6 Key Physicochemical Characteristics Influencing ADME Properties of Therapeutic Proteins

Xing Jing, Yan Hou, William Hallett, Chandrahas G. Sahajwalla, and Ping Ji

Abstract

Therapeutic proteins are a rapidly growing class of drugs in clinical settings. The pharmacokinetics (PK) of therapeutic proteins relies on their absorption, distribution, metabolism, and excretion (ADME) properties. Moreover, the ADME properties of therapeutic proteins are impacted by their physicochemical characteristics. Comprehensive evaluation of these characteristics and their impact on ADME properties are critical to successful drug development. This chapter summarizes all relevant physicochemical characteristics and their effect on ADME properties of therapeutic proteins.

Keywords

Protein therapeutics · Physicochemical characteristics · Pharmacokinetics (PK) · ADME

Abbreviations

ADA	Anti-drug antibody
ADC	Antibody drug conjugate
ADME	Absorption, distribution, metabolism, excretion
ASGPR	Asialoglycoprotein receptor
CD	Circular dichroism
cIEF	Capillary isoelectric focusing
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
DSF	Differential scanning fluorimetry
ECM	Extracellular matrix
Fab	Fragment antigen-binding domain
Fc	Fragment crystallizable domain
FcRn	Neonatal Fc receptor
FcγR	Fc gamma receptors
FDA	U.S. Food and Drug Administration
FRET	Förster resonance energy transfer
GlcNAc	N-acetylglucosamine
IgG	Immunoglobulin G
ITC	Isothermal titration calorimetry
mAb	Monoclonal antibody
ManR	Mannose receptor
MST	Microscale thermophoresis
MW	Molecular weight
PEG	Polyethylene glycol
pI	Isoelectric point
PK	Pharmacokinetics
SC	Subcutaneous
SPR	Surface plasmon resonance
$T_{1/2}$	Half-life

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T_{max}	Time to peak concentration
TMDD	Target mediated drug disposition
TNF	Tumor necrosis factor
V_d	Volume of distribution

6.1 Introduction

In recent decades, the clinical application of therapeutic proteins has revolutionized the treatment of many diseases. Since the approval of the recombinant human insulin in early 1980s, protein therapeutics have rapidly gained popularity in clinical use. To date, more than 140 therapeutic proteins have been granted approval from the U.S. Food and Drug Administration (FDA), for a wide range of indications, from alleviation of neuropathic pain to rheumatoid arthritis and lysosomal storage diseases.

Therapeutic proteins are biological products (biologics) produced from living organisms or contain component of living organisms (Dorai and Ganguly 2014). Over the past decades, the fast-evolving biotechnologies have also facilitated the development of numerous protein therapeutics such as cytokines, growth factors and replacement enzymes. The most remarkable milestone is the emergence of antibody therapeutics (Macielag 2012; Cavagna and Taylor 2014; Vidarsson et al. 2014; Wang et al. 2015).

Compared to small molecule drugs, therapeutic proteins have unique characteristics that underlie their pharmacokinetics and pharmacodynamics (PK/PD). Understanding protein characteristics that impact clinical performance of therapeutic proteins are essential for drug development of different phases. For example, product PK-influencing attributes could inform drug design and evaluation and dosing regimen selection in preclinical and clinical studies. This chapter describes all relevant physicochemical characteristics that impact ADME processes of therapeutic proteins.

Each section in this chapter briefly introduces the mechanisms governing the involvement of protein therapeutics in each ADME process. The subsequent subsections describe relevant protein physiochemical characteristics and their roles in the corresponding mechanisms. Furthermore, with data analytics for FDA-approved protein therapeutics as real examples, this chapter focuses on the established relationship between protein characteristics and PK performance, as well as the successful strategies for PK improvement. Finally, this chapter builds a comprehensive roadmap to summarize all established correlations between physiochemical characteristics of FDA approved therapeutic proteins and their ADME properties.

6.2 Impact of Physicochemical Characteristics on Absorption

The approved therapeutic proteins are mostly delivered through the subcutaneous (SC) or intravenous (IV) route of administration. As the intravenous administration route bypasses the absorption phase, this section primarily focuses on therapeutic proteins administered via the subcutaneous (SC) route. The rate and extent of absorption after subcutaneous dosing is dependent on factors such as the molecular structure, weight, size, and charge.

6.2.1 Molecular Weight and Size

The molecular weight of a therapeutic protein is its total molecular mass, which is composed of the mass of its amino acid sequence and the mass of modifications. The molecular size of a therapeutic protein, directly correlated with its molecular weight, represents its geometric dimensions. The primary structure of a therapeutic protein represents its amino acid sequence.

