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Review article

Delivery aspects of small peptides and substrates for peptide transporters ☆

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Abstract

The present summary highlight chemical strategies applied to improve plasma half-lives and oral bioavailability of peptidic drugs as well as view on intestinal and pancreatic peptidase mediated degradation of peptidic drugs. In general chemical strategies used to increase the oral bioavailability of peptidic drugs consisting of more than three amino acids is disappointing. On the other hand chemical approaches to stabilize peptidic drugs against metabolism seem promising for increasing plasma half-lives of parental peptidic drugs as well as for increasing oral bioavailability of di/tripeptidomimetics and dipeptidyl pro-moieties targeting peptide transporters.

Keywords: Proteolytic enzymes; Exopeptidases; Intestinal peptide transporters; Amide bond replacement; Peptides; Drug delivery; Prodrugs

1. Introduction

A number of peptides have therapeutic potential as drugs in treatment of a variety of diseases. They are, however, susceptible to rapid inactivation by exopeptidases and proteolytic enzymes distributed throughout the body. Chemical stabilization strategies have been successfully applied to increase plasma half-lives of parenterally administered peptidic drugs. Similar strategies have been suggested for increasing bioavailabilities of oral administered peptidic drugs. Whereas increasing bioavailability of peptidic drugs by chemical stabilization seems to be less successful strategies for peptides consisting of more than three amino acids, the approach seems promising for di/tripeptidic drugs and pro-moieties targeted the intestinal peptide transporter PEPT1. The aim of the present summary is to highlight on pancreatic and intestinal peptidases, i.e. exopeptidases and proteolytic enzymes, as well as view on chemical strategies applied to increase plasma half-lives and increase oral bioavailability of peptidic drugs and pro-moieties. Furthermore, to give a short status on the approach utilizing peptidyl bond replacement to stabilize dipeptidyl pro-moieties targeted PEPT1.

2. Intestinal digestive/absorptive process of peptides

Exogenic peptides are generally rapidly metabolised within the intestinal tract by pancreatic and intestinal proteolytic enzymes to form smaller peptides, di/tripeptides and amino acids [1,2]. Even though endocytotic uptake of peptides is described, it is generally believed that larger peptides have almost restricted entrance into enterocytes. In contrast, large amounts of amino acids and di/tripeptides are transported across the mucosal enterocytic membrane by amino acid and peptide transporters. Intestinal nutrient transporters including peptide and amino acid transporters have recently been reviewed elsewhere [3]. Most di/tripeptides are believed to be hydrolysed within the enterocytes by various enzymes; for dipeptides by enzymes such as cytosolic non-specific dipeptidase and Xaa-His dipeptidase [1,2]. Examples of enzymes involved in the pancreatic/intestinal digestive process of peptides are summarized in Table 1. These enzymes as well as transporters and the physiology of the gastrointestinal tract

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Table 1
Intestinal and pancreatic enzymes found in *Homo sapiens* involved in digestion of peptides/proteins. (AA)*n*: *n* amino acids

