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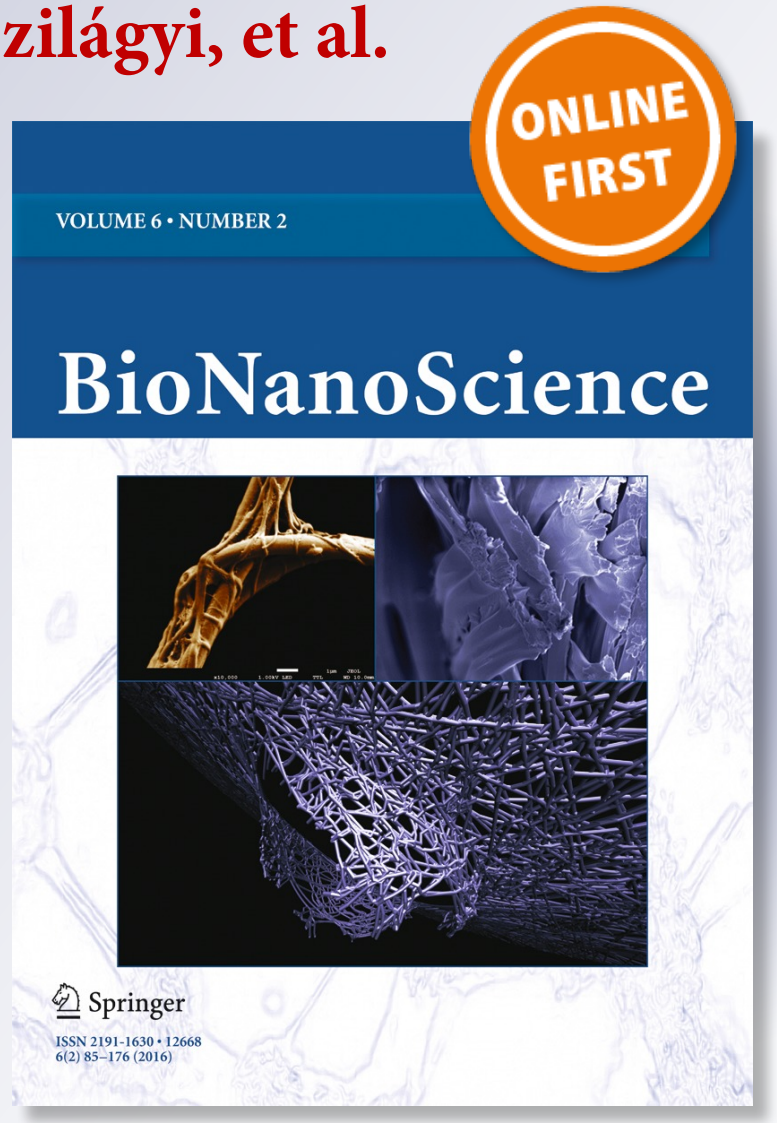

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Anti-Radical and Cytotoxic Activity of Polysuccinimide and Polyaspartic Acid of Different Molecular Weight

Diana Vitalevna Salakhieva[1] · **D. R. Gumerova**[1] · **R. A. Akhmadishina**[1] · **M. I. Kamalov**[1] · **I. S. Nizamov**[1] · **C. Nemeth**[2] · **A. Szilágyi**[2] · **T. I. Abdullin**[1]

Abstract Effect of poly(succinimide) (PSI) and poly(aspartic acid) (PASP) on free radical reactions and cell viability was assessed. Molecular weight (MW) of PASPs was determined by static light scattering technique and found as 3.9 and 8.3 kDa. Among PSIs and PASPs, only poly(aspartic acid) with higher MW was found to inhibit formation of hydroxyl-radical in Fenton's reaction, although each polymers studied were not able to eliminate diphenylpicrylhydrazyl radical. PASPs were almost non-toxic for 3 T3 fibroblasts and PC-3 cells ($IC_{50} \gg 3$ mg/mL), whereas PSIs diminished cell viability with different IC_{50} values depending on cell type and polymer MW. Our preliminary data indicate the MW dependence of bioactivity of L-aspartic acid-derived polymers designed as drug carriers and biocompatible materials.