After SC administration, therapeutic proteins are delivered to the hypodermis that is primarily composed of negatively charged extracellular matrix (ECM) (Kinnunen and Mrsny 2014; Richter et al. 2012). From the ECM, therapeutic proteins enter systemic circulation through two

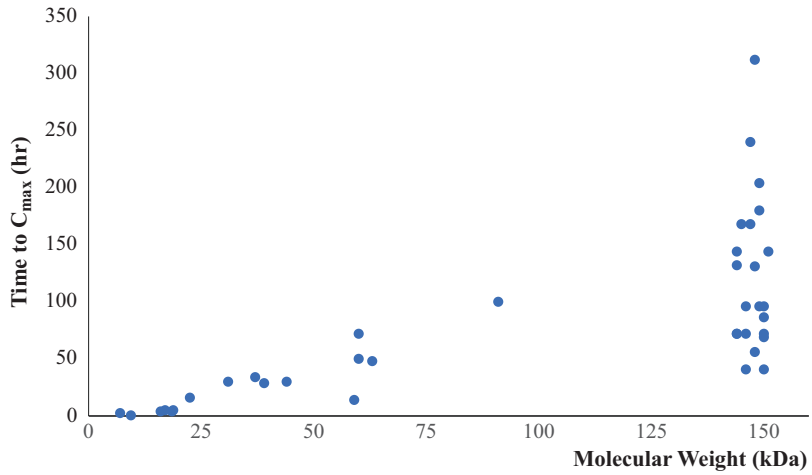

Fig. 6.1 Statistical analysis of the impact of molecular weight on T_{max} of FDA-approved therapeutic proteins

routes: blood capillaries via diffusion and lymphatic vessels via convection. The probability of absorption via convection increases with the increase in their molecular weight (MW) (Porter and Charman 2000; McLennan et al. 2005), with proteins larger than 15–20 kDa entering the circulation system primarily through the lymphatic system (Supersaxo et al. 1990). Studies have identified a correlation between T_{max} and MW (Porter and Charman 2000; Kagan 2014). Therapeutic proteins with MW from 9.4 to 18.8 kDa and 31–63 kDa generally reach T_{max} at 0.5–5 h and 14–72 h, respectively, after administration. Antibodies or derivatives with MW of 150 kDa have T_{max} values in the range of 40 h to 13 days (Fig. 6.1).

Molecular size also affects absorption (Tibbitts et al. 2016). Products with similar molecular weights but different molecular size may exhibit different T_{max} values (Fig. 6.1). Although the molecular weight of a protein correlates with its physical size, other physicochemical structural factors, such as modification and folding, could also impact its molecular size and thus the absorption profile.

6.2.1.1 Higher-Order Structure

The higher-order structure of a therapeutic protein means its three-dimension tertiary structure, which is affected by its primary structure. Moreover, higher-order structure also covers protein quaternary structure such as dimerization. Self-association of therapeutic proteins may affect absorption through its impact on MW and size. Having numerous analogs, insulin products are excellent examples illustrating the impact of dimerization on absorption (Fig. 6.2). Regular human insulin (i.e., Humulin R) is absorbed as hexamers (Palmieri et al. 2013) with a T_{max} of 4–8 h. By contrast, aspart insulin analogs (such as Novolog and Fiasp), bearing a mutation of proline28 to Aspartate in the B chain, have a T_{max} of 40–60 min. The difference in T_{max} between human insulin and aspart insulin analog is due to this single mutation that results in a change in oligomeric state (Brange and Volund 1999; Brange et al. 1988, 1990; Volund et al. 1991). Likewise, a series of other insulin mutants, identified by mutational analysis targeting the dimer interface, tend to form weaker oligomers and perform as fast-acting insulin drugs (Brange et al. 1988). For example, insulin (Humalog) with flipped lysine28/proline29 mutation and insulin glulisine (Apidra) with double mutations in B chain has a T_{max} of 30–90 min and 55 min, respectively. As another example, glargine insulin (Basaglar and Lantus) possesses two additional arginine residues and thus enhanced oligomerization, resulting in prolonged absorption with T_{max} about 12 h (Owens 2012; Hilgenfeld et al. 2014).

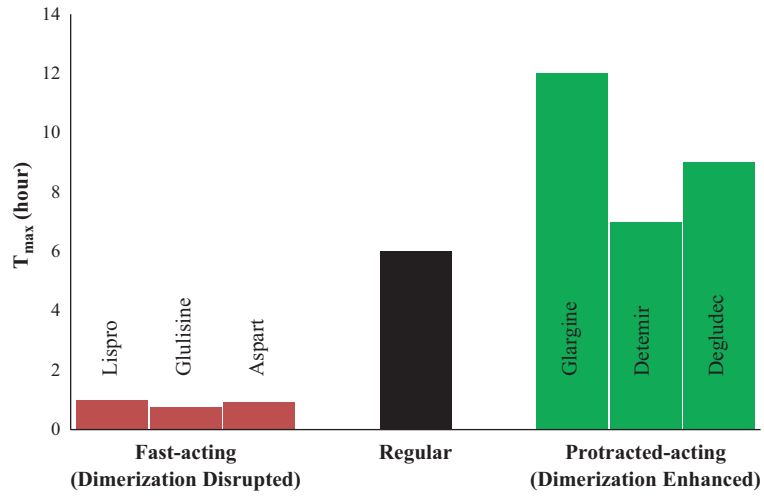

Fig. 6.2 Absorption rates of different types of approved insulin drugs with different dimerization affinity

6.2.2 Molecular Charge

The molecular charge of a therapeutic protein represents its net surface charge, commonly indicated by its isoelectric point (pI).

