Biotechnology Advances 31 (2013) 90–96

Contents lists available at SciVerse ScienceDirect

Biotechnology Advances

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

Research review paper

The coiled coil motif in polymer drug delivery systems

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ARTICLE INFO

Available online 12 January 2012

Keywords:
Coiled coil
Hydrophilic polymer
Recombinant protein
Drug targeting
Drug delivery

ABSTRACT

The coiled coil is a superhelical structural protein motif that has been thoroughly investigated in recent years. Because of the relatively well-understood principles that determine the properties of coiled coil peptides and proteins, macromolecular systems containing the coiled coil motif have been suggested for various applications. This short review focuses on hybrid polymer coiled coil systems designed for drug delivery purposes. After a short introduction, the most important features of the coiled coils (stability, association number, oligomerization selectivity and orientation of helices) are described, and the factors influencing these characteristics are discussed. Several examples of the most interesting biomedical applications of the polymer-coiled coil systems (according to the authors' opinion) are presented.

Contents

1. Introduction

The coiled coil is a common protein folding motif that has been identified in a large number of both natural proteins and de novo designed synthetic peptides. In recent years, the structural analysis of many natural proteins revealed the basic principles governing the formation of the coiled coil motif (Apostolovic et al., 2010a; Xu and Shu, 2010; Mason and Arndt, 2004; Yu, 2002).

The coiled coil motif attracts particular attention because it represents a unique example of a well-understood relationship between the peptide amino acid sequence and its three-dimensional structure. A coiled coil consists of two to six right-handed α-helices forming a left-handed superhelical bundle (Fig. 1).

The primary structure of all coiled coil peptides is based on the repetition of seven amino acid residues (heptad repeats) denoted $(a\text{-}b\text{-}c\text{-}D\text{-}e\text{-}f\text{-}g)_n$, where n is the number of heptad repeats. The positions *a* and *d* are occupied by hydrophobic residues, whereas the *e* and *g* positions are usually occupied with charged or hydrophilic residues. The choice of the hydrophobic amino acid residues at positions *a* and *d* determines the association number of the helices in the superhelix. Ionic interactions between residues in positions *e* and *g* of the two helices substantially contribute to the stability of the coiled coil and, depending on the charge of the side chains, may control the formation of either homo- or heterodimers.

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doi:10.1016/j.biotechadv.2012.01.003

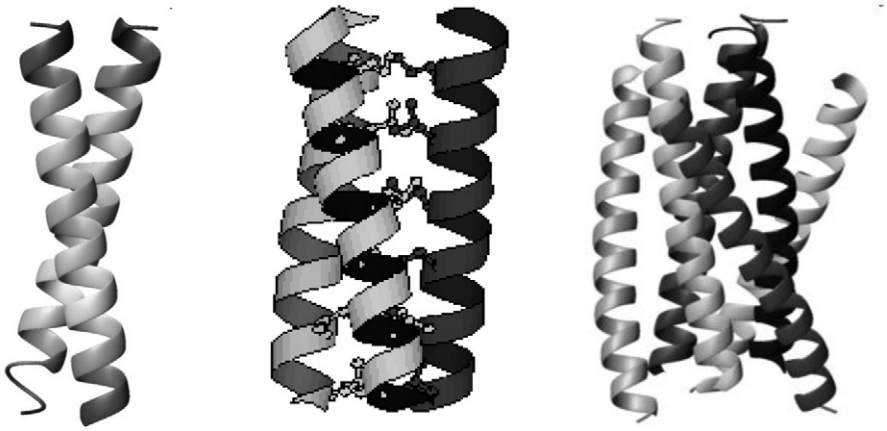

Fig. 1. Computer generated ribbon models of a coiled coil dimer, trimer and hexamer.

Hybrid copolymers consisting of both synthetic and natural macromolecules have become very attractive materials for various biomedical applications (Kopecek et al., 1998; Kopecek et al., 2001; Kopecek, 2003). Conjugation of biologically active proteins (e.g., antibodies, enzymes and antibody fragments) to synthetic hydrophilic polymer carriers often improves the pharmacokinetics of the proteins, prolongs their blood circulation, reduces unwanted immunogenicity, slows proteolytic degradation and increases the accumulation of macromolecular therapeutics in solid tumors through an enhanced permeation and retention (EPR) effect (Maeda et al., 2000). The relatively simple principles allowing the de novo design of coiled coil peptides inspired some investigators to use them for the preparation of novel, sophisticated hybrid materials based on combinations of peptides and synthetic polymers. In this review, we will focus on drug delivery systems based on hybrid materials containing the coiled coil motif.

