



Alexa 680 conjugated to bovine serum albumin

Alexa680-BSA

Arvind Chopra, PhD¹

Created: March 8, 2012; Updated: March 29, 2012.

Chemical name:	Alexa 680 conjugated to bovine serum albumin	Structure not available in PubChem .
Abbreviated name:	Alexa680-BSA	
Synonym:		
Agent Category:	Protein	
Target:	Lymphatic system	
Target Category:	Others	
Method of detection:	Optical imaging (near-infrared fluorescence (NIRF) imaging)	
Source of signal / contrast:	Alexa 680	
Activation:	No	
Studies:	<ul style="list-style-type: none"> <i>In vitro</i> Rodents 	

Background

[PubMed]

The lymphatic system collects lymph, the excess protein-rich fluid that diffuses out of blood vessels, and returns it to blood circulation (1). The flow of lymph in the system is unidirectional, and in addition to proteins it carries various antigens and antigen-presenting cells (activated) to the lymph nodes, which is where the effector cell and humoral immune responses are launched into the circulatory system (for details, see Alitalo (1)). A damaged lymphatic system (due to hereditary or accidental reasons) can lead to the development of a suboptimal immune response and/or [lymphedema](#) due to the accumulation of lymph, fat, and connective tissues at the affected site. There is currently no effective treatment for lymphedema (2). In addition, the functioning of the lymphatic system and the process of lymph flow are not well understood because there is a lack of suitable invasive or noninvasive method(s) (such as imaging) that can be used for the preclinical or clinical study of these physiological processes (1, 2). Quantitative lymphoscintigraphy is commonly used to visualize lymphatic function and lymph flow in animals and humans; however, the main disadvantages of using this method are the application of radioactive materials, limited spatial resolution of the images, and the possibility of direct radionuclide diffusion into the surrounding normal tissues through microcapillaries as a result of proteolysis, dissociation of radiotracer from the imaging agent, and vascular transport (3). Optical imaging has been used to assess the lymphatic functions in mice after direct intradermal injection of indocyanine green (ICG), a small

molecule near-infrared fluorescence dye, but quantification of lymph flow in the animals was difficult because the dye was cleared rapidly from the interstitium by the lymphatics and blood circulation in the animals (2, 4). In another study, liposome-enclosed ICG (LP-ICG) was used for the imaging of the mouse lymphatic system, and in the same study the preparation was shown to be unstable in 50% fetal bovine serum (5). This indicated that release of free ICG into the system from the LP-ICG preparation could result in an inaccurate estimation of lymph flow in the animals. In addition, free ICG was reported to alter contractility of the rat lymphatic vessels in a dose- and dilution-dependent manner, suggesting that measurement of lymph flow with ICG may not be a true representation of the process in the animals (6).

In a continued effort to develop an agent that can be used with a noninvasive technique to measure the lymph flow in rodents, Alexa 680-labeled bovine serum albumin (Alexa680-BSA) was synthesized and evaluated with near-infrared fluorescence imaging in normal mice and rats and in lymphedema (Chy) mice (2).

Related Resource Links

Alexa Fluor-related chapters in [MICAD](#)

[Clinical trials](#) related to lymphatic system

[Lymph system](#) (MedlinePlus, National Library of Medicine, NIH)

Synthesis

[PubMed]

The synthesis of Alexa680-BSA has been described by Karlsen et al. (2). The conjugated albumin was purified with size-exclusion chromatography and stored as a solution (formulation not reported) with a protein concentration of 1 mg/mL at -20°C until required. The fluorescent dye/protein ratio was reported to be 2.7. The fluorescent dye-conjugated BSA was reported to have a purity of $>99\%$ as determined with high-performance liquid chromatography (HPLC).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

HPLC analysis showed that Alexa680-BSA was stable for at least 3 months at 4°C (2). The Alexa680-BSA conjugate was reported to remain stable in mouse interstitial fluid or 100% mouse serum for up to 96 h at 37°C , and no free dye was detected in the preparation at the end of the incubation period (2).

Using an *in vitro* phantom system, a direct correlation between the concentration of Alexa680-BSA and fluorescence signal intensity was observed by Karlsen et al. ($R^2 = 0.9601$) (2).

An *in vitro* time-domain imaging study (lifetime analysis) showed that in a solution there was a linear correlation ($R^2 = 0.9529$) between the fluorescence lifetime and the albumin conjugate/free dye ratio in solution (2). This indicated that the lifetime analysis could be used to discriminate between the intact Alexa680-BSA conjugate in solution and a conjugate/free dye mixture in solution.