The negatively charged ECM may capture the positively charged portions of a therapeutic protein. Since most monoclonal antibodies (mAbs) or fusion proteins have a pI between seven and nine, the electrostatic interaction with ECM might delay their release to blood (Khawli et al. 2010; Bumbaca et al. 2012). For example, while Trulicity (dulaglutide) and Mircera (methoxy polyethylene glycol-epoetin beta) have similar MWs of ~60 kDa, they have different absorption rates (T_{max} of 48 and 72 h, respectively) which may be attributed to their difference in pIs (pI of 5.5 and 8.8, respectively).

6.2.3 Bioavailability

The extent of absorption after subcutaneous administration is dependent on pre-systemic clearance, which is dependent on multiple factors including MW, solubility and interaction with receptors, etc. (Datta-Mannan et al. 2012; Deng et al. 2012). Thus, no obvious trend could be observed between bioavailability and MW after SC administration (Fig. 6.3).

6.3 The Impact of Physicochemical Characteristics on Distribution

The volume of distribution of therapeutic proteins is affected by factors such as molecular weight, size, charge, and structure (Tabrizi et al. 2010; Tibbitts et al. 2016).

6.3.1 Molecular Weight and Size

Monoclonal antibodies (mAbs) exhibit limited distribution from the blood to the peripheral tissue primarily due to their large molecular size (Lobo et al. 2004). In general, the distribution of mAbs is restrained primarily in blood plasma and limited interstitial spaces (Boswell et al. 2010; Dostalek et al. 2013). The Fab fragments have larger volume of distribution because of smaller size (Thurber et al. 2008; Tabrizi et al. 2010). Full-length IgG therapeutics targeting TNF, on the other hand, are primarily distributed within the blood stream (Tabrizi et al. 2010).

6.3.2 Molecular Charge

Molecular charge of therapeutic proteins underlies their interactions with biological components that

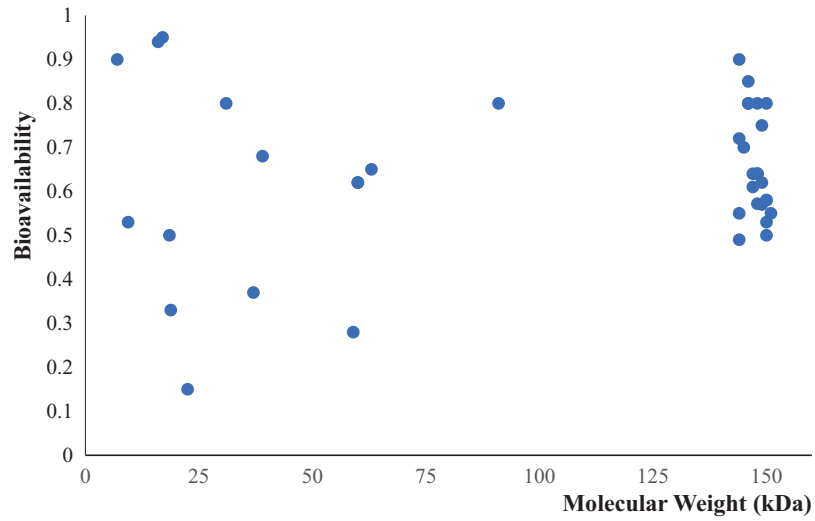

Fig. 6.3 Evaluation of the impact of molecular weight on bioavailability of FDA-approved therapeutic proteins

influence their distribution. For instance, one of the major mechanisms that govern distribution of antibody therapeutics to tumor tissues relies on receptor-mediated endocytosis and binding to the cellular antigens (Weinstein and van Osdol 1992; Tabrizi et al. 2010). Tighter binding between a mAb and tumor cells results in increased retention of an antibody at the periphery of tumor nodules, which form a binding-site barrier for antibody drugs (Graff and Wittrup 2003; Tabrizi et al. 2010). Playing a vital role in antibody elimination, the interaction between mAbs and neonatal Fc receptor (FcRn), which is essential to the FcRn-mediated endocytosis, also promotes tissue distribution of antibody therapeutics (Martins et al. 2016).

