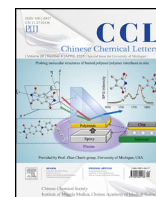
Chinese Chemical Letters 26 (2015) 426–430

Contents lists available at ScienceDirect

Chinese Chemical Letters

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



Review

Folate binding protein—Outlook for drug delivery applications

Rachel L. Merzel, Jun-Jie Chen, E. Neil G. Marsh, Mark M. Banaszak Holl *

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA

ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 5 December 2014
Accepted 8 December 2014
Available online 30 December 2014

Keywords:
Folate binding protein
Serum protein
Drug delivery
Folate conjugate
Folic acid

ABSTRACT

Serum proteins represent an important class of drug and imaging agent delivery vectors. In this mini-review, key advantages of using serum proteins are discussed, followed by the particular advantages and challenges associated with employing soluble folate binding protein. In particular, approaches employing drugs that target folate metabolism are reviewed. Additionally, the slow-onset, tight-binding interaction of folate with folate binding protein and the relationship to a natural oligomerization mechanism is discussed. These unique aspects of folate binding protein suggest interesting applications for the protein as a vector for further drug and imaging agent development.

© 2014 Mark M. Banaszak Holl. Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences. All rights reserved.

1. Introduction

In the body, drugs are transported in the blood where they can encounter over 100,000 proteins. The vast majority of these proteins are albumin (55%) and immunoglobulins (38%), such as IgG, IgA, and IgM, with smaller amounts of lipoproteins and transferrin [1]. All drugs or drug delivery scaffolds come into contact with these proteins, and the complexes formed often dominate the observed pharmacokinetics and biodistribution. These protein–drug interactions have long played a significant role in small molecule drug design and are now recognized to greatly complicate the development of new drug delivery scaffolds in the field of nanomedicine [2]. One solution to this challenge is the judicious selection of an endogenous serum protein as the delivery scaffold for a given drug, imaging agent, or theranostic combining therapy and imaging [3]. Of the serum proteins, albumin has garnered the most attention and resulted in clinical applications [4–6]. There are currently seven clinically approved drugs or imaging agents employing the albumin scaffold, with applications including the treatment of metastatic breast cancer (Abraxane®) and diabetes (Levemir®, Victoza®) and imaging of cardiovascular and cerebral circulation (^{99m}Tc-Albures, Vasovist®) and lymph nodes (^{99m}Tc-Nanocol). Albumin is currently being explored for a variety of other applications, including theranostics [7,8]. Transferrin has also been explored for drug and imaging agent delivery; however, transferrin-based systems have yet to reach the clinic [9]. Both albumin (66.5 kDa) and transferrin (78 kDa) have molecular weights above the renal clearance threshold, contributing to long circulation times. Both proteins accumulate in malignant and inflamed tissue due to the enhanced permeation and retention (EPR) effect and internalize into cells *via* receptor-specific endocytosis processes. These favorable properties, and the successes noted above, have prompted extensive research into both of these proteins, with over 4000 papers published to date.

Despite these successes, the toxicity of small molecule cancer therapeutics remains a significant challenge. Off-target dosing (uptake of the cancer therapeutic by healthy cells as well as the tumor cells) leads to a wide range of side effects, sometimes necessitating sub-optimal dosing, which can lead to worse outcomes for patients. To address this problem, researchers have worked to develop targeted therapeutics that deliver drug to tumor cells while avoiding healthy cells. Folic acid (FA) is a widely studied targeting ligand for both molecular and nanoscale cancer therapies because folate receptors (FRs) are overexpressed on the surfaces of the cancer cell membranes [10,11]. Folate is necessary for thymidine biosynthesis, and hence for *de-novo* DNA biosynthesis, and so rapidly dividing cancer cells increase the concentration of FRs on plasma membrane surfaces. To date, seven FA-targeted cancer therapeutics have advanced to clinical trials, but none have progressed to full clinical development. Even with targeted drug delivery agents, dose-limiting toxicity due to uptake by healthy cells remains a problem. Additionally, the expression of FRs on the surfaces of tumor cells is highly variable both from individual to individual and within a given cancer type. The folate

* Corresponding author.
E-mail address: mbanasza@umich.edu (M.M.B. Holl).

http://dx.doi.org/10.1016/j.cclet.2014.12.015
1001-8417/© 2014 Mark M. Banaszak Holl. Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences. All rights reserved.

metabolic pathway is also the target for inhibitors of dihydrofolate reductase (DHFR) [12–15]. Clinically approved DHFR-inhibitor drugs are used to treat a variety of cancers and autoimmune diseases (methotrexate, pemetrexed), bacterial infections (trimetrexate, piritrexim), and malaria (pyrimethamine).

Can the substantial advantages of employing an endogenous serum protein for drug delivery be combined with drugs designed to target and inhibit the folate metabolic pathway? This mini-review discusses recent advances in the understanding of soluble folate binding protein (FBP) and possible applications of this protein for drug delivery. First, we review the structure and hypothesized functions for FBP, including possible roles in folate metabolism. The approaches for isolation of the protein are also discussed. Second, we examine recent data regarding the detailed binding mechanism of FBP with FA, FA-conjugates, and antifolate (aFA)-conjugates. Third, we discuss the outlook for folate binding protein as a transport agent for therapeutics and imaging agents, including advantages and challenges of this approach.

