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Prodrugs of peptides. III. 5-Oxazolidinones as bioreversible derivatives for the α-amido carboxy moiety in peptides *

Anders Buur and Hans Bundgaard

Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, Copenhagen (Denmark)

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Summary

The hydrolysis kinetics of six *N*-acyl 5-oxazolidinones derived from various *N*-benzyloxycarbonyl amino acids and aldehydes (formaldehyde, acetaldehyde and benzaldehyde) was studied to assess their suitability as prodrug forms for the α-amido carboxy moiety occurring in peptides. The degradation of the compounds was shown to take place by hydrolytic opening of the lactone ring with the formation of an *N*-hydroxyalkyl intermediate which subsequently decomposed to the parent *N*-acylated amino acid and aldehyde. For oxazolidinones derived from formaldehyde and acetaldehyde the latter reaction was rate-limiting whereas for derivatives of benzaldehyde the hydrolysis of the lactone ring was the slowest step at neutral pH. The lactone hydrolysis was catalyzed by human plasma enzymes and influenced by substituents in the 2- and 4-positions. It is suggested that 5-oxazolidinone formation may become a useful approach to bioreversible derivatization of peptides containing an α-amido carboxy function in an effort to protect peptide bonds against enzymatic cleavage and to improve absorption characteristics through increased lipophilicity. The potential utility of the *N*-hydroxyalkyl derivatives formed upon hydrolysis of the oxazolidinones as useful prodrug forms is also discussed.

Introduction

The application of peptides as drugs is seriously hampered due to substantial delivery problems. Peptides including those consisting of only a few amino acids are rapidly metabolized by proteolysis at most routes of administration, they are in general non-lipophilic compounds showing poor membrane penetration and they possess short biological half-lives (Wiedhaup, 1981; Meisenberg and Simmons, 1983; Humphrey and Ringrose, 1986; Ferraiolo and Benet, 1985; Lee, 1986).

A possible approach to solve these delivery problems, especially in case of small peptides, may be derivatization of the bioactive peptides to produce prodrugs or transport forms which possess enhanced physicochemical properties in comparison to the parent compounds as regards delivery and metabolic stability. Thus, it may be imagined that bioreversible derivatization may protect small peptides against degradation by peptidases pre-

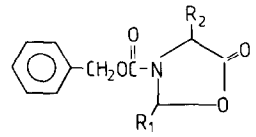
* For parts I and II, see Klixbüll and Bundgaard (1984) and Bundgaard (1986).

Correspondence: H. Bundgaard, Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

sent at the mucosal absorption barrier and render the peptides more lipophilic and hence facilitate the absorption. To be useful, however, the derivatives should subsequently be cleaved spontaneously or enzymatically in the blood following their absorption with release of the parent bioactive peptide (Bundgaard, 1986).

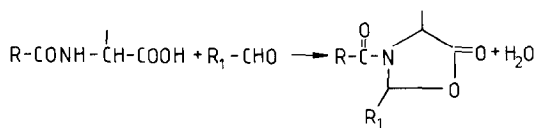
In our laboratory, studies have been initiated to develop various types of bioreversible derivatives for the functional groups or chemical entities occurring in amino acids and peptides (Klixbüll and Bundgaard, 1984; Bundgaard, 1985, 1986). In the present work, 5-oxazolidinones were evaluated as a possible prodrug type for the α-amido carboxy moiety which is found in many peptides.

5-Oxazolidinones are formed by condensing an α-amino acid or an *N*-acylated amino acid, i.e. a peptide-like structure, with an aldehyde (Scheme 1) (Ben-Ishai, 1957; Dane et al., 1959, 1960; Micheel and Thomas, 1957; Micheel and Meckstroth, 1959; Micheel and Haneke, 1959, 1962; Itoh, 1969; Davidovich et al., 1978; Freidinger et al., 1983; Seebach et al., 1983; Karady et al., 1984; Polonski, 1985; Low and Duffield, 1985; Seebach and Fadel, 1985; Laspéras et al., 1986). Whereas 5-oxazolidinones of α-amino acids are very sensitive to hydrolysis (Seebach et al., 1983; Pascal et al., 1987), *N*-acylated 5-oxazolidinones should be expected to be more stable although no kinetic data are available. The latter compounds are structurally related to *N*-acyloxyalkyl derivatives of amides or carbamates. When derived from secondary amides or carbamates as well as imides such derivatives show the normal ester stability with decomposition proceeding in two steps: chemical or enzymatic hydrolysis of the ester moiety followed by spontaneous decomposition of the *N*-hydroxyalkyl intermediate (Scheme 2) (Bundgaard and Nielsen, 1988; Bundgaard et al., 1988). In *N*-acylated 5-oxazolidinones the amide nitrogen is also substituted, the difference being the presence of a lactone ring instead of an open ester group. In order to provide information on the chemical and enzymatic reactivity of such 5-oxazolidinones we have studied the kinetics of hydrolysis of a number of oxazolidinones (**I–VI**) derived from condensation of various *N*-benzyloxycarbonyl amino acids (i.e. models of the α-amido carboxy group in peptides) with different aldehydes.