6.3.3 Primary and Higher-Order Structure

The primary structure of therapeutic proteins could also influence their distribution. For instance, compared to full-length mAbs, the Fab fragment alone may have larger volume of distribution because it has less potential for interaction with FcRn and thus can better overcome the binding-site barrier and penetrate deeper into tissues (Thurber et al. 2008; Tabrizi et al. 2010).

Additionally, the change in higher-order structure of a therapeutic protein can alter its distribution profile via change in apparent molecular size. For instance, the mean V_d of Humalog, an insulin lispro with disrupted dimerization as mentioned above, appeared to decrease with increase in dose (1.55 and 0.72 L/kg, respectively) in contrast to that of regular human insulin for which, V_d was comparable across the two dose groups (1.37 and 1.12 L/kg for 0.1 and 0.2 U/kg dose, respectively) (FDA database for labeling 2018).

6.3.4 Other Factors

Interactions with pharmacological targets on tissues or in plasma influence distribution of therapeutic proteins as well (Tabrizi et al. 2010). In addition to the abundance of the targets expressed, its interaction affinity with the drug can also have an impact on distribution. Structural factors, such as primary structure design and molecular charge, determine the interaction pattern between the drug and the drug target. More interestingly, for bispecific antibody-derived therapeutics, the relationship between binding affinity and distribution is not monotonic (List and Neri 2012; Kanodia et al. 2016). For instance, for the T-cell engagers, high affinity with T-cells will inhibit penetration to tumor tissues (List and Neri 2012). In another

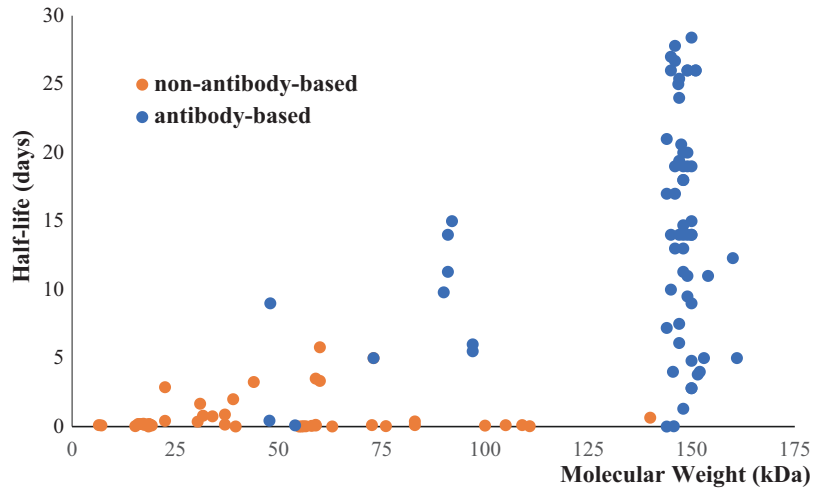

Fig. 6.4 The half-life of non-antibody (orange) vs full-length or mAb portion(s) (blue) therapeutic proteins

case, although anti-transferrin receptor (TfR)-based bispecific antibodies bind to TfR in order to pass the blood-brain-barrier, an intermediate affinity is required (Kanodia et al. 2016).

6.4 The Impact of Physicochemical Factors on Elimination

Therapeutic proteins are generally eliminated by two pathways: proteolytic catabolism and renal elimination (Shi 2014; Zhao et al. 2012), depending on its molecular weight. Kidneys may play a relevant role in the catabolism and elimination of only those biologics that have a size below the cut-off for glomerular filtration of approximately 60–70 kDa (Shi 2014; Vugmeyster et al. 2012; Tibbitts et al. 2016). For antibody-based therapeutics including monoclonal antibodies (mAbs), chimeric antibodies, antibody-drug conjugate (ADC), Fab and fusion proteins, the MW is generally much bigger than 60 kDa (Fig. 6.4), and proteolytic catabolism is the main elimination pathway.

6.4.1 Molecular Weight and Size

Clearance of therapeutic proteins can be modified through the change in weight and size such as dimerization and PEGylation. As an example, the therapeutic enzyme Fabrazyme (agalsidase beta) is a homodimer and has an apparent MW of 100 kDa. It is in equilibrium with its monomer of a MW of 50 kDa. The monomer is able to eliminate renally, promoting dissociation of the dimer. This process may help explain the short half-life of Fabrazyme (45–102 min).