2. Basic characteristics of coiled coils

The most important attributes that should be considered for design, description and comparison of coiled coils are their stability, association number (i.e., number of helices in the superhelix), oligomerization selectivity (homo- versus hetero-oligomers) and orientation of helices in the superhelix (parallel versus antiparallel). In recent years, the basic principles that determine all of these features have been thoroughly discussed in many review articles. In this review, however, we will briefly summarize only the most important characteristics concerning the design of drug delivery systems that contain the coiled coil motif.

2.1. Stability

Coiled coil stability is mostly determined by four major factors: number of heptad repeats ($(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$) (De Crescenzo et al., 2003; Litowski and Hodges, 2001; Pechar et al., 2002; Su et al., 1994), hydrophobicity of the core residues (Tripet et al., 2000; Wagschal et al., 1999) in positions *a* and *d*, electrostatic interactions (Zhou et al., 1994) between polar residues in positions *e* and *g* and the helical propensity of amino acid residues in the remaining positions (Litowski and Hodges, 2002). More heptad repeats in peptide sequences mean more opportunities for hydrophobic interactions in the core and more ionic interactions between the coiled coil-forming peptides, generally yielding a more stable coiled coil structure. The insertion of Ala residues, which have a high helical propensity, in positions *b*, *c* or *f* promotes the formation of α-helixes (and, consequently, coiled coils) even at lower numbers of heptad repeats.

The stability of coiled coils can be quantitatively characterized by the helix melting temperature (thermal stability) and binding constant. The thermal stability of the coiled coil heterodimers can be verified using circular dichroism (CD) spectroscopy by measuring the temperature dependence of the ellipticity at 222 nm. Loss of the secondary helical structure in the peptide mixture upon heating is manifested by an increase in the Θ_{222} value. The inflex point of the curve corresponds to the melting temperature of the α-helix. The binding constant can be experimentally determined using various physico-chemical methods, such as, titration with a denaturing agent (guanidinium hydrochloride) (Chao et al., 1996), isothermal titration calorimetry (Apostolovic and Klok, 2008), analytical ultracentrifugation (Gonzalez et al., 1996), CD spectroscopy (Moll et al., 2001), size-exclusion chromatography (Myszka and Chaiken, 1994), fluorescence resonance energy transfer (FRET) (Ishii et al., 1999) or fluorescence correlation spectroscopy (Brown et al., 2007).

2.2. Association number

The number of helices that forms the coiled coil superhelical bundle is called either the association number or the oligomerization degree. This number is mainly affected by the choice of residues (*a* and *d*) in the hydrophobic core. The side chains of these hydrophobic residues influence the packing geometry of the core; thus, they determine the association number. The role of residues *a* and *d,* which are buried in the core, was systematically studied on hydrophobic core mutants in the dimeric leucine zipper peptide GCN-p1 (Harbury et al., 1993). It was found that the combinations of amino acid residues IL, II and LI in the *a* and *d* positions led exclusively to the formation of dimeric, trimeric and tetrameric coiled coils, respectively. Because the only difference in the structure of the mutants was the choice of the amino acids in the hydrophobic core, the residues in the *a* and *d* positions mediated the switch in the association number of the helices.

In many cases, de novo designed coiled coils are intended for specific applications, such as, epitope display (Tang et al., 2000), physical hydrogel formation (Wang et al., 1999) or biosensor application and affinity chromatography purification (Chao et al., 1998). In particular, dimeric coiled coils are required for these specific purposes. It was found that except for the combinations of hydrophobic residues in positions *a* and *d* described above, Val and Leu placed in *a* and *d*, respectively, also resulted in dimeric coiled coils (Chao et al., 1998; Graddis et al., 1993; Pechar et al., 2002; Wang et al., 2001; Xu et al., 2005; Yang et al., 2006b; Yang et al., 2006a).