Degradation Kinetics of Three Gonadorelin Analogues: Developing a Method for Calculating Epimerization Parameters

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Purpose. To develop a method for calculating epimerisation parameters, find out if the kinetics of the independent reactions can be established, and elucidate primary structure-chemical degradation relationships in the degradation kinetics of three gonadorelin analogues.
Methods. The influences of pH, temperature, and buffer concentration on the degradation of the three gonadorelin analogues buserelin, goserelin, and triptorelin were investigated using RP-HPLC. A method was developed to calculate epimerisation and hydrolysis rate constants independently.
Results. Explicit structure-degradation mechanism relations were found in the degradation of all three compounds. The L-serine residue was found to be involved in both a solvent-catalysed backbone hydrolysis and a hydroxyl-catalysed epimerisation whereas, the O-tertiary butyl D-serine residue was only involved in proton-catalysed ether hydrolysis. The kinetics of identical reactions in different analogues were generally comparable.
Conclusions. The degradation of the gonadorelin analogues is located at a relatively small number of chemical residues and prediction of the degradation mechanisms and kinetics of other peptides with similar structural elements appears to be possible.

KEY WORDS: buserelin; goserelin; triptorelin; peptides; degradation; epimerization.

INTRODUCTION

Knowledge is limited about chemical degradation of peptides and its kinetics of. Most information has been obtained from studies of oligopeptides and these studies generally have three restrictions. First, the identities of the degradation products are determined in the minimum of the pH-log(k_{obs}) profile, in which proton-, solvent-, and hydroxyl-catalysed mechanisms, may all contribute in a similar extent to the total degradation (1,2). Consequently, it is not possible to distinguish the products resulting from the various types of mechanisms.

Second, if investigated, the measured degradation kinetics are often the result of a number of parallel reactions. Separate determination of the kinetics for each reaction is, in most cases, not performed. As a result, the reaction kinetics of each individual mechanism remain unknown. Third, in general, only one peptide is investigated. Because no structural analogues are included in the study, structure-reactivity relationships cannot be established.

These restrictions result in information that is too limited to be useful for the prediction of the reactivity of other peptides with a similar structure. This is a lost opportunity since peptides are composed of a set of standard elements (amino acid residues). The possibilities of applying structure-properties relationships are extensive, as demonstrated in the field of pharmacology (3). If information about the reactivity is acquired in a systematic manner it may be possible to create a library with kinetic data that can be applied to predict the reactivity of a new peptide.

In this study, the kinetics of the degradation of three gonadorelin analogues, buserelin, goserelin, and triptorelin, have been investigated systematically. The degradation products of these gonadorelin analogues have been determined at varying conditions in a previous study (4). Interestingly, epimerisation was found to be an important reaction for the degradation of all analogues, as is the case for a significant number of 'conventional' medicinal compounds (5). Our objectives here are to develop a method for calculating epimerisation parameters, find out if the kinetics of the independent reactions can be established, and, finally, elucidate primary structure-degradation relationships in the degradation kinetics of the three analogues.

MATERIALS AND METHODS

Chemicals

Buserelin acetate was obtained from Hoechst-Marion-Roussel BV (Hoevelaken, The Netherlands), goserelin acetate from Zeneca Pharma BV (Ridderkerk, The Netherlands), and triptorelin acetate from Ferring (Hoofddorp, The Netherlands). All other chemicals used were of analytical grade and deionised water was applied throughout the study.

RP-HPLC System

A Gynkotech Model 300 precision pump (Separations, H.I. Ambacht, The Netherlands) was connected to a ISS 100 sampling system (Perkin-Elmer Corporation, Norwalk, Connecticut, USA). For the analysis of all three LH-RH analogues, a mobile phase consisting of 0.1% trifluoroacetic acid (TFA) (v/w) in a mixture of 22% acetonitrile and 78% water (w/w) was used. The column was a Lichrospher 100 RP-18 (5 μm) 125 × 4 mm i.d. (Merck, Darmstadt, Germany). Detection was performed using an Applied Biosystems 785A programmable absorbance detector (Separations, H.I. Ambacht, The Netherlands) operating at 214 nm and 20 μl sample was injected.

pH Measurement

All pH measurements were performed on a Consort P514 meter (Salm & Kipp, Breukelen, The Netherlands) equipped with a Slim-trode electrode (Hamilton, Darmstadt, Germany). The pH was measured before and after degradation. All pH values were determined at the appropriate degradation temperature.

