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Review Article

Albumin-binding as a universal strategy for half-life extension

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Abstract

With the development of recombinant DNA technology, a growing number of peptides and proteins are being applied to clinical treatment of diseases. However, a huge challenge needed to be addressed for many therapeutic peptides or proteins is that their half-lives in circulation is short due to rapid renal clearance and enzymatic degradation. To overcome the challenge, half-life extension strategies have been extensively developed. Albumin-binding strategy, whether covalent or non-covalent, represent a widely applied and highly successful half-life extension strategy, and many products have been marketed or tested in clinical trials. This review focuses on the utilization of albumin for half-life prolongation and emphasizes on the albumin-binding drugs already on the market or in the clinical stage. We summarize the related techniques including genetic fusion, chemical conjugation, non-covalent binding fatty acid and protein moieties.

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Keywords

half-life extension, albumin, covalent binding, non-covalent binding.

Introduction

Therapeutic peptides and proteins, characterized by high target specific, high bioactivity, high solubility and low toxicity, are undergoing rapid development and have become the focus of global drug research and development [1]. Since the approval of human insulin in 1982, a new chapter in biopharmaceuticals has been opened. Over the past 40 years, more than 300 therapeutic peptides and proteins have been approved by the Food and Drug Administration (FDA), including antibodies, hormones, cytokines, coagulation factors, enzymes and so on [2].

However, direct clinical application of these peptides and proteins faces a huge challenge, poor pharmacokinetic profiles. Except antibodies with longer half-lives, therapeutic peptides and proteins are typically possessing short half-lives in circulation, from several minutes to hours, resulting in constant infusion or repeat injections [3,4]. On one hand, therapeutic peptides or proteins with low molecular weight below 50 kDa (threshold of kidney filtration) can be rapid cleared by the kidney. One the other hand, enzymatic degradation of peptides and proteins directly leads to the loss of bioactivities in vivo [4, 5]. To overcome the above two major factors, many strategies have been developed to improve the pharmacokinetic properties of therapeutic peptides and proteins. Altering unstable or susceptible amino acids, increasing the molecular size and utilizing neonatal Fc receptor (FcRn)-mediated recycling system are proven to be effective for half-life extension [6].

Polyethylene glycol (PEG) modification is the first technique to produce long-acting therapeutic drugs by chemical conjugation. The PEGylated drugs exhibit longer half-lives due to the significant increased hydrodynamic volumes, reducing renal clearance [7,8]. However, high polydispersity of production, poor degradability in body, reported neutralizing anti-PEG antibodies and loss of activity have drawn increasing attention on the application of PEG molecules [9,10]. Therefore, several alternative strategies have been developed, such as PEG mimetics (XTEN [11], PAS [12], ELP polypeptides [13]), Fc-fusion [14] and albumin-binding [15].

Albumin-binding for half-life extension

Human serum albumin (HSA, Figure 1A) with a molecular weight of 66.5 kDa, is the most abundant protein in plasma at a concentration of 35-50 mg/mL, corresponding to about 0.5-0.7 mM [16]. The half-life of albumin in circulation is approximately 19 days [17]. The extremely long half-life of albumin is mainly attributable to the binding to FcRn, protecting the albumin from lysosomal degradation via the FcRn-mediated recycling mechanism (Figure 1E) [18,19]. In view of the specific mechanism of albumin, the half-life of a peptide or protein can be markedly extended by binding to albumin through covalent or non-covalent manner. Therefore, albumin has been developed into a universal carrier for half-life extension (Table 1).

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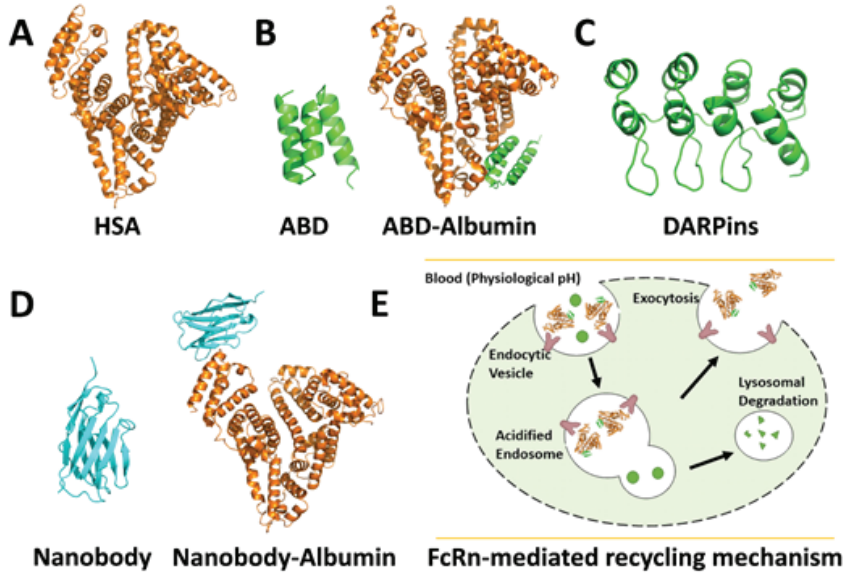