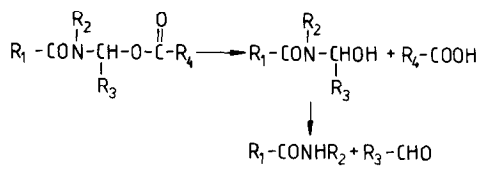


	R_1	R_2
I	-H	-H
II	$-CH_3$	-H
III	$-C_6H_5$	-H
IV	-H	$-CH_3$
V	-H	$-CH(CH_3)_2$
VI	-H	$-CH_2C_6H_5$

Formulae I–VI



Scheme 1



Scheme 2

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was done with a system consisting of a Waters Pump Model 6000A, a variable wavelength UV detector (Waters Type Lambda Max 480), a Rheodyne 7125 injection

valve with a 20-μl loop and a Chrompack column (100 × 3 mm) packed with Chromspher C-8 (5-μm particles).

Synthesis of 5-oxazolidinones

The N-carbobenzoxy-5-oxazolidinones **I–VI** were prepared by reacting the *N*-benzyloxycarbonyl (*Z*) derivatives of glycine (**I–III**), DL-alanine (**IV**), L-valine (**V**) and L-phenylalanine (**VI**) with the appropriate aldehyde according to previously described procedures: **I**, mp 83.5–84°C (Ben-Ishai, 1957); **II**, mp 57–58°C (Micheel and Meekstroth, 1959); **III**, mp 102–103°C (Micheel and Meekstroth, 1959); **IV**, mp 64–65°C (Ben-Ishai, 1957); **V**, 53–54°C (Ben-Ishai, 1957) and **VI**, mp 82.5–83°C (Ben-Ishai, 1957). The melting points agreed with those reported in the references listed.

Kinetic measurements

The decomposition of the *N*-acyl 5-oxazolidinones **I–VI** was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate and borate were used as buffers; a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The total buffer concentration was generally 0.02 M.

The rates of hydrolysis of the 5-oxazolidinones were determined by using a reversed-phase HPLC procedure capable of separating the 5-oxazolidinones from the degradation products. For compounds **I** and **II** a mobile phase system of 50% v/v methanol in 0.01 M acetate buffer of pH 5.0 was used. The flow rate was 1.2 ml/min and the column effluent was monitored at 220 nm. The retention times for **I** and **II** were 1.7 and 2.3 min, respectively. In the analysis of products formed upon degradation of compound **II** a mobile phase system of phosphoric acid–acetonitrile–methanol–water (1 : 5 : 30 : 64 v/v) was used. For compounds **III–VI**, a mobile phase system consisting of phosphoric acid–methanol–acetonitrile–water (1 : 5 : 45 : 49 v/v) was used. The flow rate was 1.0 ml/min (**III**) or 1.2 ml/min (**IV–VI**) and the column effluent was monitored at 223 nm (**III**) or 220 nm (**IV–VI**). Under these conditions the compounds showed the following retention times: **III**, 3.3 min; **IV**, 1.9 min; **V**, 2.4 min; **VI**, 3.1 min. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 100 μl of a stock solution of the derivatives in acetonitrile to 10.0 ml of buffer solution, pre-equilibrated at 37°C, in screw-capped test tubes, the final concentration of the derivatives in the reaction solution being about 10^{-4} M. The solutions were kept in a water-bath at 37 ± 0.2°C and at appropriate times samples were taken and immediately chromatographed. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual 5-oxazolidinone against time.