Keywords Poly(succinimide) · Poly(aspartic acid) · Molecular weight · Free radicals · Cytotoxicity

1 Introduction

Synthetic, amino acid-based poly(carboxylic acid)s, poly(aspartic acid), and poly(glutamic acid) are promising materials in several industrial, agricultural, medical, and pharmaceutical applications. These homopeptides combine low toxicity and biodegradability with special physicochemical properties such as pH- and ionic strength-dependent solubility, and due to these beneficial features, they are potential alternatives of commonly used poly(carboxylic acids), e.g., polyacrylates. Despite to poly(glutamic acid), poly(aspartic acid) (PASP) can be produced by a simple synthesis, which does not involve N-carboxyanhydride-based methods and does not result in by-products. PASP can be readily synthesized by thermal polycondensation of L-aspartic acid in the presence of acid catalyst as well as by the reaction of maleic anhydride and ammonia [1].

Potential biomedical applications of PASP and its derivatives include the development of drug carriers for anti-cancer compounds [2] and gene therapeutics [3] as well as stimuli-responsive and in situ forming hydrogels for tissue engineering and drug delivery [4]. Synthesis of bioactive PASP conjugate with γ-aminobutyric acid was recently reported [5]. These applications listed above require optimization and control of molecular weight of PASP-based materials to be able to provide proper activity and reproducibility for these polymeric systems. Polycarboxylic structure of PASP and its conjugates with amino acids imparts metal complexation and chelation ability to polymers [6, 7], which may result in multiple biological outcomes including antioxidant activity. In our study, a potential anti-radical effect of PASP and poly(succinimide) (PSI) with different molecular weight as well as their cytotoxic activity was estimated. Results could be considered in the development of bio- and nanomaterials based on poly(aspartic acid).

2 Material and Methods

Poly(succinimide) was synthesized by thermal polycondensation of L-aspartic acid (Acros Organics) in the presence of phosphoric acid under argon atmosphere as described

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elsewhere [8]. Two PSI samples denoted as PSI1 and PSI2 were prepared by 3 and 1.5 h reaction time, respectively. PSIs were hydrolyzed to the corresponding PASPs (sodium salt) denoted as PASP1 and PASP2. Fourier transform infrared spectroscopy (FTIR) spectra were recorded using Bruker Tensor 27 infrared spectrophotometer (Bruker Optik Gmbh) on KBr pellets pressed (4000–400 cm^{-1}; ν = stretching vibration, δ = deformation vibration, s = strong, w = weak, m = medium vibrations, br = broad band). Refractive index of aqueous PASP solutions with the concentration of 0.5–10.0 mg/mL was measured on RX-5000CX refractometer (Atago). Polymers molecular weight was determined by static light scattering technique on Zetasizer NanoZS analyzer (Malvern Instruments) in quartz cuvette. Hydroxyl

