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# Targeting of the kynurenic acid across the blood–brain barrier by core-shell nanoparticles

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ABSTRACT

Core-shell nanoparticles (CSNPs) were developed to get over therapeutic amount of kynurenic acid (KYNA) across the blood–brain barrier (BBB). Bovine serum albumin (BSA) was used as core for encapsulation of KYNA and the BSA/KYNA composite was finally encapsulated by poly(allylamine) hydrochloride (PAH) polymer as shell. In the interest of the optimization of the synthesis the BSA and KYNA interaction was studied by two-dimensional surface plasmon resonance (SPR) technique as well. The average size of d ~ 100 nm was proven by dynamic light scattering (DLS) and transmission electron microscopy (TEM), while the structure of the composites was characterized by fluorescence (FL) and circular dichroism (CD) spectroscopy. The *in vitro* release properties of KYNA were investigated by a vertical diffusion cell at 25.0 °C and 37.5 °C and the kinetic of the release were discussed. The penetration capacity of the NPs into the central nervous system (CNS) was tested by an *in vitro* BBB model. The results demonstrated that the encapsulated KYNA had significantly higher permeability compared to free KYNA molecules. In the neurobiological serial of *in vivo* experiments the effects of peripherally administered KYNA with CSNPs were studied in comparison with untreated KYNA. These results clearly proved that KYNA in the CSNPs, administrated peripherally is suitable to cross the BBB and to induce electrophysiological effects within the CNS. As the neuroprotective properties of KYNA nowadays are proven, the importance of the results is obvious.



## 1. Introduction

Many potential therapeutic agents that are used in biological systems are limited because they can't get through the BBB, thanks to their properties, for example size, hydrophilic character, charge, *etc*. This barrier can be circumvented by specific transporters, chemical modifications, and interactions with other molecules or application of composites (Gabathuler, 2010). The kynurenine pathway is the main route of tryptophan (Trp) metabolism in mammalian brain, which involves several neuroactive compounds. KYNA is a product of the Trp metabolism, it has a neuroprotective and neuroinhibitory properties (Stone, 1993; Marosi et al., 2010; Sas et al., 2007). It exerts its effect mainly by antagonizing ionotropic glutamate receptors, its neuroprotective capacity can be attributed mainly to the inhibition of the N-methyl-D-aspartate (NMDA) receptors. KYNA can also influence the presynaptic glutamate release by antagonizing the α7-nicotinic-actylcholine receptors (Carpenedo et al., 2001; Zadori et al., 2009). KYNA might play important roles in modulating neurotransmission in the CNS (Fukushima et al., 2007). Alterations of the kynurenine metabolites have been demonstrated in several neurological disorders such as Huntington's disease, migraine or stroke (Darlington et al., 2007; Vécsei et al., 2013). Elevating the level of the neuroprotective KYNA in the brain has been demonstrated to be of therapeutic value in animal models of Parkinson's disease, Huntington's disease and migraine (Fejes et al., 2011; Silva-Adaya et al., 2011). However, systemic administration of KYNA is not reasonable, because its penetration through the BBB is very poor. In our recent studies, we synthesized a new compound, glucosamine-kynurenic acid (KYNA-NH-GLUC), which in peripheral administration was able to cross the BBB, and to induce reductions

*Abbreviations:* BSA, bovine serum albumin; BBB, blood–brain barrier; CD, circular dichroism; CNS, central nervous system; CSNPs, core-shell nanoparticles; DLS, dynamic light scattering; FL, fluorescence spectroscopy; HPLC-MS, high performance liquid chromatography-mass spectrometry; IEP, isoelectric point; KYNA, kynurenic acid; L-KYN, L-kynurenine; PAH, poly(allylamine) hydrochloride; PBS, phosphate buffer; RBECs, rat brain endothelial cells; SEPs, somatosensory evoked responses; SPR, surface plasmon resonance; TEM, transmission electron microscopy; Trp, tryptophan.

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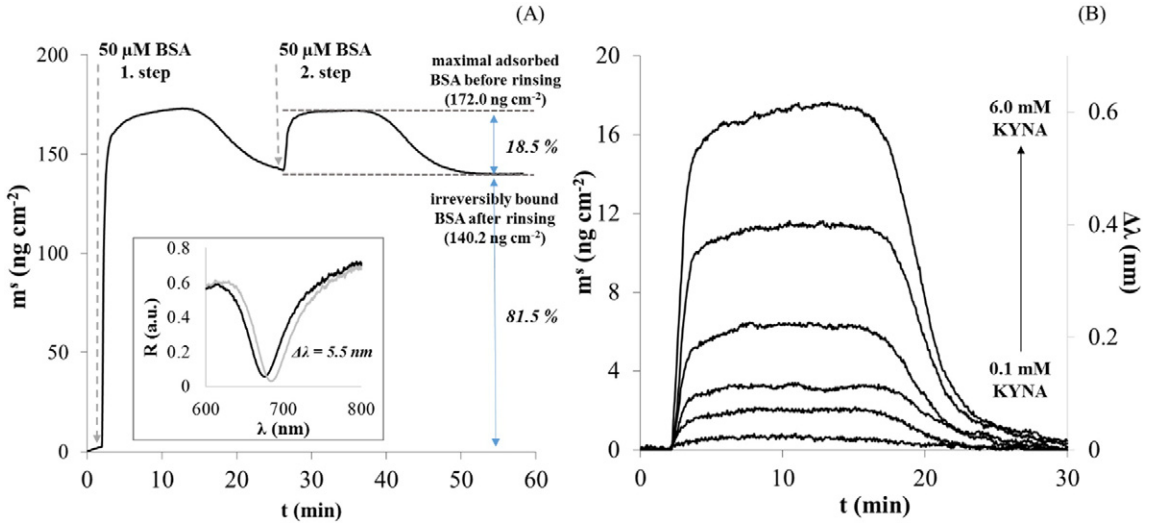