The hydrolysis of compound **III** was also followed spectrophotometrically by recording the increase in absorption at 255 nm due to liberation of benzaldehyde. The reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding about 20 μl of a stock solution of compound **III** in acetonitrile, giving a final concentration of about 5×10^{-5} M. Pseudo-first-order rate constants were determined from plots of log ($A_\infty - A_t$) vs time, where A_∞ and A_t are the absorbance readings at infinity and time t, respectively.

Hydrolysis in human plasma solutions

The hydrolysis of the derivatives **I–VI** was also studied in 0.01 M phosphate buffer (pH 7.4) containing 80% human plasma at 37°C. Initial concentrations of the derivatives were about 10^{-4} M. At appropriate times samples of 250 μl were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in methanol–water (1 : 1 v/v) in order to deproteinize the samples. After mixing and centrifugation for 3 min at 13,000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of hydrolysis in aqueous solution

The kinetics of hydrolysis of *N*-acyl 5-oxazolidinones **I–VI** was studied in aqueous buffer solu-

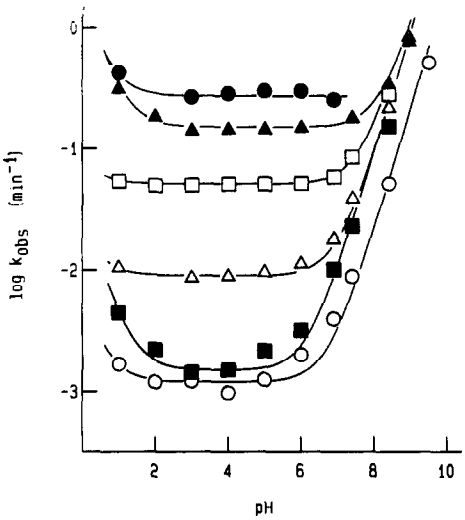


Fig. 1. The pH–rate profiles for the hydrolysis of various *N*-acyl 5-oxazolidinones in aqueous solution ($\mu = 0.5$) at 37 °C. **I** (□); **II** (●); **III** (▲); **IV** (△); **V** (○); **VI** (■).

tions at 37 °C and $\mu = 0.5$ over a wide range of pH. At constant pH and temperature the disappearance of the oxazolidinones displayed strict first-order kinetics over several half-lives and all reactions went to completion.

The rates of decomposition were found to be subject to catalysis by the buffer substances used to maintain constant pH. Buffer-independent pseudo-first-order rate constants (k) were determined by extrapolation of linear plots of the observed pseudo-first-order rate constants against total buffer concentration to zero concentration.

The influence of pH on the overall degradation rate of the 5-oxazolidinones is shown in Fig. 1 where the logarithms of k are plotted against pH. The observed pH-rate relationships indicate that the overall hydrolytic reaction can be described in terms of a water-catalyzed or spontaneous reaction and specific acid- and base-catalyzed reactions according to the following rate expression:

$$k = k_H a_H + k_0 + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.62 \quad (2)$$

Values of the specific rate constants k_0, k_H and k_{OH} obtained from the pH-rate profiles and Eqn. 1 are listed in Table 1.

The rate data obtained for the various derivatives show that the stability is maximal in the pH range 2–5 but that even at such pH values the stability is limited. The most stable derivative is compound **V** which at pH 2–4 and 37 °C possesses a half-life of 11.5 h. The much greater reactivity of the *N*-acyl 5-oxazolidinones in comparison with related linear *N*-acyloxyalkyl derivatives (Bundgaard and Nielsen, 1988) can be ascribed to a ring strain of the lactone ring. The carbonyl infrared absorption of *N*-acyl 5-oxazolidinones occurs in the 1800–1815 cm^{-1} region (Ben-Ishai, 1957), indicating a high reactivity of the carbonyl group. In accordance with the facile hydrolysis *N*-acyl 5-oxazolidinones are known to behave as active esters in aminolysis reactions (Ben-Ishai, 1957; Micheel and Meckstroth, 1959; Itoh, 1969).