PEGylation is a widely used to modify the physical size of small therapeutic proteins. It modifies biologics by covalent conjugation with polyethylene glycols (PEGs). In general, PEGylation can improve drug solubility, decrease immunogenicity, prolong residence time in body, and decrease degradation by metabolic enzymes, resulting in the improved PK and PD properties. A variety of biologics have been PEGylated with different PEGs to improve PK properties (Veronese and Pasut 2005; Hamidi et al. 2006; Jevsevar et al. 2010). First, attachment of PEG moieties greatly increases MW of therapeutic proteins. For instance, the PEGylaiton of asparaginase increases its MW from 34.5 to 380–450 kDa by attaching 69–82 molecules of mono-PEG (5 kDa each), resulting in a prolonged half-life of 5.8 days of Oncaspar (pegaspargase). The PEGylation of uricase (34 kDa) with approximately 40 PEG moieties increases the MW to 540 kDa and significantly increases its half-life (Richette et al. 2014). Cimzia (Certolizumab Pegol Injection), with a half-life of 14 days, is composed of an antibody

Fab fragment (50 kDa) conjugated to a 40 kDa PEG moiety. Second, PEGylation can increase the hydrodynamic radius. PEG exhibits a much greater molecular volume due to the extended conformation of the PEG polymer per unit of mass (Caliceti and Veronese 2003). Pegfilgrastim (39 kDa) consists of a single 20 kDa linear PEG molecule attached to filgrastim (18.8 kDa). The half-life of pegfilgrastim is much longer than filgrastim (15–80 h compared to 3.5–9 h, respectively) because the attached PEG moiety significantly increases the hydrodynamic radius (Yang and Kido 2011). Finally, PEGylation can increase the stability and reduce the catabolic elimination. The highly hydrated polyether backbone of the PEG moiety helps Pegasys (Peginterferon alfa-2a) forms a water shield, preventing the degradation by proteolytic enzymes and thus increasing the half-life of the parent protein (Jevsevar et al. 2010).

6.4.2 Charge

More negatively charged small proteins are less likely cleared by renal filtration because of the negatively charged framework of the kidney (Porter and Charman 2000). For instance, both Proleukin (Aldesleukin) (15.3 kDa) and Kalbitor (Ecallantide) (7.1 kDa) are cleared renally, but Aldesleukin has a shorter half-life compared to Ecallantide (13–85 min vs. 2 h, respectively). The small difference in half-life might be partly attributed to the difference in pI between Aldesleukin (pI: 6.83) and Ecallantide (pI: 5.58).

Total or local charge of therapeutic proteins can be modified by glycosylation. Aranesp (Darbepoetin alpha), for instance, is a 165-amino acid protein (37 kDa) that differs from Epogen/Procrit (Epoetin alfa) (30 kDa) by containing five N-linked oligosaccharide chains instead of three. While the two additional carbohydrate chains increase the MW of the glycoprotein by only 7 kDa, darbepoetin alpha has a threefold longer terminal half-life than epoetin alfa. The two additional carbohydrate molecules provide darbepoetin alpha significantly more negative charges as compared to epoetin alfa, thus elongating the half-life (Egrie and Browne 2002). Another case example of where glycosylation impacts clearance of a product is for Extavia (Interferon beta-1b) (18.5 kDa) and Rebif (interferon beta-1a) (22.5 kDa). The glycosylation of interferon beta-1a increases its solubility and stability in contrast to unmodified interferon beta-1b, resulting in a longer half-life for interferon beta-1a (69 h vs. 8 min to 4.3 h, respectively) (Song et al. 2014).

6.4.3 Elimination of Antibody-Based Therapeutics

Antibody-based therapeutics include mAbs, chimeric antibodies, antibody-drug conjugate (ADC), Fab and fusion proteins. As shown by Fig. 6.4, in general, therapeutic antibodies exhibit significantly longer half-lives than smaller non-antibody therapeutic proteins. The elimination of these therapeutics mainly occurs via intracellular catabolism (proteolysis) through two major catabolic pathways: Fc receptor mediated clearance and target mediated clearance (Lobo et al. 2004; Zhao et al. 2012).

The Fc receptor family is composed of cell surface receptors. Two major types of human Fc receptors that bind Fc domain of IgG are Fc gamma receptors (FcγR) and neonatal Fc receptor (FcRn) (Pechtner et al. 2017). The function of FcRn and the mechanism governing its protective role in antibody drug elimination have been well established. Briefly, FcRn protects IgG from lysosomal degradation and recycling IgG back into the circulation via specific binding to Fc domain of IgG. This recycling pathway preserves serum antibody level of IgG (Liu 2018; Lencer and Blumberg 2005; Sockolosky and Szoka 2015; Wang et al. 2008; Zhao et al. 2012; Pechtner et al. 2017). Changes in IgG-FcRn binding affinity resulted in altered clearance rate and half-lives of antibody drugs (Ghetie et al. 1997; Dall'Acqua et al. 2002, 2006; Vaccaro et al. 2005; Zalevsky et al. 2010). The structural properties of antibodies play an essential role in their interaction with FcRn and therefore FcRn-mediated recycling. Underlying the IgG-FcRn

binding are two major structural factors of antibody therapeutics: surface charge and primary structure.