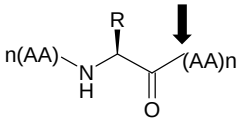
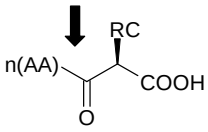
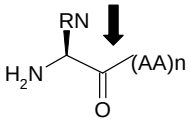
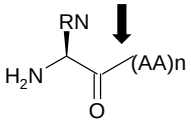
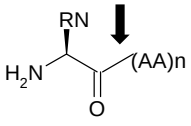
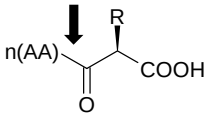
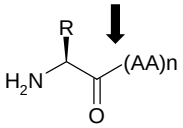
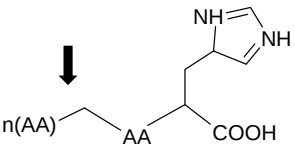
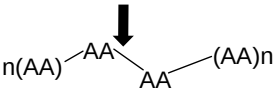
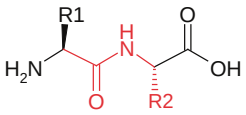
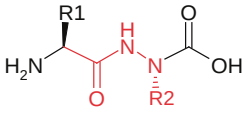
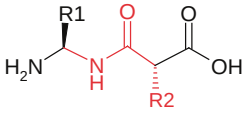
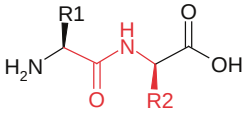
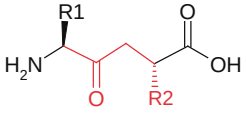
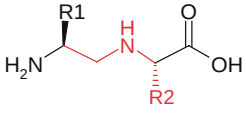
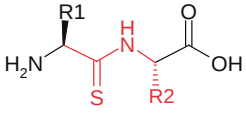
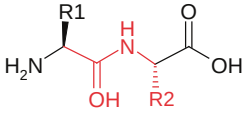
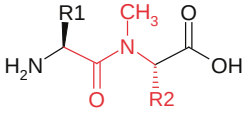
Peptidyl bond	IUBMB nomenclature, recommended name, tissue and gene: GO ontology number	Released AA and peptides
 R1: aromatic	EC 3.4.21.1 chymotrypsin, secreted in pancreas as chymotrysinogen activated by trypsin, endopeptidase, GO: 0004263	Cleave at C-terminal at aromatic AA. Small peptides
 R3: aromatic	EC 3.4.17.1, carboxypeptidase A, secreted in pancreas. Exopeptidase, GO: 0004182	Prefer aromatic AA in C-terminal. Tyr, Trp, Phe and small peptides. (Little or no activation with -Asp, -Glu, -Arg, -Lys, or -Pro)
 R1: preferment Ala	EC.11.2, membrane alanyl aminopeptidase, luminal enterocytic membrane, GO: 0004179	Neutral amino acids, Ser, Cys, Gly, Ala, Gln, Asn, Leu, Val and small peptides
 R1: preferment Cys	EC 3.4.11.3, cysteine aminopeptidase, secreted by pancreas. Exopeptidase. No GO number known	Neutral amino acids, Ser, Cys, Gly, Ala, Gln, Asn, Leu, Val and small peptides
 R1: preferently Leu	EC 3.4.11.1, leucyl aminopeptidase, secreted by pancreas. Exopeptidase, GO: 004178	Neutral amino acids, Ser, Cys, Gly, Ala, Gln, Asn, Leu, Val and small peptides
 R3: cationic	EC 3.4.17.2, carboxypeptidase B, secreted in pancreas. Exopeptidase GO: 0050425	Cationic amino acids, Lys, Arg and small peptides
 R1: cationic	EC 3.4.21.4, trypsin, secreted as trypsinogen in pancreas, exopeptidase, GO: 0004295	
	EC 3.4.13.18, cytosolic non-specific dipeptidase, cytosolic in enterocytes, GO: 0042315	Pro Anionic AA Glu, Asp Cationic and neutral AA (see above)
	EC.3.4.13.3, Xaa-His dipeptidase, cytosolic in enterocytes as well as secreted by pancreas. Exopeptidase, GO number not known	AA-His
	EC. 3.4.21.36, pancreatic elastase, secreted in pancreas as proelastase, GO: 0008125	Small peptides

Table 2
Chemical structures of various peptidyl bond replacement (in red) that stabilizes against peptidases

Chemical structure	Name
	(1) L,L-Dipeptide
	(2) L,L-α-Aza dipeptide
	(3) L,L-Retro-dipeptide
	(4) L,D-Dipeptide
	(5) Ketomethylene L,L-dipeptide
	(6) Caba-replaced amidic carbonyl in L,L-dipetide
	(7) Thioamidated L,L dipeptide
	(8) Hydroxymethylated L,L, dipeptide
	(9) *N*-methylated L,L dipeptide

R1, side chain in N-terminal amino acid; R2, side chain in C-terminal amino acid.

should be considered in an integrative manner when designing peptidic drugs for oral administration.

3. Peptidyl bond replacement to stabilize peptides against exopeptidases and proteolytic enzymes

Peptidyl bond replacement is used to stabilize peptidic drugs against exopeptidases and proteolytic enzymes (Table 2). Examples are thioamidation (7) or *N*-ketomethylene formation (5) [4,5]. Others are carba-replacement of the amidic carbonyl (6), α-aza peptide formation (2), *N*-methylation (9), replacing single amino acids by corresponding D-amino acids (4), or by performing retro-amides (3) [6,7]. Together with S–S bridging between two cysteine moieties these examples of well-known peptidyl bond replacements are applied alongside chemical protection strategies at N- and C-terminals of peptides.