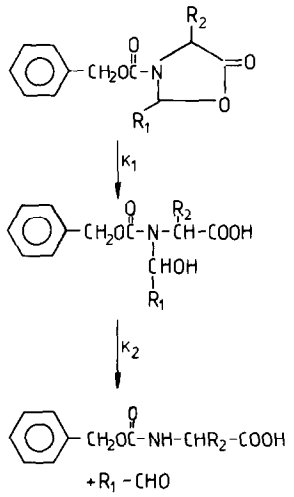
The stability is seen to be highly affected by substituents in the 2- and 4-positions of the 5-oxazolidinone ring. Substitution of hydrogen in the 2-position (R_1) with methyl or phenyl causes an increase in the rate of lactone hydrolysis whereas alkyl or aralkyl substituents in the 4-position (R_2) decrease the reaction rate. The latter effect is most likely mainly due to steric reasons.

TABLE 1

Rate data for the hydrolysis of various N-acyl 5-oxazolidinones in aqueous solution at 37 °C and $\mu = 0.5$

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})
I	0.037	0.051	5.0×10^{4}
II	1.5	0.27	n.d.
III	2.0	0.15	3.9×10^{4}
IV	0.014	9.0×10^{-3}	3.7×10^{4}
V	5.0×10^{-3}	1.2×10^{-3}	8.9×10^{3}
VI	0.032	1.5×10^{-3}	3.9×10^{4}

The rate data refer to the k_1-reaction in Scheme 3.

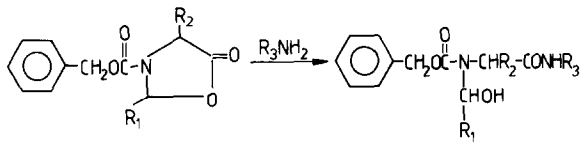


Scheme 3

These results thus indicate that as regards prodrug formation it is possible to vary the stability of the derivatives by selecting different aldehydes.

Mechanism of degradation

In accordance with the behaviour of linear *N*-acyloxyalkyl derivatives of secondary amides or carbamates (Bundgaard and Nielsen, 1988) the degradation of *N*-acyl 5-oxazolidinones appears to take place via a two-step reaction as shown in Scheme 3: initial hydrolytic opening of the lactone ring followed by a spontaneous decomposition of the intermediary *N*-hydroxyalkyl derivative (**VII**) to give the parent *N*-acyl amino acid and aldehyde. Support for such a reaction sequence was provided by the observation of an intermediate in the overall degradation as evidenced by HPLC monitoring of the degradation. This was studied in greater detail for the compounds **II**, **III** and **VI**. Using the HPLC mobile phase systems described before it was possible to monitor the disappearance of these compounds as well as the time courses for the degradation products. Fig. 2 illustrates the degradation of compound **III** at pH 5.0 and 37 °C. The disappearance of **III** is accompanied by the formation of a new peak in the chromatograms which subsequently disappears more slowly. Furthermore, the disappearance of **III** is accompanied by the formation of the parent *N*-benzyloxycarbonyl amino acid (*Z*-Gly), and its formation is seen to pass through a lag period. It was further observed by HPLC analysis that benzaldehyde was formed exactly concurrently with *Z*-Gly. These observations are fully in accord with the reaction scheme proposed (Scheme 3). Although the intermediate degradation product was not isolated its identity as the *N*-hydroxybenzyl derivative of *Z*-Gly is strongly indicated by its conversion to benzaldehyde and *Z*-Gly. Furthermore, treatment of *N*-benzyloxycarbonyl 5-oxazolidinones with one equivalent of benzylamine has previously been shown to result in the formation of an *N*-hydroxyalkyl amide (Scheme 4) (Ben-Ishai, 1957; Micheel and Meckstroth, 1959).



Scheme 4

The rate-determining step in the liberation of the parent *N*-acylated amino acid from the 5-oxazolidinones depends on the relative stabilities of the latter and the intermediate *N*-hydroxy-