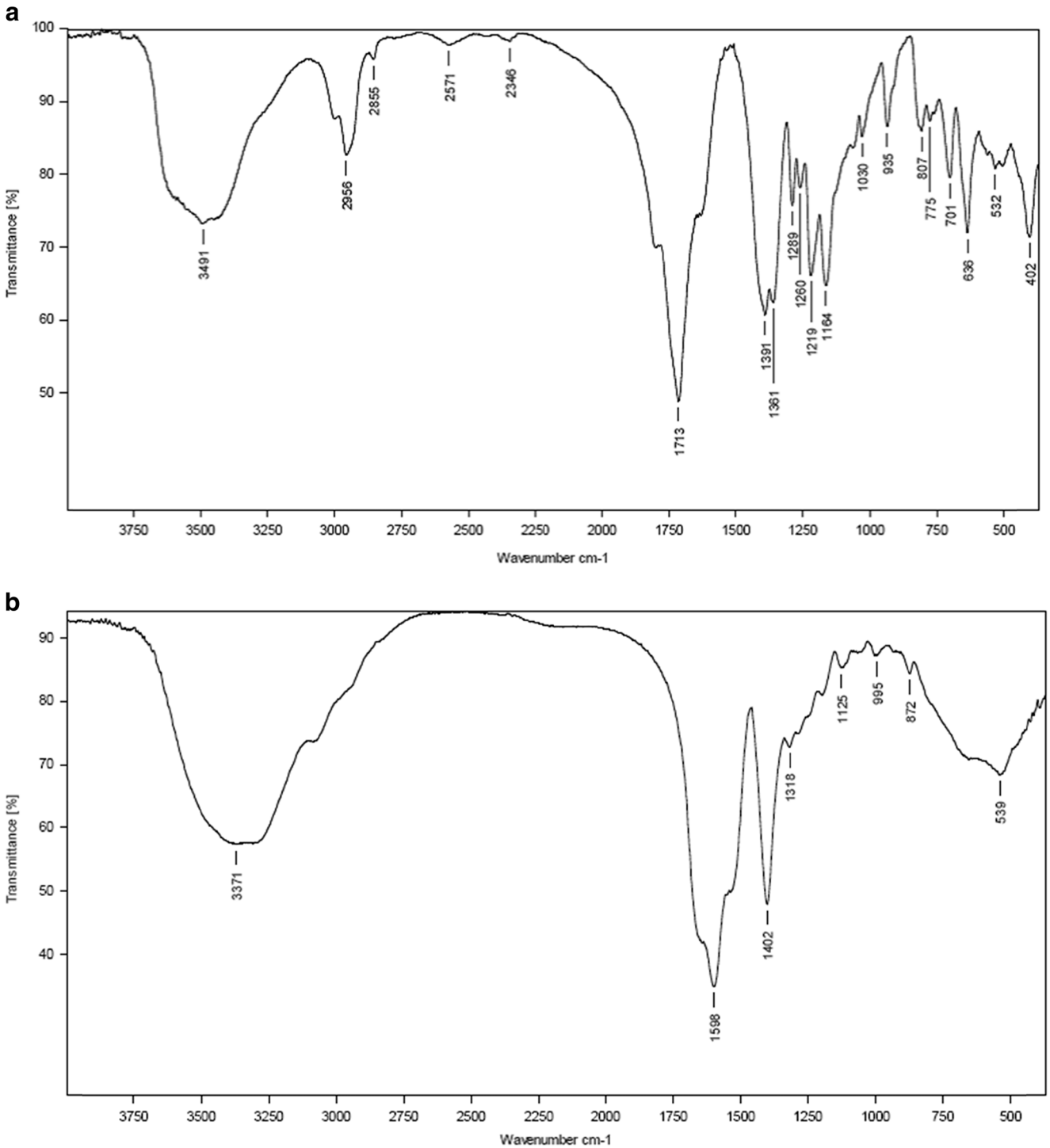

Fig. 1 FTIR/ATR spectra of PSI1 (**a**) and PASP1 (**b**)

radical was generated in Fenton's type reaction in the presence of cobalt (II) chloride and hydrogen peroxide in phosphate buffer saline and detected with the aid of 2′,7′-dichlorofluorescein diacetate (DFC) fluorescent probe (Acros Organics) on Infinite M200 microplate analyzer (Tecan). 2,2-Diphenylpicrylhydrazyl (DPPH) assay was carried out using a stable chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich) as described earlier [9]. Cytotoxicity of polymers was studied on PC-3 prostate cancer cell line and NIH 3 T3 mouse embryonic fibroblasts (ATCC) using MTT assay (Promega).

3 Results and Discussion

The structure of the synthesized PSIs and PASPS was confirmed by FTIR spectroscopy. FTIR spectrum of PSI contains strong characteristic absorption band at 1713 cm^{-1} which corresponds to C=O stretching vibrations of imide bonds (Fig. 1a). Further typical bands of the polymer were also detected at 3491 cm^{-1} s br (νN-H), 2956 m, 2855 w ($\nu_{as,s}CH_2$, νCH), 1391 s, 1361 s ($\delta_s CH_2$), and 1219 cm^{-1} s (νC–O–C). In FTIR spectrum of PASP, characteristic absorption band of the amide bond (amide I) was detected at 1598 cm^{-1}, while broad band at 3371 cm^{-1}assigned to the N–H bond (Fig. 1b) was also appeared. Strong band at 1402 cm^{-1} corresponds to deformation vibrations of CH_2 group bonds adjacent to C=O group (fragment CH_2(C=O); Fig. 1b).

Molecular weight (MW) of polymers was varied by changing duration of polycondensation reaction of L-aspartic acid (3 h for PSI1 and 1.5 h for PSI2). Static light scattering techniques was applied to estimate average MW of PASPs. Refractive index increment (dn/dc) of PASP was found as ~0.17 mL/g, which is similar to dn/dc values of protein solutions (~0.185 mL/g). Average MW of PASP1 and PASP2 produced from corresponding PSI was almost 8.3 and 3.9 kDa, respectively, indicating that increase of the reaction time from 1.5 to 3 h results in twofold increase in the MW of the PSI and the corresponding PASP samples.

