sections similarly to other conventional myelin stains such as LFB. At 100 μM, CMC selectively stained intact myelin tracts in the myelinated corpus callosum region. Among the coumarin derivatives we have screened, CMC exhibited the highest contrast (Figures 4A and 4C) in wild-type brain. CMC stained myelin sheaths relative to the levels of myelination. For example, in the plp-Akt-DD mouse, in which the size of the corpus callosum is enlarged, CMC readily revealed this hypermyelination. Compared with control sections, plp-Akt-DD mouse brain sections showed increased CMC staining that was comparable to the size of the corpus callosum (Figures 4B and 4D). For correlation, MBP immunostaining was also conducted in adjacent sections (Figure 4E). The CMC staining pattern was consistent with the MBP immunostaining. Accordingly, the enlargement of the hypermyelinated corpus callosum was also confirmed by MBP immunostaining using adjacent brain sections (Figure 4F).

Quantitative analysis indicated that the fluorescence intensity was proportional to the level of myelination. As shown in Figure 5, MBP antibody staining showed fluorescence intensity in the plp-Akt-DD model that was 1.31-fold higher than that in the wild-type control brain. CMC staining exhibited a similar ratio of fluorescence intensity between the two types of mice. In the plp-Akt-DD mouse brain, the fluorescence intensity of CMC was 1.27-fold higher than that in the wild-type mouse brain, which is consistent with the MBP immunostaining. Interestingly, these results are also in line with a previously developed measurement method.

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![Figure 1](image) Structures of coumarin derivatives that have been screened for myelin staining. CMC, case myelin compound.
based on X-ray diffraction (Avila et al. 2005). According to this assay, the amount of myelin in the \textit{plp}-Akt-DD mice was also found to be 1.3-fold or 30\% higher than that in the wild-type mice at 2 months of age (Flores et al. 2008).

In Situ Staining of CNS Nerves

Next, we investigated the ability of CMC to label myelin ex vivo in the mouse brain. A dose of 0.5 mg CMC (25 mg/kg) was injected via the tail vein into wild-type mice and \textit{plp}-Akt-DD mice. One hr postinjection, mice were perfused, and the brains were removed and sectioned. CMC staining of myelin was then directly examined with fluorescence microscopy. As shown in Figures 4G–4J, CMC readily entered the brain and selectively labeled myelin sheaths of the corpus callosum and cerebellum in both wild-type mice and \textit{plp}-Akt-DD mice, suggesting that CMC stains CNS myelin in situ.

To determine the time course of the in situ myelin staining by CMC, we examined brain tissue sections at different time points after injection of 1.0 mg (25 mg/kg) of CMC (Figure 6). As early as 10 min postinjection, strong fluorescence was observed in the corpus callosum. Similar fluorescence intensity was observed as late as 4 hr postinjection. After 24 hr, however, the fluorescence due to CMC disappeared and became almost invisible.

The distribution of CMC binding to myelin was revealed by comparing CMC staining with immunohistochemical staining for MBP in the cerebellum, caudate putamen, and temporal cortex (Figure 7). In the cerebellum, CMC staining was confined to myelin tracts, whereas MBP staining was observed in the granule cell layers. In the caudate putamen, CMC selectively stained myelin fibers, whereas MBP also
Figure 4  In vitro CMC staining of the entire brain section (A) and corpus callosum (C) in wild-type mouse brain. In vitro CMC staining of the entire brain section (B) and corpus callosum (D) in plp-Akt-DD mouse brain. Myelin basic protein (MBP) immunohistochemical staining of wild-type mouse brain (E) and plp-Akt-DD mouse brain (F). In situ CMC staining of myelin sheaths in the corpus callosum of wild-type mouse (G) and plp-Akt-DD mouse (H), and cerebellum of wild-type mouse (I) and plp-Akt-DD mouse (J).
stained oligodendrocyte cell bodies and processes. In the temporal cortex, no CMC staining was observed, whereas MBP staining revealed the presence of fine myelinated fibers in the gray matter. These studies suggest that CMC selectively binds to large myelinated fiber tracts and nerves.