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Standard Degradation Conditions

Degradation experiments were performed in flame-sealed 1 ml glass ampoules, kept in a thermostated water bath at the appropriate temperature. Sampling of the ampoules was done over a range of 2 estimated half lives. For each degradation curve 10 measurement points were used and all experiments were performed in duplicate. Standard degradation conditions were 70°C, a gonadorelin analogue acetate salt concentration of 10 μg/ml, an ionic strength of 0.1, adjusted with sodium perchlorate, and a buffer concentration of 25 mM. Buffers used were perchloric acid below pH 2.5, acetate in the pH range 3.5–6, phosphate in the pH range 6–7.5, borate in the pH range 7.5–9, carbonate in the pH range 9–10, and sodium hydroxide above pH 10. All experiments were performed under standard degradation conditions, unless otherwise noted.

Calculation of k_{obs}, k_i, k_h, and K_e

The overall degradation rate constant k_{obs} was provided by the slope of plots of the natural logarithm of the residual parent concentration against the degradation time. The intermediate formation rate constant k_i and the equilibrium constant K_e were calculated from plots of the residual analogue concentration divided by the sum of the analogue concentration and the epimer concentration, against the degradation time, which was fitted with equation (1) using an in-house developed non-linear least square fitting computer program:

$$[L] = p_L + (1 - p_L) \cdot e^{-k_i t} \quad (1)$$

in which [L] is the parent concentration ranging from 0–1 (0–100%), p_L is the fraction of the L configuration resulting from the reaction of the intermediate with water, and t represents the reaction time. K_e is calculated using the equation:

$$K_e = \frac{(1 - p_L)}{p_L} \quad (2)$$

The hydrolysis rate constant k_h was calculated by the slope of plots of the natural logarithm of the sum of the analogue and epimer concentration against the degradation time.

Standard Deviation in the Degradation Parameters

The standard deviations in the rate constants were determined at pH 2 and 8.3.

Influence of Buffers on k_{obs}, k_i, and k_h

The influences of acetate, phosphate, borate and carbonate on k_{obs}, k_i, and k_h were determined. The experiments with triptorelin and phosphate buffers were performed at 80°C and $\mu = 0.4$, all other experiments under standard conditions.

Influence of the pH on k_{obs}, k_i, k_h, and K_e

The influence of the pH on the k_{obs} of buserelin, goserelin and triptorelin was determined in the pH range 1–12 . The influence of the pH on k_i, k_h, and K_e of buserelin and triptorelin was measured in the pH range 5.8–11.3.

Influence of the Temperature on k_{obs}, k_i, k_h, and K_e

The influence of the temperature on k_{obs} of buserelin, goserelin and triptorelin was determined at pH 2, 4.9 and 8.3. The influence of the temperature on k_i, k_h, and K_e was measured at pH 8.3. Data were fitted with the Arrhenius equation:

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \quad (3)$$

in which A represents the frequency factor, E_a is the energy of activation, R is the gas constant, and T is the absolute temperature.

RESULTS AND DISCUSSION

The chemical structures of buserelin, goserelin, and triptorelin are shown in Figure 1. For the kinetic analysis RP-HPLC systems were developed. In the RP-HPLC systems trifluoroacetic acid was used for ion pairing formation and to maintain a low pH. Tbe stability-indicating properties of the RP-HPLC systems have to be evaluated for all degradation reactions, since any product might co-elute with the parent compounds. Useful information can be obtained from liquid chromatography-mass spectrometrical (LC-MS) characterisation of degraded samples, studied earlier (4), because the earlier applied LC system closely resembles the kinetic RP-HPLC systems used here. Differences are restricted to a smaller column diameter and the acetonitrile percentage. It is to be expected that the chromatographic behaviour of the LC-MS and RP-HPLC systems are similar.

Analysis of the LC-MS data indicates no degradation products co-elute with the parent compounds. However, the MS detector cannot discriminate between the parent and its epimers because of their identical mass to charge (m/z) ratio. Epimers mainly arise through hydroxyl-catalysed mechanisms and therefore, are likely to occur at higher pH values. The LC-MS and RP-HPLC systems were both found to be able to separate parent and D-serine[4] epimer compounds.

Calibration curves of buserelin, goserelin, and triptorelin in the concentration range 5–25 μg/ml are linear with correlation coefficients (R^2) > 0.99.