We studied an effect of PSI and PASP on Fenton's type reaction, which generates hydroxyl radical, one of the most prevalent reactive oxygen species [10]. Co^{2+}/H_2O_2 reaction was previously optimized for reproducible generation of hydroxyl radical detected by DCF probe and tested using model free radical scavengers, e.g., ascorbic acid. Figure 2a shows concentration/signal curves for polymers relative to control reaction in the absence of effectors. We found that PASP1 induced noticeable inhibition of hydroxyl radical formation with half maximal effective concentration of 90.8 ± 1.4 μg/mL, though other polymers did not. However, each polymer studied including PASP1 did not react with DPPH chromogenic radical, thus not resulting in decrease of DPPH optical absorbance (Fig. 2b).

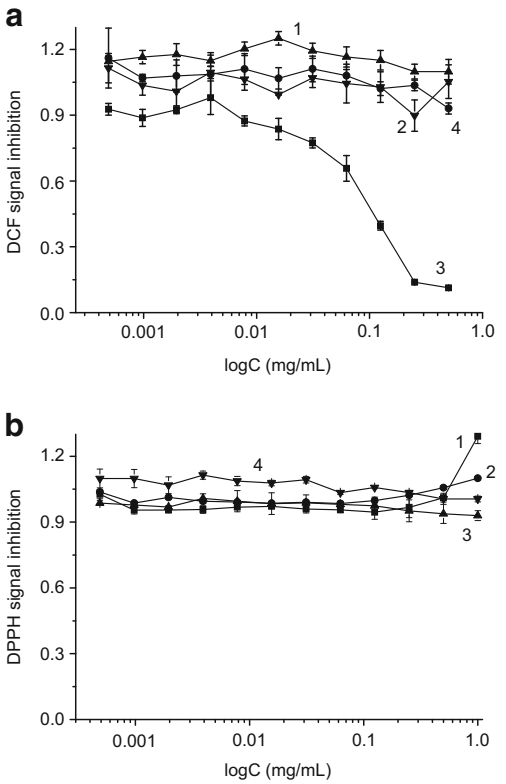

Fig. 2 Effect of PSI1 (*1*), PSI2 (*2*), PASP1 (*3*), and PASP2 (*4*) on **a** Fenton's reaction with DCF fluorescent probe and **b** DPPH radical optical absorbance. The signal inhibition was calculated relative to control reactions in the absence of effectors (100 %)

These data suggest that both PASP and PSI do not possess direct free radical scavenging activity, and therefore, the inhibition of Fenton's reaction (Fig. 2a) could be explained by capability of PASP to complex or chelate transition metal ions [6]. The fact that only PASP1 possesses hydroxyl radical eliminating activity indicates a potential role of MW of synthetic poly(aspartic acid)s which should be studied elsewhere.

Cytotoxicity of synthesized polymers was studied on PC-3 prostate cancer cells and 3 T3 mouse fibroblasts (Table 1). We found that both poly(aspartic acid)s PASP1 and PASP2 did not have any cytotoxic activity at concentrations as high as 3 mg/mL, demonstrating the high biocompatibility of these polypeptides. PSI decreased cell viability with different half maximal inhibitory concentrations (IC_{50}). Increase of PSI MW was accompanied by the enhancement of polymer cytotoxicity for PC-3 cells and its decrease for 3 T3 cells by a factor ~1.5 (Table 1). Anti-tumor activity of

Table 1 Cytotoxic concentrations of PSI and PASP (IC_{50}, μg/mL) according to MTT assay

Polymers	PC3 cells	3 T3 cells
PSI1	430.6 ± 71.8	868.6 ± 41.7
PSI2	615.5 ± 87.8	581.8 ± 8.2
PASP1	>3000	>3000
PASP2	>3000	>3000

poly(succinimide) was recently reported [11]. Enhanced uptake of amphiphilic polymers by cancer cells [12] could provide a route to selective MW-dependent anti-cancer activity of poly(succinimide)s.

4 Conclusions

Our results demonstrate the presence of molecular weight dependent anti-radical and cytotoxic properties of poly(succinimide) and poly(aspartic acid) designed as potential drug carriers and biocompatible materials.

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