**Fig. 1.** Representative SPR sensorgrams of the binding of BSA onto gold sensor surface (A) and the binding of KYNA onto the BSA-functionalized gold surface (B) ($c_{BSA}$ = 50 μM, I = 150 mM (NaCl), pH = 7.4 (PBS, T = 25.0 °C, the KYNA concentrations are as follows: 0.1; 0.5; 1.0; 2.0; 4.0; 6.0 mM).

in the amplitudes of the evoked responses, similar to those of KYNA administrated intracerebroventricularly (Füvesi et al., 2004). Afterwards, other effective KYNA derivatives were synthesized which were able to cross the BBB and to induce neuroprotective effects in case of peripheral administration too (Nagy et al., 2011; Fülöp et al., 2012).

Application of novel, nanotechnology-based carrier systems may promote drug delivery into the brain, which may offer future therapeutic options for several neurological disorders. Different nanocarrier systems have been developed and early *in vitro* and preclinical studies yielded promising results to achieve drug delivery through the BBB (Wong et al., 2012; Lu et al., 2014). Nowadays, the application of CSNPs has become an area of intense growing interest. The albumin-based NPs are considered to be an attractive opportunity as carrier systems because many binding sites are reachable to various drug molecules. The albumins have several specific advantages in nano-scale range, such as biodegradability, biocompatibility and non-toxicity (Elzoghby et al., 2012) so the BSA is widely used for drug delivery, using carriers such as microparticles, nanospheres, NPs and gels (Wang et al., 2008). The secondary structure of the BSA can be examined by CD spectroscopy since it has two negative bands (208 nm, 222 nm) in the far UV region; the first band is a characteristic for α-helical structure of the protein (Kelly et al., 2005) which can be calculated from the observed ellipticity values (Mandal et al., 2010). To encapsulation of different molecules frequently polymers or polyelectrolytes, such the poly(lactic-*co*-glycolic acid) (PLGA) (Rafati et al., 2012), polyethylene glycol (PEG) (Ashjari et al., 2012), chitosan (Bowman and Leong, 2006) or the PAH are used. The PAH is a synthetic cationic polyelectrolyte which is water-soluble and biodegradable, so it is used in nanotechnology and nanomedical field as well (Zhou and Li, 2004; Wytrwal et al., 2014).

In this study, BSA-based CSNPs were developed for KYNA encapsulation. Size and structural information were gained by DLS, TEM and CD methods. *In vitro* measurements were performed to study the release properties and the release mechanism of the KYNA. An *in vitro* BBB model was applied to assess the penetration capacity of the encapsulated KYNA compared to free KYNA. In the *in vivo* neurophysiological studies, the effects of intraperitoneally (i.p.) administered KYNA, its prodrug L-kynurenine (L-KYN), BSA/PAH and BSA/KYNA/PAH were studied.

## 2. Materials and methods

### 2.1. Materials

The BSA (fraction V), the KYNA, the PAH with $M_W$ of 15.000 g mol$^{-1}$, the components of the phosphate buffer (PBS), the sodium phosphate dibasic hexadecahydrate ($Na_2HPO_4 \times 16H_2O$) and the sodium phosphate monobasic monohydrate ($NaH_2PO_4 \times H_2O$) were purchased

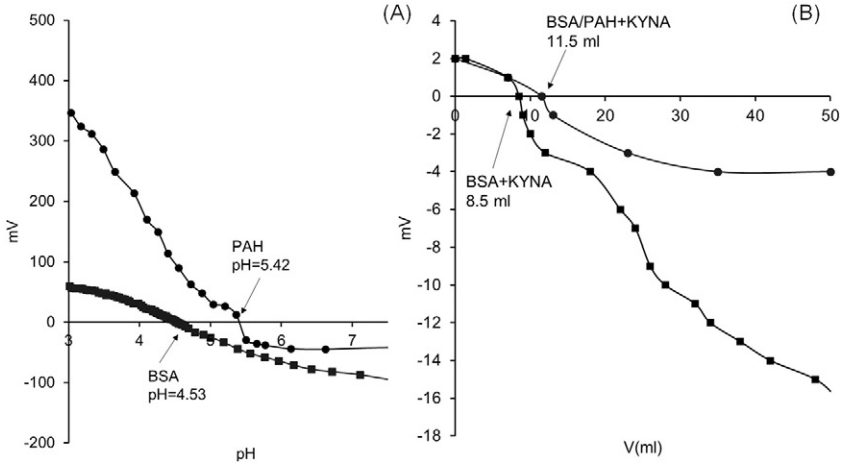


**Fig. 2.** The streaming potential of BSA and PAH as a function of pH (A) and the titration of BSA and the BSA/PAH composites with KYNA (B).