6.4.4 Primary Structure

Given that all FDA-approved antibody drugs are developed based on IgG1, IgG2 and IgG4 subtypes, these antibodies should potentially have an elimination half-life of approximately 21 days (Zhao et al. 2012; Dall'Acqua et al. 2002; Ghetie et al. 1997; Levêque et al. 2005). However, elimination half-lives of all marketed IgG1-based antibodies differ widely (Fig. 6.4). One of the major physiochemical features that underlie this diversity is the degree of humanization of antibody therapeutics, which include human IgG, human-murine chimeric and humanized IgG. Human and rat FcRn receptors share only 65% amino acid sequence homology, causing the differences in IgG-FcRn affinity and, in turn, FcRn-mediated elimination of mAbs between the two species (Kuo et al. 2010; Dostalek et al. 2013). Indeed, among all antibodies approved by FDA, there exists a correlation between half-life and the degree of antibody humanization. Elimination half-life increases with the increase in humanization of the antibody therapeutics (Fc absence < murine < chimeric < humanized < human), a trend consistent with the previously suggested order (Zhao et al. 2012; Dostalek et al. 2013). Abciximab and idarucizumab, for instance, have a short half-life of 0.5 and 10.3 h, respectively, because they contain only the Fab fragments of IgG thus lack of Fc-FcRn interaction. This shortened half-life is consistent with an animal study showing that IgG in mice without FcRn is catabolized significantly faster than wild-type mice (Lobo et al. 2004; Zhao et al. 2012). One FDA-approved bispecific antibody derivative, Blincyto (Blinatumomab), represents another striking case for the impact of Fc absence on elimination. Blinatumomab exhibits a half-life of only 2.1 h because it is comprised of only Fab domains.

Although possessing the human Fc domain could theoretically result in chimeric antibodies with similar elimination rates as humanized or human IgG, the observed difference in half-life indicates that the intact human IgG structure may be required to fully restore the native IgG-FcRn interaction. The antigen binding (Fab) portion of native IgG may either contribute to FcRn binding or reduce the elimination of IgG through other mechanisms. In addition to direct therapeutic functions, full-length IgG as well as IgG fragments are effective carriers of therapeutic agents such as small molecules or proteins. Small molecule therapeutics such as tumor toxins are covalently attached to mAbs to form antibody-drug conjugates (ADC) (Lambert 2005; Senter 2009; Wu and Senter 2005). Similarly, some protein therapeutics can be also linked to mAb platform by DNA recombinant technology to form fusion proteins (Strohl 2015; Pechtner et al. 2017). Even though ADCs and fusion proteins possess the Fc domain and thus capable to utilize FcRn-mediated recycling, some of these therapeutics exhibit much shorter half-life than expected. For example, Fc fusion drugs, abatacept, aflibercept, and etanercept, have elimination half-lives of 5–6 days, even though they are not cleared via renal filtration due to their molecular weights being greater than 70 kDa. Similarly, the currently marketed ADC drugs exhibit elimination half-lives of 1.3–6 days. These short half-lives indicate that the conjugated molecules or fused proteins may interfere with the Fc-FcRn interaction, therefore inhibiting FcRn mediated recycling pathway.

6.4.5 Impact of pI on FcRn-Dependent and FcRn-Independent Elimination

The molecular charge or pI of therapeutic IgGs also plays a significant role in their elimination. Firstly, the molecular charge is essential to the electrostatic interaction between IgG and FcRn and hence the FcRn-mediated elimination. FcRn-IgG interaction is strictly pH-dependent. Possessing a pI of 7–9, IgG binds FcRn in slightly acidic pH, but not in neutral pH, via electrostatic interaction between titratable histidine residues in

CH2-CH3 domains with acidic residues on the α2-domain of FcRn (Lencer and Blumberg 2005; Dall'Acqua et al. 2006; Qiu et al. 2016). This dependence upon physiological conditions underlies the IgG preservation mechanism that governs the release of IgG from FcRn-bound form within acidic lysosomes back to the systemic circulation (Ghetie and Ward 2000). Mutational analyses have mapped the IgG-FcRn binding sites. The pH-dependent electrostatic interaction of IgG is mainly contributed by histidine310 and histidine435, while isoleucine253 is required for the hydrophobic interaction with FcRn (Sockolosky and Szoka 2015; Martin et al. 2001).

Secondly, multiple studies have demonstrated that the pI of IgG therapeutics impacts their FcRn-independent elimination. For instance, within an animal study, lowering the pI of variable domain of an antibody significantly reduced the its clearance without affecting its binding affinity with FcRn (Igawa et al. 2010). Another mutational analysis targeting anti-hepatitis C antibody suggested that adding negative charges to the variable domains of antibodies with high pI increases their clearance, independent of FcRn binding (Li et al. 2014). These results suggest that the elimination reduction, by lowering pI, is due to the decrease in fluid phase pinocytosis, because the negative charge on the antibody inhibits its binding to cells. Alternatively, adding positive charges on the high pI antibody at the lower pH of lysosomes could cause a faster rate of degradation of the antibody when internalized via pinocytosis (Igawa et al. 2010; Li et al. 2014).