One of the very few successful examples where chemical approaches have been applied to partly stabilize a peptidic drug molecule against gastrointestinal peptidases is desmopressin that is used in the treatment of diabetes mellitus. Desmopressin (Fig. 1), is partly protected against proteolytic degradation by carboxypeptidases protecting the carboxy-terminals via amidation. Furthermore, desmopressin is stabilized by insertion of D-Arg instead of L-Arg. The N-terminal is protected against aminopeptidases by mercaptopropionic acid (Mpa). However, desmopressin is still partly degraded by proteolytic enzymes such as chymotrypsin even though approaches have been taken to avoid this by S–S bridging between Mpa and Cys. The bioavailability of desmopressin after oral administration is never the less limited to 0.1% which partly can be ascribed to proteolytic degradation, partly to the physico-chemical properties of desmopressin, i.e. its limited lipophilicity, its relatively large molecular weight, and its large number of H-bond acceptor and donors that limit passive permeability across the enterocytic membrane [8]. Nevertheless, due to the high potency and limited side effects of desmopressin, an oral bioavailability of 0.1% is therapeutically acceptable. Another examples where a peptidic drug have been

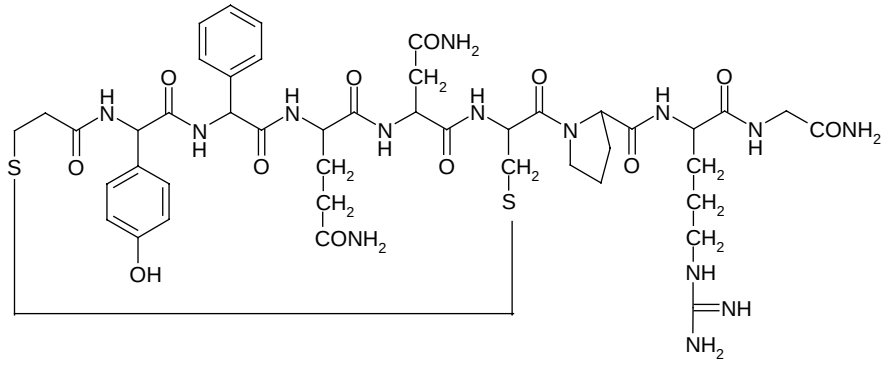


Fig. 1. Chemical structure of desmopressin Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH_2.

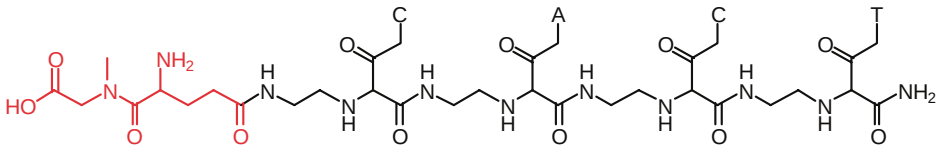


Fig. 2. Chemical structure of the terameric PNA CACT conjugated Glu-Sar. Glu-Sar is in red. (For interpretation of the reference to colour in this legend, the reader is referred to the web version of this article.)

stabilized against enzymatic degradation is atosiban (Tractocile®) where a cyclized octapeptide is performed by S–S bridging between the cysteine moiety and the mercaptoetanoic acidification of N-terminal tyrosine. In the same molecule the C-terminal glycine have been protected by amidation. Atosiban, which is administered parenterally, has improved plasma stability and thereby longer elimination half lives over the corresponding natural octapeptide.

Prolonged action after parental delivery is also obtained in human insulin in which fatty acid acylation of the ε-amino group in Lys B29, i.e. insulin detemer, results in protraction due to albumine binding [9].

Another suggested chemical approach to increase oral bioavailability is to introduce pro-moieties that ideally should chemically mask or steric hinder peptidase catalyzed degradation as well as lipophilize the molecule and thereby improve transport properties across the enterocytic membrane by transiently reducing amounts of hydrogen bond donor and acceptor moieties. However, the usefulness of the prodrug concept as such in which derivatives are prepared on single functional groups to stabilize and lipophilize the parent peptide has been limited. No oral peptide prodrug formulation with a peptidyl backbone larger than three amino acids is to our knowledge on the market. On the other hand, in an acylated desmopressin derivative described by Wang and co-workers as well as in terlipressin several chemical approaches have been applied alongside, i.e. the prodrug, the S–S bridging and the terminal-protection concepts [10]. Terlipressin or Glypressin® is the triglycyl-prodrug of lysin vasopressin. It is marketed in Europe as an injection concentrate for parental delivery and is described to have prolonged duration of action, i.e. longer elimination half live, compared to parent lysin vasopressin. The lysin vasopressin part of the molecule seems to be stabilized against enzymatic degradation by C-terminal amidation, by S–S bridging between the two Cys-moieties, and by etherfication of the phenolic moiety in terminal tyrosine.

4. Dipeptidyl prodrug approach

In the dipeptidyl prodrug approach two approaches are applied alongside, i.e. the stabilization of the pro-moiety and targeting of peptide transporters PEPT1/2. The dipeptidyl pro-moiety is stabilized by well-known peptidyl bond replacements such as *N*-ketomethylene formation (5), D-amino acids (4), *N*-methylation (9), or thioamidation (7) (Table 2) [11–13].

