International Journal of Biological Macromolecules 92 (2016) 515–522

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Conjugation of biogenic and synthetic polyamines with serum proteins: A comprehensive review

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ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 13 July 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Polyamine
Serum protein
Conjugation
Drug delivery
Modeling

ABSTRACT

We have reviewed the conjugation of biogenic polyamines spermine (spm), spermidine (spmd) and synthetic polyamines 3,7,11,15-tetrazaheptadecane.4HCl (BE-333) and 3,7,11,15,19-pentazahenicosane.5HCl (BE-3333) with human serum albumin (HSA), bovine serum albumin (BSA) and milk beta-lactoglobulin (b-LG) in aqueous solution at physiological pH. The results of multiple spectroscopic methods and molecular modeling were analysed here and correlations between polyamine binding mode and protein structural changes were estabilished. Polyamine-protein bindings are mainly *via* hydrophilic and H-bonding contacts. BSA forms more stable conjugates than HSA and b-LG. Biogenic polyamines form more stable complexes than synthetic polyamines except in the case of b-LG, where the protein shows more hydrophobic character than HSA and BSA. The loading efficacies were 40–52%. Modeling showed the presence of several H-bonding systems, which stabilized polyamine-protein conjugates. Polyamine conjugation induced major alterations of serum protein conformations. The potential application of serum proteins in delivery of polyamines is evaluated here.

1. Introduction

Polyamines are ubiquitous cellular components and are essential for cell growth and differentiation [1–5]. While biogenic polyamines are involved in several cell functions such as cell growth, differentiation, modulating gene expression, enzyme activities, activation of DNA synthesis and facilitating protein-DNA interactions [1,2,6–8], synthetic polyamines (Scheme 1) exert antitumor activity in multiple experimental models, including breast and lung cancer, and are being used in clinical trials [9–15]. Polyamine analogues can mimic some of the self-regulatory functions of biogenic polyamines but are unable to substitute for natural polyamines in their growth promoting role [16–18]. Conjugation of polyamines enhances the antibacterial and anticancer activity of chloramphenicol [19]. The potential applications of polyamine-DNA conjugation in cancer therapy and gene delivery are active areas of research [20–24]. Design and development of a carrier for delivering polyamine analogues to the target molecules is of major biomedical importance. Recently, synthetic polymers, including dendrimers and poly (ethylene glycol) are used for encapsulation of biogenic and synthetic polyamines [25–27]. Conjugation of serum proteins with biogenic and synthetic polyamines are reported [28–31]. Serum albumins are emerging as versatile protein carriers for drug targeting and for improving the pharmacokinetic profile of peptide or protein-based drugs [32–36].

Serum proteins contain multiple binding sites with different affinity and can transport drugs, fatty acids, steroid hormones and many other lipophilic compounds [37–40]. Carrier proteins such as HSA, BSA and b-LG, show different hydrophobicity profile [41–43] and exhibit different affinity towards drug interactions.

In this review, we compared the conjugation of biogenic and synthetic polyamines (Scheme 1) with human and bovine serum albumins as well as milk beta-lactoglobulin, using the results of multiple spectroscopic methods and molecular modeling. This study provides useful information for developing serum proteins in the delivery of antitumor drugs polyamine analogues.

Abbreviations: Spm, spermine; Spmd, spermidine; BE-333, 3,7,11,15-tetrazahepta-decane.4HCl; BE-3333, 3,7,11,15,19-pentazahenicosane.5HCl; HSA, human serum albumin; BSA, bovine serum albumin; b-LG, beta-lactoglobulin; FTIR, Fourier transform infrared.

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http://dx.doi.org/10.1016/j.ijbiomac.2016.07.049
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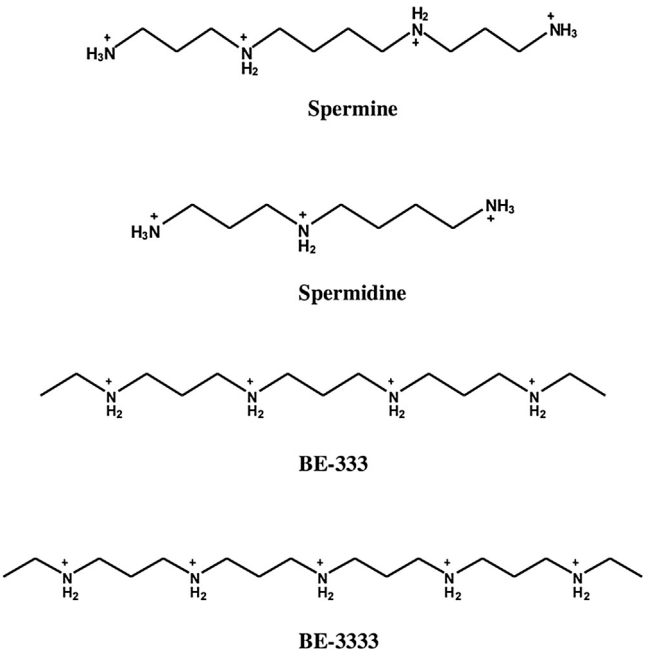

Scheme 1. Chemical structures of biogenic and synthetic polyamines.

2. Experimental

2.1. Molecular modeling

The structure of free HSA (PDB id: 1AO6, chain A) obtained by X-ray crystallography was used as a template [44]. The structure of BSA was predicted by automated homology modeling using SWISS-MODEL Workspace from the amino acid sequence NP-851335 [45–47]. The two proteins share 78.1% of sequence identity, which is sufficient to obtain reliable sequence alignment. The b-LG structure was obtained from the literature report [43]. The docking studies were performed with ArgusLab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, Seattle,WA, http://www.arguslab.com). Three dimensional structures of polyamines were generated from PM3 semi-empirical calculations, using Chem3D Ultra 6.0 [30].