Figure 1. **Crystal structures of albumin-binding moieties and schematic representation of FcRn-mediated recycling mechanism.** *A) Crystal structure of human serum albumin (HSA, PDB: 1AO6). B) Crystal structures of ABD and ABD-albumin complex (PDB: 1TF0). C) Crystal structure of DARPins (PDB: 4GRG). D) Crystal structures of nanobody and nanobody-albumin complex (PDB: 5VNW). E) The half-life of a therapeutic peptide or protein can be significantly extended by covalent or non-covalent binding to albumin through FcRn-mediated recycling system. The cells internalize albumin molecules, followed by binding to FcRn in the endosome at pH 6.0. Then, the albumin molecules are recycled back to the plasma at pH 7.4, resulting in half-life extension. The serum proteins without binding to FcRn are suffered from lysosomal degradation.*

Table 1. *Therapeutic proteins in market or in clinical trials using albumin as a carrier for half-life extension.*

Drug/molecule	Peptide/protein	Stage/phase	Half-life unmodified (human)	Half-life modified (human)	Half-life extension method
Albiglutide	GLP-1	In market	1-2 minutes	5 days	Genetic fusion
Eftrenonacog-α	Factor IX	In market	20 hours	90-104 hours	Genetic fusion
CJC-1134-PC	Exendin-4	Phase 2	2.4 hours	8 days	Chemical conjugation
Liraglutide	GLP-1	In market	1-2 minutes	13 hours	Lipidation
Semaglutide	GLP-1	In market	1-2 minutes	165 hours	Lipidation
Insulin detemir	Insulin	In market	5-10 minutes	5-7 hours	Lipidation
Insulin degludec	Insulin	In market	5-10 minutes	25 hours	Lipidation
ABY-035	Affibody molecule	Phase 2	Unpublished	Unpublished	ABD
MP0250	DARPins binding VEGF-A and HGF	Phase 2	Unpublished	14 days	DARPins
MP0274	DARPins binding HER2	Phase 1	Unpublished	5 days	DARPins
ALX-0061	Nanobody againsts IL-6R	Phase 2	4.3 hours (Monkey)	6.6 days (Monkey)	Nanobody
ATN-103	Nanobody againsts TNF-α	Phase 3	Unpublished	9.5-13.5 days	nanobody
ALX-0761/M1095	Nanobody againsts IL-17A and IL-17F	Phase 1	Unpublished	Unpublished	Nanobody
ALX-0141	Nanobody againsts RANKL	Phase 1	Unpublished	8.9-20.6 days	Nanobody
GSK2374697	Exendin-4	Phase 1	2.4 hours	6-10 days	AlbudAb

Covalent binding to albumin

A peptide or protein binds to albumin covalently mainly through genetic fusion to the C- or N-terminus of albumin or chemical conjugation to free cysteine (C34) [6]. Two albumin-fusion proteins have been approved by the FDA. Albiglutide, approved for the treatment of type 2 diabetes mellitus, is constructed by two tandem modified GLP-1 molecules genetically fused to the N-terminus of albumin resulting in a half-life of 5 days in human with once-weekly administration [20]. Albutrepenonacog alfa is generated by genetically fusing a human coagulation factor IX to albumin, leading to a half-life of 90-104 hours in human, 5 times longer than the native protein, approved for the treatment of hemophilia B [16]. In addition, the half-lives of factor VIII, factor VIIa, growth hormone and G-CSF, which have discontinued or terminated after clinical trials, were prolonged to 2-8 days by genetic fusion to albumin [5, 6]. It is noteworthy that genetic fusion of proteins to albumin may lead to a large loss of bioactivity because of the increased steric hindrance caused by the large molecular size of albumin [21]. Only 1.4% of the specific activity retained for the albumin-IFN-α2b fusion protein compared with the unmodified IFN-α [22].