2. The structure, function, and isolation of folate binding protein

Folate binding protein (FBP) is a ~30 kDa glycoprotein containing 222 amino acids present in 1–2 nmol/L concentrations in human serum and other body fluids and 100 nmol/L concentrations in milk [16–20]. The functions of FBP in the body are not well understood, but it has been hypothesized to regulate the trafficking and homeostasis of folate, protect against folate degradation, and shield against bacterial utilization of folate. FBP is closely related to two isoforms of membrane-bound FRs: FR-α and FR-β, both of which are connected to plasma cell membranes *via* glycosylphosphatidylinositol (GPI) anchors [17,21]. A third isoform, FR-γ, is a secreted protein and lacks the signal for modification with a GPI anchor. Soluble FBP likely originates from FR-α that has undergone cleavage of the GPI anchor and from FR-γ, which inherently lacks a GPI modification. X-ray crystal structures of the FA-bound protein were recently reported (Fig. 1) [14,15]. FBP is obtained on the gram scale by purification of whey protein [20,22–26], although engineered proteins have been expressed in Chinese Hamster Ovary (CHO) cells [14].

Glycosylation of the protein is not required for the FA-binding activity of soluble FBP [27–29]. The quaternary structure of FBP changes as a function of FA-binding, consistent with a slow-onset, tight-binding interaction. At micromolar concentrations, the binding of FA to FBP also induces a self-assembly/aggregation process that has been examined *in vitro* [30,31]. Interestingly, the aggregation of FR-α in the cell membrane has been shown to be an integral part of FA-binding and cellular internalization [32,33]. Recently, it was discovered that FBP internalizes into cells *via* a megalin-mediated endocytosis pathway [34], suggesting the possibility of megalin playing a direct role in folate metabolism.

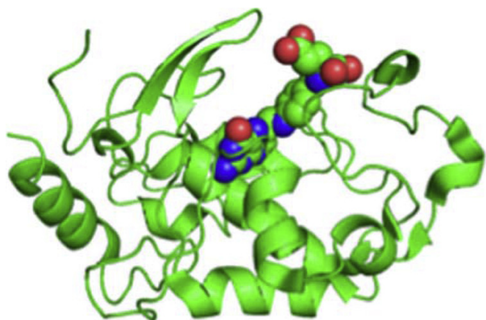


Fig. 1. The X-ray structure of folate receptor α with folic acid in the binding site.

This observation is particularly interesting for the use of FBP in chemotherapeutic targeting.

3. The binding mechanism of folic acid to folate binding protein

Folic acid binds to FBP *via* a slow-onset, tight-binding mechanism (Eq. (1)) [35]. The initial FBP interaction with folic acid is followed by a reorganization of the protein structure, leading to the observed nanomolar FA–FBP binding constant. The induction of structural change in the FBP upon binding FA is characterized by quenching of the inherent tryptophan fluorescence in FBP [30,36]. The change in structure is hypothesized to lead to reduction of the number hydrophobic residues on the protein surface, resulting in a FA-ligand induced aggregation of the protein [37,38]. At pH 7.4, the degree of aggregation (n) is dependent on FBP concentration. As measured by gel-filtration, at concentrations of 1–10 nmol/L, FBP–FA is monomeric, whereas a tetramer $(FBP–FA)_4$ and a nonamer $(FBP–FA)_9$, were observed for concentrations of 1.0 μmol/L and 10 μmol/L, respectively. Ultracentrifugation experiments indicated that oligomers as high as $(FBP–FA)_{30}$ were present for 100 μmol/L solutions of FBP. High performance liquid chromatography (HPLC) and sodium dodecyl sulfate-poly(acrylamide) gel electrophoresis (SDS–PAGE) both indicate the formation of three new species upon FA binding to FBP [39]. The HPLC studies are particularly interesting as they provide a ready method for quantifying the relative amounts of each species present in solution. More work is needed to understand how these three species relate to tetramers, nonamers, and other species reported in the fluorescence, gel-filtration, and ultracentrifugation studies. These data indicate that a monomer structure is anticipated to be the dominant form of the protein in most biological tissues where the FBP concentration is 1–2 nmol/L; however, these reported oligomerization properties may play an important role in the binding and aggregation of FR-α in the cell membrane prior to internalization. A stopped-flow kinetic study examined the relative binding strengths of folic acid to FBP and to albumin [36]. This comparison is of particular interest since albumin has a concentration of ~0.6–0.7 mmol/L in blood, or a factor ~500,000 more concentrated than FBP. FBP binds FA tightly with a $K_d < 1.3$ nmol/L, whereas albumin exhibits much weaker binding with a K_d of 21 ± 2.1 μmol/L. For human plasma, this indicates that FBP will be fully bound by folate, with the remaining 7–30 nmol/L of folate present more weakly associated with albumin. The values of K_d suggest that about 3% of folate in human serum will be present in free form. Lowering the pH from 7.4 to the more acidic values commonly found in endosomes activates deoligomerization of the FBP and release of the bound FA.

$$FA + FBP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} FA - FBP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} FA - FBP* \quad (1)$$

4. The binding mechanism of folic acid conjugates to folate binding protein

There has been a substantial amount of interest in using FA-conjugates for targeted drug and imaging agent delivery [10,11,40–50] and for targeted polymer vectors [51–55]. Based on the FBP and albumin concentrations present in human serum [36], FA-conjugates employed at a micromolar concentration would be expected to interact extensively with albumin as well as to saturate all available soluble FBP. The rate of binding to FBP already present in serum would be determined by the k_{off} of existing bound FA and the production of new FBP. This suggests that both FBP and albumin could play useful roles in

Download English Version:

https://daneshyari.com/en/article/1254055

Download Persian Version:

https://daneshyari.com/article/1254055

Daneshyari.com