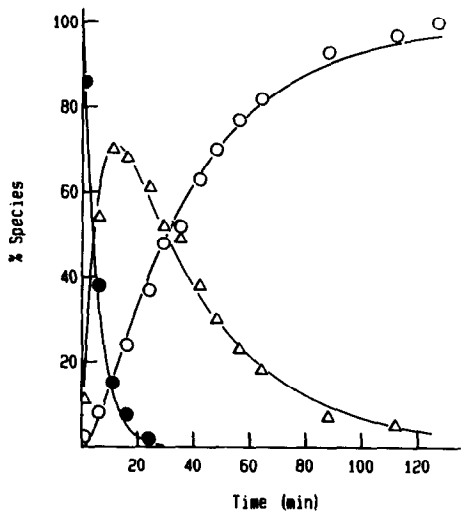


Fig. 2. Time-courses for compound **III** (●), the *N*-hydroxybenzyl derivative of *Z*-glycine (△) and *Z*-glycine itself (○) in the degradation of **III** in aqeuous solution of pH 5.0 at 37 °C. The curve for the *N*-hydroxybenzyl derivative was constructed from data based on HPLC measurements and the identity: % derivative = 100 − (**III** + *Z*-Gly)%.

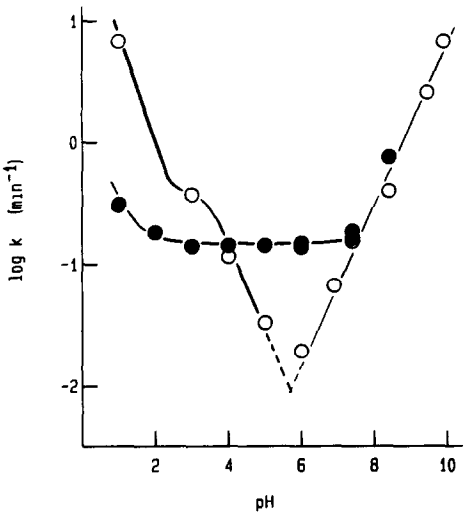
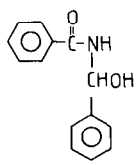


Fig. 3. The pH–rate profiles for the k_1-reaction (●) and k_2-reaction (○) for compound **III** in aqueous solution at 37 °C.

alkyl derivative. This, in turn, depends on pH of solution. Fig. 3 shows the pH-rate profiles for the k_1- and k_2-reactions (cf. Scheme 3) for compound **III**. At pH-values lower than 4 and higher than about 7.5 the slowest and hence rate-determining step in the formation of *Z*-Gly is the k_1-reaction, i.e. hydrolysis of the lactone ring. In the pH-range 4–7.5 the k_2-reaction becomes the slowest reaction. Except for the curvature at pH 3–4 the pH-rate profile for the k_2-reaction is similar to that previously described for an analogous compound, *N*-(α-hydroxybenzyl)benzamide (**VII**) (Bundgaard and Johansen, 1984). Also, the actual rates of decomposition of the *N*-hydroxybenzyl derivative formed from compound **III** are of the same order as those previously reported for compound **VII** (e.g., $t_{1/2} = 6.5$ min at 37 °C). The curvature observed at pH 3–4 in the log k_2-pH profile can be accounted for in terms of a higher reactivity of the ionized species than the form with an unionized carboxy group.



Formula VII

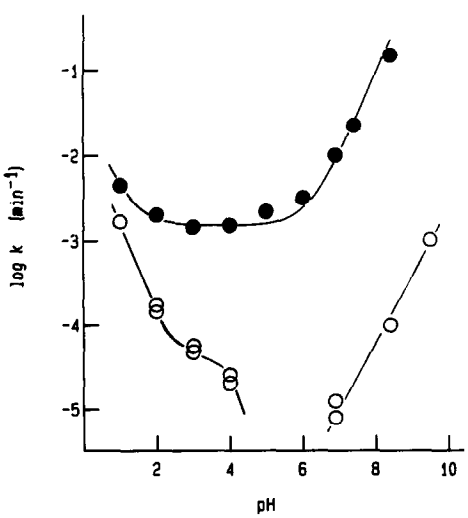


Fig. 4. The pH-rate profiles for the k_1-reaction (●) and k_2-reaction (○) for compound **VI** in aqueous solution at 37 °C.

For compound **VI** derived from formaldehyde and *Z*-Phe the slowest step in its overall degradation is decomposition of the intermediate *N*-hydroxymethyl derivative (the k_2-reaction) irrespective of pH (Fig. 4).