**In Situ Staining of Sciatic Nerves**

Next, the ability of CMC to stain myelinated nerves in the PNS was investigated. We first examined in situ fluorescent staining of sciatic nerves by CMC under the same conditions as in situ staining of CNS nerves (Figure 8A). For in situ staining in live animals, mice were first anesthetized, and the large sciatic nerve, along with a small sciatic nerve branch, was exposed (Figure 8B). A dose of 1.0 mg CMC (25 mg/kg) was then administered to wild-type mice via tail vein injection. At 10 min after injection, strong fluorescence appeared in the exposed nerves. As shown in Figure 8C, CMC readily perfused across the tissues and selectively stained both large and small PNS nerves in the midst of the surrounding tissues.

**In Vivo Visualization of PNS Nerves**

To examine the ability of CMC to visualize PNS nerves in vivo, CMC was administered to C57BL/6 wild-type mice, and the fluorescence of peripheral nerves in mouse ears was directly monitored under UV light in comparison with normal light (Figure 9). To minimize autofluorescence, the mice were fasted for 72 hr (Figure 9A), and then a dose of 1.0 mg (50 mg/kg) of CMC was administered via tail vein injection. Immediately after injection, bright fluorescence was observed in the ear region (Figure 9B). To eliminate the possibility that the fluorescence came from blood vessels in the same region, we perfused the entire mouse with saline. The fluorescence intensity remained the same (Figure 9C). As expected, no difference was observed under normal light (Figures 9D–9F). In addition, no adverse pharmacological effects on the mice in terms of body movement, tail motion, or functions of internal organs were observed after systemically injecting CMC at various doses up to 100 mg/kg, which suggested that the dose required for staining myelinated fibers is consistent with the long-term survival of the animals. These studies indicated that CMC allowed selective visualization of myelinated nerves in situ.
Discussion

Here, we have demonstrated that CMC is an appropriate fluorescent probe for staining myelinated nerve fibers. The advantage of CMC over other conventional myelin stains such as LFB, fluoromyelin, and Black-Gold II is that it readily penetrates tissue barriers, including the blood-brain barrier, and selectively binds to myelin in situ. The pattern of fluorescent CMC
staining in the CNS was comparable to that of immunohistochemical staining for MBP. High-magnification views of both types of staining in different brain regions suggested that CMC staining was confined to compact myelinated fibers, whereas MBP staining also labeled individual myelinated fibers. Further studies in a hypermyelinated mouse model indicated that CMC was a sensitive myelin probe that localized in myelinated regions in proportion to the degree of myelination.

To determine the nature of CMC binding to myelin, we screened a series of coumarin derivatives (Figure 1). None of them exhibited the same staining intensity and sensitivity as CMC. Comparison of these structures suggested that the 2-aminophenyl group is essential for myelin staining. As shown in Figure 2, CMC shares the same pharmacophore as stilbene derivatives that we previously developed as myelin-imaging agents (Wu et al. 2008). The backbone of CMC also consists of two phenyl groups conjugated by a C=C bond (i.e., stilbene). We thus hypothesize that the stilbene backbone interacts with unidentified myelin component(s).

CMC is a fluorescent molecular probe, and its UV excitation and emission wavelengths were examined in three solvents with different polarities, i.e., methylene chloride, DMSO, and methanol. We found that the emission peak was affected by the polarity of the solvent. As shown in Figure 3, the emission peak shifted toward shorter wavelengths in a less polar solvent such as methylene chloride and toward longer wavelengths in a more polar solvent such as methanol. Therefore, the appearance of CMC is expected to vary in different tissue environments. In DMSO solution, for example, an emission peak of 551 nm resulted in a green appearance. After tissue distribution, the emission of CMC shifted toward shorter wavelengths, resulting in an aqua-blue appearance.

As a sensitive myelin-imaging probe, CMC can be used to quantify myelination in situ, as demonstrated in the plp-Akt-DD mouse model of hypermyelination (Figure 4). For example, CMC readily differentiated the plp-Akt-DD mice from normal controls by labeling the enlarged, hypermyelinated corpus callosum. We
found that CMC was relatively resistant to fading in tissue, and thus, the fluorescence intensity can be quantified to determine changes in myelin content (Figure 5). Using the corpus callosum as a region of interest, the ratio of hypermyelination to normal myelination as determined by CMC staining was found to be consistent with that determined by in vitro immunohistochemical staining or previously developed X-ray diffraction.