6.4.6 Target Mediated Drug Disposition (TMDD)

TMDD causes the non-linear clearance (Mager 2006; Keizer et al. 2010; Dostalek et al. 2013). If subject to TMDD upon binding to targets on cell surface, the therapeutic proteins are internalized into the cells and subsequently degraded in lysosomes (Mellman and Plutner 1984; Press et al. 1988; Coffey et al. 2004; Lammerts et al. 2006; Keizer et al. 2010). Therefore, the structural characteristics that impact the interaction between therapeutic proteins and their pharmacological targets will inevitably influence their TMDD mediated elimination. For antibodies, the relevant structural characteristics include the molecular chargewhich involves the electrostatic interaction, and proper glycosylation required for antigen or target recognition. During drug development for a product with a given mechanism of action, the principle of primary structure design is to provide proper higher-order structural properties such as hydrophobicity, molecular charge and correct glycosylation, in order to facilitate the interaction between the drug product and its target.

6.4.7 Glycosylation

Glycosylation on the Fc domain of antibodies may impact their clearance. Although human-like native glycosylation may not account for the long half-life of IgG, the absence of influence is only relevant when the glycosylation is buried (Liu 2015; Bumbaca et al. 2012; Higel et al. 2016). In certain situations, attached terminal carbohydrate moieties are exposed and available to bind glycan receptors, causing faster clearance through the glycan receptor mediated elimination pathway (Winkelhake and Nicolson 1976; Wright et al. 2000). Glycan receptors that are involved in the elimination of glycoproteins include mannose receptor (ManR) and asialoglycoprotein receptor (ASGPR), both of which are specific to certain glycan types. It has been demonstrated that high-mannose containing IgG or Fc-fusion proteins are cleared faster than those with other glycosylation patterns (Liu 2015; Wright and Morrison 1994; Kanda et al. 2007; Liu et al. 2011; Yu et al. 2012). Similarly, antibodies carrying the terminal N-acetylglucosamine (GlcNAc) or galactose also exhibit fast clearance facilitated by ASGPR that recognizes GlcNAc (Winkelhake and Nicolson 1976; Beck and Reichert 2011; Stefanich et al. 2008). The other type of glycan, sialic acid (NANA), on the other hand, is critical to reduce the clearance of antibodies or Fc-fusion proteins, because NANA is able to cap the galactose and to block the recognition by ASGPR (Schwartz 1991; Liu 2015).

6.5 Immunogenicity and Anti-drug Antibodies (ADA)

Immunogenicity, an unwanted immune response to therapeutic proteins, involves the generation of anti-drug antibodies (ADA) that compromise drug efficacy and raise safety concerns. Factors influencing the immunogenicity of biologics can be classified into disease-, patient-, or product-related. For example, the dose, route, frequency and duration of administration are important for the immunogenicity response and ADAs. ADA induction can affect PK profiles of therapeutic proteins via influencing their elimination (Lobo et al. 2004). Besides of the inherent characteristics (e.g., pI and glycosylation) of therapeutic proteins, ADA-binding represents a factor that influences the product interactions with biological components such as Fc receptors (Davies et al. 1993; Strohmeier et al. 1995). The effect of ADA-binding on elimination may depend on the number of antigenic sites available on a given therapeutic protein. If there are one or two binding sites found on a therapeutic protein, ADA can contribute to the increase in its half-life. If there are more than two binding sites, on the other hand, the ADA-binding may result in faster clearance of the product (Rehlaender and Cho 1998; Lobo et al. 2004). In addition, the degree of aggregation of manufactured therapeutic proteins also influences the induction of ADA (Ratanji et al. 2014).

6.6 Characterizing Physicochemical Properties Affecting ADME of Therapeutic Proteins

6.6.1 Structure

The primary structure of a therapeutic protein represents its amino acid sequence. The higher-order structure of therapeutic proteins means its three-dimension tertiary structure, which is affected by its primary structure. Moreover, higher-order structure also covers protein quaternary structure such as dimerization. Protein folding is the process in which a protein forms three-dimension structure from the primary structure. Protein stability means the ability of a protein to maintain its three-dimension structure.

Circular Dichroism (CD) is used extensively to evaluate protein structure. Even though CD does not provide 3-D structure information, it can monitor the extent and rate of structural variation and ligand binding. In pharmaceutical industry, CD is also used to assess the stability of the designed proteins (Kelly and Price 2000). Differential Scanning Calorimetry (DSC) and Differential Scanning Fluorimetry (DSF) are two fundamentals tools for assessing thermal stability of proteins that have been widely applied by the pharmaceutical industry. These two similar methods semi-quantitatively measure stability via determination of melting temperatures of proteins (Johnson 2013; Bernhards et al. 2009).