In considering the potential utility of 5-oxazolidinones as prodrug forms for the parent *N*-acylated amino acid it is thus important to note that the rate of formation of the latter may be strongly limited by the conversion of an *N*-hydroxyalkyl intermediate despite a facile hydrolysis of the oxazolidinone moiety. In order to accelerate the rate of decomposition of the *N*-hydroxyalkyl intermediate the oxazolidinones should be prepared from other aldehydes than formaldehyde, e.g. acetaldehyde or benzaldehyde. Thus,

TABLE 2

Half-lives for the decomposition of various N-hydroxyalkyl derivatives formed upon initial hydrolysis of N-acyl 5-oxazolidinones in aqueous solution at 37 °C

Compound **VII**		$t_{1/2}$		
R_1	R_2	pH 3.0	pH 7.4	pH 9.90
H	$CH_2C_6H_5$	231 h	1080 h	16.5 h
CH_3	H		195 min	
C_6H_5	H	1.9 min	4.5 min	0.1 min

The rate data refer to the k_2-reaction in Scheme 3.

TABLE 3

Rate data for the hydrolysis of various N-acyl 5-oxazolidinones in 0.01 M phosphate buffer of pH 7.4 and in 80% human plasma (pH 7.4) at 37 °C

Compound	$t_{1/2}$ (min)	
	Buffer	Plasma
I	8.2	0.2
II	2.7	<1
III	4.1	1.0
IV	18	0.7
V	78	20
VI	30	4.3

The rate data refer to the k_1-reaction in Scheme 3.

whereas the intermediates derived from compounds **II** and **III** show a half-life of 195 and 4.5 min, respectively, at pH 7.4 and 37 °C, that derived from compound **VI** has a half-life of 45 days (Table 2). This is in accordance with previous studies showing that *N*-hydroxyalkylated amides derived from benzaldehyde, acetaldehyde, chloral or glyoxylic acid derivatives are much more unstable than those derived from formaldehyde (Bundgaard and Johansen, 1984; Bundgaard and Buur, 1987).

Hydrolysis in human plasma

The susceptibility of the 5-oxazolidinones to undergo enzymatic hydrolysis was studied in 80% human plasma (pH 7.4) at 37 °C. Under these conditions strict first-order kinetics was observed. As it appears from the rate data obtained (Table 3) plasma enzymes catalyze the rate of hydrolysis considerably. As revealed by HPLC analysis the enzymatic catalysis was restricted to the hydrolytic opening of the lactone ring. The rate of decomposition of the intermediate *N*-hydroxyalkyl derivatives was not affected by the presence of plasma which is similar to the behaviour of other *N*-hydroxyalkyl compounds (Bundgaard and Johansen, 1984; Bundgaard and Buur, 1987).

Conclusion

The results obtained suggest that 5-oxazolidinones can serve as potential prodrug forms for the α-amido carboxy moiety occurring in most peptides. The derivatives studied are hydrolyzed quantitatively into the parent *N*-acylated amino acid in aqueous solution and in plasma solutions at rates dependent on the 2- and 4-substituents in the oxazolidinone as well as on the stability of the initial product of hydrolysis, the corresponding *N*-hydroxyalkyl derivative. The stability of the latter can be controlled by selection of appropriate aldehydes or ketones for the oxazolidinone formation.

Due to masking of the carboxylic acid function in the acylated amino acids the 5-oxazolidinones are considerably more lipophilic than the parent compounds at physiological pH and this may result in an increased ability to penetrate biomembranes. The same could possibly be achieved by simple C-terminal esterification of peptides but by involving the α-amido group in the derivatization it might also be possible to protect this peptide bond against cleavage by peptidases and proteolytic enzymes at absorption sites, although this remains to be studied. Thus, it has previously been shown that conversion of the C-terminal carboxylic acid function in Met-enkephalin into a CH_2OH-group stabilizes this pentapeptide to proteolysis (Roemer et al., 1977; Roemer and Pless, 1979).