Besides genetic fusion, chemical conjugation is also an effective method to carry a short half-life payload. Seventeen disulfides exist in the structure of albumin, however, only one unpaired cysteine (Cys-34), which was high conserved in most of mammalian species [23]. The free HS-group in Cys-34 can be used to covalently conjugate a payload with a maleimide group specifically and rapidly [17]. CJC-1134-PC is designed by conjugation of a maleimide modified exendin-4 to the Cys-34 of albumin via a chemical linker, extending the half-life of exendin-4 from 2.4 hours to 8 days in human. CJC-1134-PC is in phase 2 clinical trials [5,24]. Bak et al. reported three albumin-conjugated GLP-1 variants constructed by conjugating the GLP-1 molecules with p-azido-l-phenylalanine (AzF) incorporated at position V16, Y19 or F28, to the DBCO-PEG4-MAL modified albumin, resulting in approximately 160-fold longer half-life compared with native GLP-1 [25]. Furthermore, the Cys-34 conjugation method was also utilized to prolong the half-life of YY peptide, G-CSF, GLP-1 and DARPin domain [26].

Non-covalent association with albumin

Albumin needs to be expressed in the yeast or mammalian cells, whether through genetic fusion or chemical conjugation, leading to a high production cost and long production period. To take advantage of high concentrations of endogenous albumin, chemical molecules or small proteins that target albumin with a high affinity have also been developed to extend the half-life through indirect association with FcRn system, such as fatty acid, albumin-binding domain (ABD), DARPins, nanobodies and AlbudAb.

Lipidation is a technique that applies fatty acid side chains to bind albumin. Lipidation has been well established for half-life extension and several biopharmaceuticals have been marked by Novo Nordisk. The half-life of semaglutide is extended to up to 165 hours in human by a C18 fatty acid side chain conjugated to Lys26 of GLP-1 molecule via a γGlu-2XOEG linker [27]. The half-life of semaglutide is much longer compared with liraglutide, the first generation of lipidated once-daily GLP-1R agonist with a half-life of 13 hours, mainly due to the substitution of C18 for C16 fatty acid and replacement of Ala8 with Aib, increasing the affinity for albumin and resistance to DPP-4 [28]. Additionally, the lipidation method is also used to modify insulin to generate insulin detemir and insulin degludec, resulting in a once-daily injection improving the compliance of patients [29]. Morevore, a recent study demonstrated that a fatty acid modified Fab derived from adalimumab exhibited a 15.2-fold longer half-life in mice compared with the native Fab [30].

Lipidation requires additional chemical cross-linking and subsequent further purification processes, while genetic fusion eliminates these steps at a lower cost. Albumin-binding domain (ABD) is a naturally occurring small protein, 46 amino acids, expressed by gram-positive bacteria, exhibiting nanomolar affinity for albumin (Fig. 1B) [31,32]. ABD035 [31] and ABDCon [33] are two evolved proteins with higher albumin affinity and thermal stability than wild-type ABD (G148-GA3), through phage display and consensus sequence design methods, respectively. The most advanced ABD-fusion protein is ABY-035, which has entered a phase 2 clinical trial for the treatment of psoriasis. ABY-035 is composed of three domains exhibiting a sandwich structure, two Affibody molecules binding to both subunits of IL-17A and one ABD binding to albumin for half-life extension [34]. In addition to this, ABD has been applied to a variety of peptides or proteins. The half-life of HER2-specific immunotoxin ZHER2-PE38 was significantly prolonged from 13.5 minutes to 330.8 minutes in mice through genetic fusion to ABD035 [35]. ABD could significantly prolong the half-life of GP-1 by genetic fusion to G148-GA3, ABD035 or ABDCon in mice [36]. Genetic fusion to ABD also significantly prolonged the half-lives of TRAIL [37], Interferon-α [21], scDb [38], H-Ferritin nanoparticle [39], αvβ3-integrin-binding protein [40], exendin-4 [41] and anti-HER3 affibody [42]. It is worth noting that ABD is an exogenous protein to the human body, so the immunogenicity of ABD needs to be evaluated carefully. ABD094, a variant of ABD without immunogenic potential in T-cell proliferation assays, has been developed and tested in clinical trials by Affibody AB company [32,34].