6.6.2 Size and Self-Association

The molecular size of a therapeutic protein, directly correlated with its molecular weight, represents its geometric dimension. Dynamic light scattering (DLS) analyses are routinely used in detection of protein aggregation, the size of proteins and complexes or to monitor the binding of ligands. Due to the limitation of currently protein manufacturing technologies, DLS has been used quite often to assess the quality of manufactured therapeutic proteins (Lorber et al. 2012). Analytical gel-filtration is a chromatography-based method to assess the hydrodynamic size of therapeutic proteins.

The dimerization of therapeutic proteins can be quantitatively measured with Förster resonance energy transfer (FRET) (Jing et al. 2010). This method can be extensively used for insulin drugs which have multiple analogs with different dimerization states.

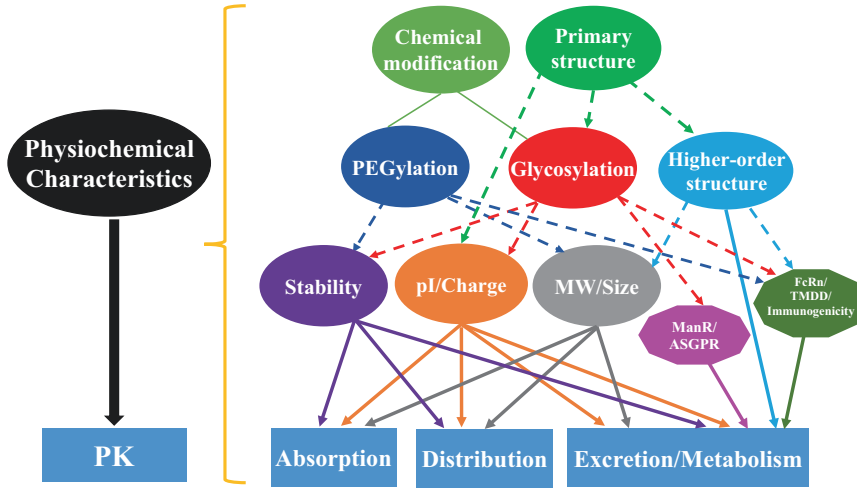

Fig. 6.5 Relationship between protein characteristics and their impact on ADME. A solid arrow line represents a direct influence of a protein characteristic or a mechanism on ADME. A dashed arrow line represents the influence of one protein characteristic to another or to a mechanism. The oval circles represent physiochemical features of therapeutic proteins. The two polygons represent mechanisms that correlate with physiochemical features and ADME properties of therapeutic proteins

6.6.3 Molecular Charge

The molecular charge of a therapeutic protein represents its net surface charge, commonly indicated by its isoelectric point. The simplest way to estimate the charge of a protein is to calculate pI from its amino acid sequence. However, this calculation may be inaccurate since it does not consider the folding of the target protein. Capillary isoelectric focusing (cIEF) is a high-resolution method that is widely applied to experimentally assess protein charge in pharmaceutical industry, especially for characterization of mAb drugs (Righetti 2004; Pergande and Cologna 2017).

6.6.4 Protein-Protein Interaction

Protein interactions are crucial to both therapeutic capabilities and PK of therapeutic proteins. Commonly used biophysical technique to characterize protein-protein interactions include Surface Plasmon Resonance (SPR) (Fabini and Danielson 2017), Isothermal Titration Calorimetry (ITC) (Pierce et al. 1999), Förster resonance energy transfer (FRET), and DLS (Hanlon et al. 2010).

6.7 Conclusions

In biological system, proteins are the major molecules responsible of executing biological functions because they possess advanced structures and biophysics. This concept underlies not only the therapeutic power of proteins in clinical therapy, but also more sophisticated correlation between their molecular characteristics and pharmacokinetic behaviors and clinical success.

This chapter describes structural characteristics and factors that impact either directly or indirectly the ADME properties of therapeutic proteins (Fig. 6.5). The molecular size of proteins, reflecting the combination of molecular weight and shape, determines not only their absorption route but also the elimination pathways. The molecular charge of therapeutic proteins, affecting their interactions with a wide range of biological components such as ECM

framework, kidney, Fc receptors and pharmacological targets, has a significant impact on every step of ADME. The post-translational modification of therapeutic proteins is critical for their biological activities and, more importantly, affect a multitude of their structural characteristics such as molecular size, molecular charge, stability and protein-receptor interactions. Further advances in protein engineering technologies and development of more sophisticated bioanalytical tools will facilitate the understanding of the relationship between structure and ADME and promote successful development of novel therapeutic proteins.

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