The intermediately formed *N*-hydroxyalkyl derivatives should also be considered as prodrug forms having the potential to afford protection of the amide bond against proteolytic cleavage. *N*-Alkylation of peptide bonds usually make them resistant to enzymatic attack (Farmer, 1980; Veber and Freidinger, 1985; Thaisrivongs et al., 1986) but whereas *N*-methyl and similar alkyl derivatives are not readily bioreversible, the *N*-hydroxyalkyl compounds are spontaneously converted to the parent amide at physiological pH, the rate of conversion being dependent on the nature of the alkyl group. As shown above half-lives ranging from a few minutes to several days at pH 7.4 can be obtained. Although *N*-hydroxyalkylation other than *N*-hydroxymethylation of secondary amides such as peptide bonds is not easy or even impossible (Bundgaard and Johansen, 1984), 5-oxazolidinone formation may be a clue to the preparation of such derivatives. Studies are in progress to

prepare *N*-hydroxyalkyl derivatives of small peptides in this way and to investigate the influence of *N*-hydroxyalkylation of peptide bonds on their proteolytic resistance.

References

Ben-Ishai, D., Reaction of acylamino acids with paraformaldehyde. *J. Org. Chem.*, 79 (1957) 5736–5738.

Bundgaard, H., Design of prodrugs: bioreversible derivatives for various functional groups and chemical entities. In H. Bundgaard (Ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 1–92.

Bundgaard, H., Bioreversible derivatization of peptides. In S.S. Davis, L. Illum and E. Tomlinson (Eds.), *Delivery Systems for Peptide Drugs*, Plenum, New York, 1986, pp. 49–68.

Bundgaard, H. and Buur, A., Prodrugs as drug delivery systems. 65. Hydrolysis of α-hydroxy- and α-acyloxy-*N*-benzoylglycine derivatives and implications for the design of prodrugs of NH-acidic compounds. *Int. J. Pharm.*, 37 (1987) 185–194.

Bundgaard, H., Buur, A., Hansen, K.T., Larsen, J.D., Møss, J. and Olsen, L., Prodrugs as drug delivery systems. 77. Phthalidyl derivatives as prodrug forms for amides, sulfonamides, carbamates and other NH-acidic compounds. *Int. J Pharm.*, (1988) in press.

Bundgaard, H. and Johansen, M., Hydrolysis of *N*-(α-hydroxybenzyl)benzamide and other *N*-(α-hydroxyalkyl)amide derivatives: implications for the design of *N*-acyloxyalkyl-type prodrugs. *Int. J. Pharm.*, 22 (1984) 45–56.

Bundgaard, H. and Nielsen, N.M., Prodrugs as drug delivery systems. 74. Facile hydrolysis of *N*-(acyloxymethyl) amide derivatives and implications for the design of prodrugs of NH-acidic compounds and of carboxylic acids. *Acta Pharm. Suec.*, 24 (1988) 233–246.

Dane, E., Heiss, R. and Schäfer, H., Peptid-Synthesen unter Verwendung von Chloral. *Angew. Chem.*, 71 (1959) 339.

Dane, E., Heiss, R. and Schäfer, H., Verfahren zur Herstellung von Peptiden. *Ger. Offen.* 1, 079, 648 (1960).

Davidovich, Y.A., Pavlova, L.A., Volkonskii, A.Y. and Rogozhin, S.V., Synthesis of 2-trichloromethyloxazolidin-5-ones by reaction of chloral with *N,O*-trimethylsilyl derivatives of amino acids. *Chem. Heterocycl. Comp.*, 11 (1978) 1194–1195.

Farmer, P.S., Bridging the gap between bioactive peptides and nonpeptides: some perspectives in design. In Ariëns, E.J. (Ed.), *Drug Design, Vol. X*, Academic, 1980, pp. 119–143.

Ferraiolo, B.L. and Benet, L.Z., Peptides and proteins as drugs. *Pharm. Res.*, (1985) 151–156.

Freidinger, R.M., Hinkle, J.S., Perlow, D.S. and Arison, B.H., Synthesis of 9-fluorenylmethyloxycarbonyl-protected *N*-alkyl amino acids by reduction of oxazolidinones. *J. Org. Chem.*, 48 (1983) 77–81.

Harned, H.S. and Hamer, W.J., The ionization constant of water in potassium chloride solution from electromotive forces of cells without liquid junctions. *J. Am. Chem. Soc.*, 53 (1933) 2194–2206.

Humphrey, M.J. and Ringrose, P.S., Peptides and related drugs: a review of their absorption, metabolism and excretion. *Drug Metab. Rev.*, 17 (1986) 283–310.

Itoh, M., Peptides. I. Selective protection of α- or side-chain carboxyl groups of aspartic and glutamic acid. A facile synthesis of β-aspartyl and γ-glutamyl peptides. *Chem. Pharm. Bull.*, 17 (1969) 1679–1686.