2.2. UV spectroscopy

The UV–vis spectra were recorded on a Perkin-Elmer Lambda spectrophotometer with a slit of 2 nm and scan speed of 400 nm min^{-1}. Quartz cuvettes of 1 cm were used. The absorbance measurements were performed at pH 7.4 by keeping the concentration of protein constant (100 μM), while increasing polyamine concentrations (5 μM–300 μM). The binding constants of polyamine-protein conjugates were obtained according to the published method [48].

2.3. FTIR spectroscopy

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model), equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter, using AgBr windows. Solution of polyamine was added dropwise to the protein solution with constant stirring to ensure the formation of homogeneous solution and to reach the target polyamine concentrations of 0.125, 0.25 and 0.5 mM with a final protein concentration of 0.25 mM. Spectra were collected after 2 h incubation of HSA, BSA or b-LG with polyamine solution at room temperature, using hydrated films. Interferograms were accumulated over the spectral range 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 100 scans. The difference spectra [(protein solution + polyamine solution) − (protein solution)] were generated using water combination mode around 2300 cm^{-1}, as standard [49]. When producing difference spectra, this band was adjusted to the baseline level, in order to normalize difference spectra [28–31].

2.4. Analysis of protein secondary structure

Analysis of the secondary structures of HSA, BSA and b-LG and their polyamine conjugates were carried out as reported [50,28–31]. The curve-fitting analysis was performed using the GRAMS/AI Version 7.01 software of the Galactic Industries Corporation.

3. Results and discussion

3.1. Location of polyamine binding sites on HSA, BSA and b-LG by docking

Docking results showed that in the spm-HSA conjugates, spermine is surrounded by Ala-291, ***Arg-222 (H-bond)**, Arg-257, ***His-242 (H-bond)**, Ile-264, Ile-290, Leu-219, Leu-234, 238-Leu, Leu-260, Phe-23 and ***Tyr-150 (H-bond)** with the free binding energy of −8.6 kcal/mol (Fig. 1A and Table 1). In the spmd-HSA complex, spermidine is located near Arg-145, ***Asp-108 (H-bond)**, Pro-147, Tyr-148, Arg-197, ***Ser-193 (H-bond)**, Ala-194, Ala-191, Gln-159, ***Lys-190 (H-bond)** and His-146 with the free binding energy of −7.60 kcal/mol, while in the BE-333-HSA adduct, BE-333 is surrounded by Asn-429, Gln-459, Val-456, Ala-191, Val-455, Lys-195, Leu-198, Lys-199, Trp-214, Asp-451, Tyr-452 and Lys-436 with a free binding energy of −6.19 kcal/mol (Fig. 1A and Table 1). In the polyamine-BSA conjugates, spermine is located close to Pro-247, Ser-295, Ser-296, ***Lys-297 (H-bond)**, ***Ile-321 (H-bond)**, Ala-320, ***Leu-298 (H-bond)**, ***Ala-249 (H-bond)** and Asp-319 with the free binding energy of −8.16 kcal/mol (Fig. 1B and Table 1). In spmd-BSA, spermidine is linked to ***Ala-314**, Cys-288, ***Glu-315,** ***Glu-317**, Leu-242, Lys-299, Phe-246 and Val-316 with free binding energy of −7.28 kcal/mol, whereas in the BE-333-BSA conjugate, BE-333 is surrounded by Asp-142, Asp-153, Glu-159, Glu-154, Leu-146, Lys-151, Phe-150, Phe-157, Trp-158 and Tyr-161 with a free binding energy of −6.10 kcal/mol (Fig. 1B and Table 1). Similarly, in the polyamine-b-LG adducts, spermine is located near Ile-56, Ile-71, Ile-84, Leu-10, Leu-39, Leu-46, Leu-54, Leu-58, Leu-103, Lys-60, Met-107, Phe-105**, Pro-38* (H-bond),** Val-15, Val-41, Val-43, Val-92 and Val-94 with the free binding energy of −7.47 kcal/mol (Fig. 1C and Table 1). In the spermidine-b-LG, spermidine is close to Ile-12, Ile-56, Leu-10, Leu-46, Leu-54, Leu-58, Leu-103, Leu-122, Phe-82, Phe-105, Val-15, Val-41, Val-43, Val-92 and Val-94 with the free binding energy of −6.69 kcal/mol, while in the BE-333-b-LG, BE-333 is surrounded by Ile-12, Ile-56, Ile-71, Ile-84, Leu-10, Leu-39, Leu-46, Leu-54, Leu-58, Leu-103, Leu-122, Lys-60, Met-107, Phe-105, Pro-38, Val-15, Val-41, Val-43, Val-92 and Val-94 with the free binding energy of −7.81 kcal/mol (Fig. 1C and Table 1). As it is shown in Fig. 1 and Table 1, polyamines are surrounded by both hydrophilic and hydrophobic amino acids with marked similarities in the bound amino acid residues. It is interesting to note that several H-bonding systems are established in the biogenic polyamine-protein conjugates that are not present in the synthetic polyamine-protein complexes (Fig. 1 and Table 1). This can be related to easier H-bond formation with primary amine than secondary amine groups since primary amine has more basicity than secondary amine. The free binding energy showed that BSA forms more stable conjugates than HSA and b-LG, with biogenic polyamines forming more stable conjugates except in the case of