To determine the time course of CMC distribution, we conducted in situ staining at different time points following tail vein injection. As shown in Figure 6, myelin staining was observed as early as 10 min post-injection and lasted at least 4 hr without significant reduction in fluorescence intensity. After 24 hr postinjection, however, the fluorescence effectively disappeared. This method is compatible with other immunohistochemical staining and processing methods. As shown in Figure 7, after CMC staining, the same tissue sections can be used for immunohistochemical staining for MBP. In addition, the probe remained bound and retained fluorescence during paraffin embedding as well as during storage in cryoprotectant before and after frozen sectioning. After tissue harvesting, the sections were mounted with fluorescence mounting medium. In this way, CMC remained bound and fluoresced for several months without significant fading or diffusion, allowing re-analysis at later times. The fluorescence apparently survives much longer (Figure 4) following in vitro tissue staining and processing than in situ staining. This observation suggests that the relatively rapid depletion of fluorescence following in situ staining may be due to reversible binding of CMC to myelin sheaths in vivo. Thus, bound CMC may dissociate from myelinated regions and subsequently be cleared from the brain at later time points.

Comparison of high-magnification views of CMC staining and MBP staining was conducted in tissue sections harvested from different brain regions, i.e., cerebellum, caudate putamen, and temporal cortex, which represent different amounts of myelination. As shown in Figure 7, CMC staining was confined to compact myelinated fibers in the white matter, whereas MBP staining also labeled individual myelinated fibers in the gray matter. Further studies are underway to develop novel agents that will stain all myelin, including small nerve bundles.

A unique aspect of this work is the ability of CMC to stain myelinated nerve fibers in situ in both the CNS and PNS. Here, we demonstrated that CMC can be used as a fluorescent imaging agent to map nerves in the PNS. We first examined the selective distribution of CMC in regions surrounding sciatic nerves (Figure 8). Following tail vein injection, both large and small sciatic nerves were readily visualized under UV light, with very little nonspecific accumulation in the surrounding tissues.

To further evaluate the potential of CMC staining of myelinated nerves in vivo, we monitored the fluorescence intensity in mouse ears following injection. Owing to intrinsic attenuation of fluorescence by the body, only mouse ears are transparent to fluorescence and can be used directly to monitor the in vivo distribution of CMC. As shown in Figure 9, the autofluorescence was reduced to a minimum when the mice were fasted for 72 hr. As early as 10 min after injection of CMC, strong fluorescence was observed. To eliminate the possibility that CMC would accumulate in the blood vessels, the mice were then perfused with saline. Removing the blood resulted in no change in fluorescence intensity, indicating that CMC selectively stained the myelinated nerves in the ear region.

Thus, this method can potentially be used for nerve mapping, which is often required in intra-operative procedures in which surgeons need to identify myelinated nerves prior to dissecting diseased tissues to preserve functions of normal organs. In previous clinical trials, coumarin derivatives have been given orally over a wide range of doses. A dose of 7 g daily was determined to be the maximal tolerated dose (MTD) (Marshall et al. 1994). In our preclinical studies, a dose of 10–25 mg/kg was often used in mice, which is equivalent to 1.5 g in humans. This dose is well below the MTD of coumarin. In addition, for intra-operative fluorescent imaging applications, for example, it is also possible to administer CMC locally to selected organs of interest, for which a fraction of the dose required for the whole body can be used.

Because CMC can be administered via intravenous injection, it first distributes throughout the entire body and then selectively stains myelinated nerves. This property will make it possible to examine myelin changes in different regions of interest. With current methods of colorimetric histochemical staining and immunostaining using antibodies, brain tissues must be sectioned first, and it is difficult to stain all the sections. Because only few empirically selected sections will be stained, myelin changes, if any, may be overlooked when they occur in regions that are not included in the sections to be examined. Using in situ staining, however, all myelinated regions can be stained at the same time and can potentially be examined. As a direct application, in situ staining is especially useful for the newly developed cryoimaging by Nguyen (Nguyen et al. 2008), a technique designed to conduct automated sectioning of brain tissues after in situ staining and that provides molecular fluorescence images of the whole brain using section-and-image technology. The system consists of a mouse-sized, motorized cryo-microtome interfaced with a modified fluorescence microscope and a robotic xyz imaging system positioner, all of which are fully automated by a control system. In addition, CMC as a myelin-imaging
agent could potentially be used in laser capture analysis and electrophysiological studies.

Compared with other myelin-imaging agents that we have previously developed, coumarin derivatives are unique because they exhibit negligible toxicity, as demonstrated in both preclinical (Luszczki et al. 2009) and clinical settings (Casley-Smith 1999), making them candidates for further development as myelin-imaging agents that are suitable for clinical imaging modalities and translational studies.

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Literature Cited