Application of pro-moieties such as Gly-Sar, D-Glu-Ala or the ketomethylene isosters Aspψ[$COCH_2$]Gly and Pheψ[$COCH_2$]Asp, which all have intrinsic affinity to PEPT1, has been investigated by Steffansen and co-workers and by Luthman and co-workers [5,12–14]. In this approach the β or the γ position of one of the two amino acids is used to form various dipeptidyl ester prodrugs and model prodrugs. The model prodrugs D-Glu-(Obn)Ala, Asp(Obn)ψ[$COCH_2$]Gly and Pheψ[$COCH_2$]Asp (Obn) has been proven to be substrates for PEPT1, i.e. they are transported across Caco-2 cells in a hPEPT1 dependent manner; whereas a prodrug such as L-Glu(acyclovir)Sar is most likely an inhibitor of hPEPT1 [12,14,15]. The oral bioavailability of acyclovir when given as the L-Glu(acyclovir)Sar prodrug to rats is, in consistence with the inhibitory nature of the prodrug, less than 2%

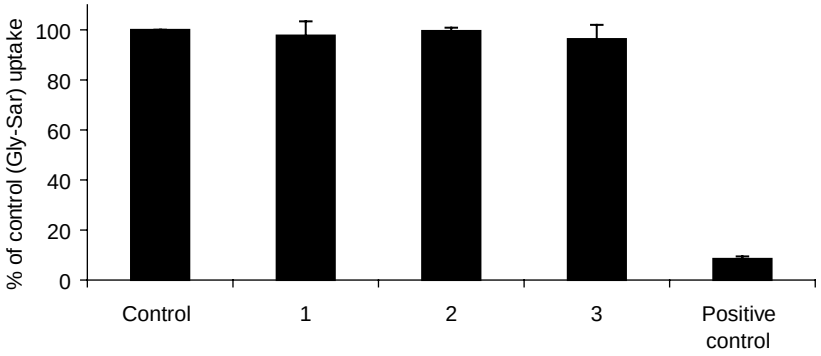


Fig. 3. The uptake of [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar) was measured in Caco-2 cells in the presence and absence of tetra, hepta or decameric PNA's linked to Gly-Sar. The concentration of: (1) Glu[CACT]-Sar, (2) Glu[GATCACT]-Sar and (3) Glu[GTAGATCACT]-Sar were 2.8, 2.6 and 0.7 mM, respectively, and as positive control 2.5 mM L-Glu(benzylamide)-Sar was used. Values are average ±SD from two independent experiments in two to three different Caco-2 cell passages.

compared to a bioavailability of 81 and 15% when administered, respectively, as val-ganciclovir or acyclovir itself [15]. As intuitively expected the transport activity of PEPT1 does not seem useful for delivering large molecules such as peptide nucleic acids (PNAs). This is illustrated in Figs. 2 and 3 where respectively, the chemical structures and the uptake of [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar) in Caco-2 cells in the presence and absence of tetra, hepta or decameric PNA linked Glu-Sar, as well as in the presence of L-Glu(benzylamide)Sar is given. These studies indicate that the benzyl amide derivative significantly decreases the [^{14}C]Gly-Sar uptake whereas the PNA derivative does not interact with PEPT1 at the investigated concentrations.

Various small di/tripeptidomimetic drugs and prodrugs such as some angiotensine converting enzyme (ACE) inhibitors, some β-lactam antibiotics (penicillins and cephalosporins), bestatin, the valine ester prodrugs of acyclovir and ganciclovir have been shown to target peptide transporters, PEPT1/2, in the apical membrane of intestinal and renal epithelial cells; thus increased bioavailability of some of these compounds may be ascribed to PEPT1/2. Recent advances in therapeutic applications of human peptide transporters have recently been reviewed [10,11]. In summary, these studies indicate that PEPT1/2 targeted di/tripeptidomimetics and dipeptidyl prodrugs may be a very promising delivery approaches for di/tripeptidomimetics and small drug molecules, respectively, whereas the approach seems to be of limited use for delivering larger peptides or macromolecules such as PNAs.

5. Conclusions

Peptides have a large potential as drug. Whereas several stabilized di/tripeptidomimetics such as some cephalosporins and ACE-inhibitors are registered as oral formulations, few oral formulations of larger peptides are marketed. This may be ascribed to limited in vivo stability and limited transport properties of these compounds. A promising approach to increase oral bioavailability of di/tripeptidomimetic-drugs and prodrugs may be to stabilize the molecules against pancreatic and intestinal peptidases by peptidyl bond replacement and at the same time targeting intestinal nutrient peptide transporters.

Acknowledgements

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