Karady, S., Amato, J.S. and Weinstock, L.M., Enantioretentive alkylation of acyclic amino acids. *Tetrahedron Lett.*, 25 (1984) 4337–4340.

Klixbüll, U. and Bundgaard, H., Prodrugs as drug delivery systems. 30. 4-Imidazolidinones as potential bioreversible derivatives for the α-aminoamide moiety in peptides. *Int. J. Pharm.*, 20 (1984) 273–284.

Laspéras, M., Pascal, R., Taillades, J. and Commeyras, A., Préparation de *N*-alkyloxazolidinones-5 à partir de *N*-alkylaminoamides. Application à la synthese de dipeptides *N*-alkyles. *Nouv. J. Chim.*, 10 (1986) 111–117.

Lee, V.H.L., Peptide and protein drug delivery: opportunities and challenges. *Pharm. Int.*, (1986) 208–212.

Low, G.K.-C. and Duffield, A.M., Negative ion chemical ionization mass spectra of α-amino acid oxazolidinone derivatives. *Biomed. Mass Spectr.*, 12 (1985) 348–354.

Meisenberg, G. and Simmons, W.H., Peptides and the blood–brain barrier. *Life Sci.*, 32 (1983) 2611–2623.

Micheel, F. and Haneke, H., Peptidsynthesen nach dem Oxazolidonverfahren. II. Peptide der L-Glutaminsäure. *Chem. Ber.*, 92 (1959) 309–312.

Micheel, F. and Haneke, H., Weitere peptidsynthesen nach dem oxazolidon-Verfahren. IV. *Chem. Ber.*, 95 (1962) 1009–1015.

Micheel, F. and Meckstroth, W., Peptidsynthesen nach dem Oxazolidon-Verfahren. III. *Chem. Ber.*, 92 (1959) 1675–1679.

Micheel, F. and Thomas, S., Eine neue peptid-Synthese. *Chem. Ber.*, 90 (1957) 2906–2909.

Pascal, R., Laspéras, M., Taillades, J. and Commeyras, A., Hydrolyse des α-aminoamides catalysée par le formaldehyde en milieu neutre ou faiblement basique. Participation nucléophile du groupe voisin hydroxyle au niveau de l'adduit carbinol-amine. Catalyse acido-basique. *Nouv. J. Chim.*, 11 (1987) 235–244.

Polonski, T., Optical activity of lactones and lactames. III. Circular dischroism spectra of 5-oxazolidinones. *Tetrahedron*, 41 (1985) 603–609.

Roemer, D., Buescher, H.H., Hill, R.C., Pless, J., Bauer, W., Cardinaux, F., Closse, A., Hauser, D. and Huguenin, R., A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature (Lond.)* 268 (1977) 547–549.

Roemer, D. and Pless, J., Structure activity relationship of orally active enkephalin analogues as analgesics. *Life Sci.*, 24 (1979) 621–624.

Seebach, D., Boes, M., Naef, R. and Schweizer, W.B., Alkylation of amino acids without loss of the optical activity:

preparation of α-substituted proline derivatives. A case of self-reproduction of chirality. *J. Am. Chem Soc.*, 105 (1983) 5390–5398.

Seebach, D. and Fadel, A., *N,O*-Acetals from pivalaldehyde and amino acids for the α-alkylation with self-reproduction of the center of chirality. Enolates of 3-benzoyl-2-(tert-butyl)-1,3-oxazolidin-5-ones. *Helv. Chim. Acta*, 68 (1985) 1243–1250.

Thaisrivongs, S., Pals, D.T., Harris, D.W., Kati, W.M. and Turner, S.R., Design and synthesis of a potent and specific renin inhibitor with a prolonged duration of action in vivo. *J Med. Chem.*, 29 (1986) 2088–2093.

Veber, D.F. and Freidinger, R.M., The design of metabolically-stable peptide analogs. *Trends Neurosci.*, 8 (1985) 392–396.

Wiedhaup, K., The stability of small peptides in the gastrointestinal tract. In Breimer, D.D. and Speiser, P. (Eds.), *Topics in Pharmaceutical Sciences*, Elsevier, Amsterdam, 1981, pp. 307–324.