Animal Studies

Rodents

[PubMed]

It has been shown that there was a linear correlation between the amount of Alexa680-BSA injected into the mouse paw skin (~15–90 pmol) and the intensity of the fluorescent signal ($R^2 = 0.9232$) obtained from the site of injection (2).

A lifetime analysis of the fluorescence signal generated by the probe injected into the hind limbs of C57BL/6 mice ($n = 4$ animals/group) from 1 h to 6 h postinjection (p.i.) showed that the average lifetime of the signal was 1.61 ± 0.01 ns at each time point (2). Without blood or lymph circulation, any dye that has dissociated from the probe would accumulate in the skin of dead animals. Therefore, euthanized mice were injected with the fluorescent probe as described above, and the average lifetime of the signal at each time point was determined. The lifetime of the signal from the euthanized animals was reported to be 1.61 ± 0.0005 ns. Because the fluorescence lifetime of the signal in the live and euthanized animals was identical, the investigators concluded that the conjugate was not degraded *in vivo* and was probably suitable to investigate the flow of lymph in rodents (2).

To investigate the lymphatic clearance of Alexa680-BSA in C3H and C57BL/6 mice ($n = 6$ animals/time point per strain), the rodents were given an intradermal injection of the tracer in the hind leg paw skin (2). The rodents were imaged every 1 h for a total of 6 h, and the dye removal rate constant, k , that defines the lymph flow was calculated from the images as explained by Karlsen et al. (2). The fluorescent conjugate was removed from the injection site significantly faster ($P = 0.01$) in the C57BL/6 mice ($k = -0.40 \pm 0.03\%/min$) than in the C3H mice ($k = -0.30 \pm 0.02\%/min$). These observations indicated that the lymph flow in the animals was strain-dependent.

In another study, the investigators studied the effect of local vasoconstriction on the lymph flow in C57BL/6 mice (2). The animals were given an intradermal injection of the probe in the paw, and 1 h later the mice were injected with endothelin-1 (ET-1; a potent vasoconstrictor; 100 pmol) at a site close to the probe injection site to induce local vasoconstriction. Control animals received an injection of normal saline. At 60 min after the ET-1 injection, the average k value for the probe injection site was $-0.04 \pm 0.03\%/min$, which was significantly less ($P = 0.001$) than the value in the control animals ($-0.22 \pm 0.03\%/min$). After the initial inhibition of clearance of the probe at the site of the ET-1 injection, the clearance of Alexa680-BSA was shown to be restored to control levels for at least 1 h (k values were $-0.25 \pm 0.02\%/min$ and $-0.23 \pm 0.03\%/min$ for the ET-1 and the control mice, respectively) (2).

The effect of overhydration on lymph flow was investigated in anesthetized Sprague-Dawley rats (2). The animals were injected intradermally with Alexa680-BSA on the right hind limb paw. The experimental rats ($n = 6$ animals) had their kidneys ligated to prevent water excretion, and these animals were intravenously infused with isotonic saline (15% of body weight) to facilitate volume expansion. Control animals ($n = 5$ rats; anesthetized) were not subject to surgery or infused with saline. The paw was scanned every 30 min; from the images the average k value for the control rats was calculated to be $-0.09 \pm 0.07\%/min$ compared with a significantly higher value of $-0.37 \pm 0.06\%/min$ for the experimental animals ($P = 0.012$; approximately four-fold higher). These observations indicated that the fluorescent conjugate was cleared rapidly from the injection site in the test rats. This showed that Alexa680-BSA could also be used to measure changes in lymph flow in these rodents.

Chy lymphedema mice lack lymphatics in the dermis of the paw, but have a normal lymphatic system in the skeletal muscle of the hind limbs (2). The washout of Alexa680-BSA from the paw skin and thigh muscle of the Chy mice ($n = 6$ animals) was studied by acquiring images over a 6-h period. As a control, the washout of the probe was similarly studied in normal C3H mice ($n = 6$ animals). The k value averaged $-0.16 \pm 0.01\%/min$ in the paw skin of the Chy mice, which was significantly lower ($P < 0.001$) than the k from the paw skin of the C3H mice ($-0.30 \pm 0.02\%/min$). The k values from the hind limb muscle of the Chy and C3H mice were $-0.28 \pm 0.03\%/min$ ($n = 7$ animals) and $-0.32 \pm 0.02\%/min$ ($n = 6$ animals), respectively.

From these studies, the investigators concluded that Alexa680-BSA can be used to study the lymph flow in rodents (2).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

References

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