Designed ankyrin repeat proteins (DARPins) are a class of binding proteins evolved from naturally occurring ankyrin repeat domains via ribosome display and phage display (Figure 1C) [43]. The DARPins are composed of two to four 33-residue repeats flanked by a N-capping repeat and a C-terminal repeat, with a molecular weight of 14-21 kDa [44]. The DARPins exhibit many advantages, such as high solubility, high production and high thermal stability. These favorable properties render DARPins ideal scaffold [45]. The albumin-binding DARPins have been selected by ribosome display methods and have been applied to prolong the half-lives of payloads [46]. The albumin-binding DARPins exhibited a half-life of 12 days in monkey, approximately 1500 times longer than non-albumin-binding DARPins, only 11 min [46]. MP0250 contains four DARPin domains, one binding VEGF-A, one binding HGF and two binding albumin for half-life prolongation [47]. The half-life of MP0250 was approximately 2 weeks in human in the phase 1 clinical trial. MP0250 is in phase 2 clinical trial [45]. MP0274, which is in phase 1 clinical trial, is also a multi-domain fusion protein designed to bind to two different epitopes on HER2 and to albumin for an extended half-life, contributing to a half-life of more than 5 days in monkeys [48, 49]. A recent study showed that the half-life of GLP-1 was extended to 18.0 hours and 52.3 hours in mice by one or two tandem albumin-binding DARPins, respectively [50].

Nanobodies are a class of variable domain of heavy chains, derived from the antibodies devoid of light chains produced in Camelidae (including camels and llamas) with a molecular weight of 15 kDa (Figure 1D) [51]. Compared with the conventional monoclonal antibodies, the nanobodies are more stable, low toxicity and easy to produce [51]. The nanobodies targets specific molecules

can be selected from a phage or yeast display library. Just like the DARPins, the nanobodies with high affinity for albumin have been utilized to prolong the half-life of fusion partners. Vobarilizumab (formerly ALX-0061), a bispecific nanobody targeting IL-6 receptor and albumin, was found to have a half-life of 6.6 days in monkey [52]. Ozoralizumab (formerly ATN-103), designed simultaneously binding to TNF-α and albumin, has been reached phase 3 clinical trial with a half-life of 9.5-13.5 days in human [53, 54]. Furthermore, ALX-0761/M1095 and ALX-0141, both containing albumin-binding nanobody domain, have entered phase 1 clinical trials [6].

Additionally, some other albumin-binding proteins are under development. Albumin-binding domain antibody (AlbudAb) platform, developed by GlaxoSmithKline (GSK), is composed of a variable domain of heavy or light chain derived from the immunoglobulins [55]. The structure of AlbudAb is very similar to a nanobody. The AlbudAb platform has been extensively used to improve the pharmacokinetic properties of exendin-4 [55], GLP-1 [56], IFN-α2b [57] and IL-1 receptor antagonist [58]. Recent study demonstrated that apelin-13 (Pyr1) and MM202, an apelin mimetic peptide, could conjugate to AlbudAb moiety through a PEG linker, resulting in an extended plasma half-life [59]. Recently, some novel albumin-binding peptides or protein domains have been screened by phage or yeast display. Anti-albumin VNAR (variable binding domain of IgNAR), selected from *squalus acanthias* immunized by HSA, markedly prolonged the half-life of native VNAR domain from 0.15 hour to 25-33 hours in mice [60]. In a recent report, an albumin-specific repebody composed of LRR (Leucine-rich repeat) modules selected by Kim et al., prolonged the half-life of GLP-1 to 10 hours in mice [61]. Albumin-binding aptide (APTHSA), a constant β-hairpin peptide, resulted in a 4 times longer half-life of exendin-4 in mice [62].

Conclusions

Covalent or non-covalent association with albumin has proven to be an effective strategy for half-life extension of therapeutic peptides and proteins. Compared with covalent binding via genetic fusion or chemical conjugation to albumin, non-covalent binding through albumin-binding moieties has many advantages in application, such as low molecular weight suitable expression in E.coli, low cost, high production, simple purification process, high thermal stability and storage stability. With the development of biotechnology and structural biology, more albumin-binding modules may be discovered in the future. However, still there is a challenge needed to be addressed. Immunogenicity may be induced by the albumin-binding moieties derived from other microorganisms or animals, and needs to be evaluated carefully.

Conflict of interest

The authors declare no conflict of interest.

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