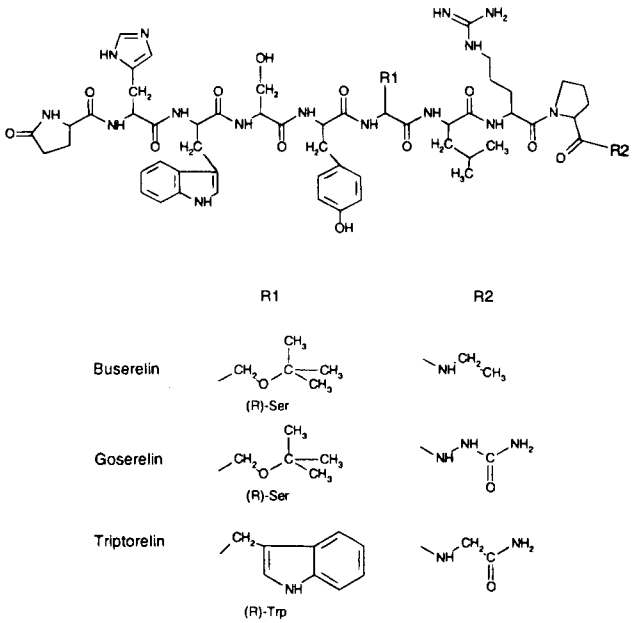

Fig. 1. Chemical structures of buserelin, goserelin and triptorelin.

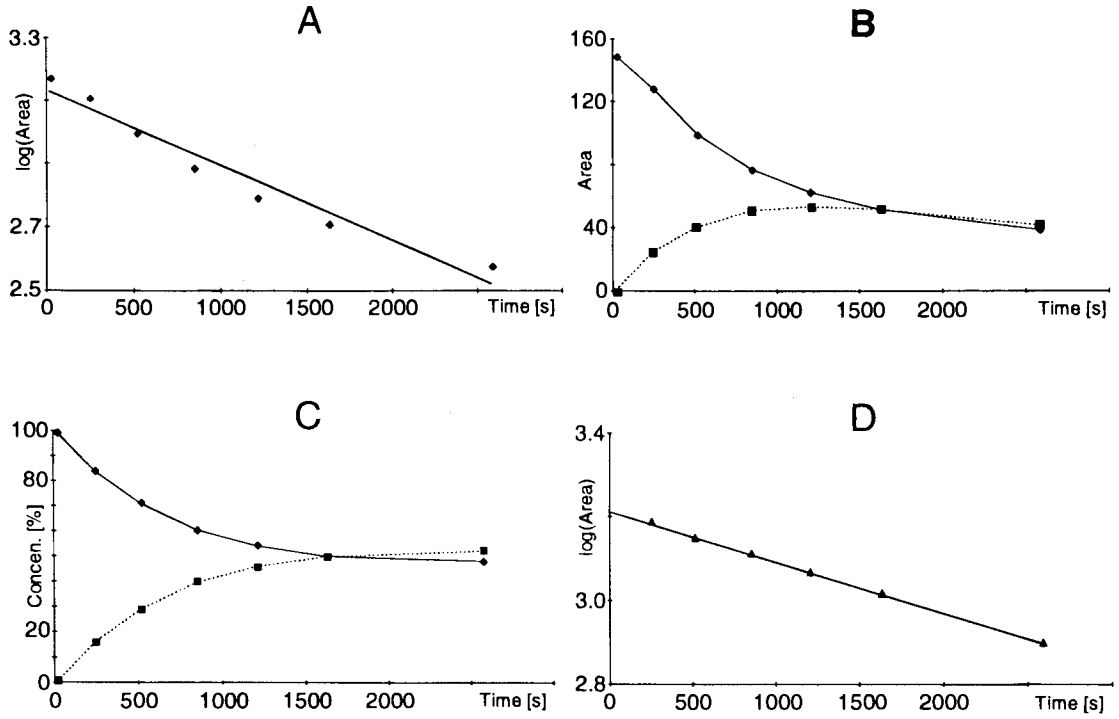

Fig. 2. Degradation plots of triptorelin at pH 10 and 70°C. The x-axis represents the degradation time in all 4 subfigures. In(A), the logarithmic value of the peak area of triptorelin is put on the y-axis fitted with linear regression. In B and C, the concentration of triptorelin (—◆—) and its D-serine[4] (-- ■ --)epimer are plotted in peak area, respectively percentage of the sum of triptorelin and epimer. In D, the logarithm of the sum of the peak area of triptorelin and its epimer is set on the y-axis, fitted with linear regression.

Degradation below pH 6 obeys (pseudo) first-order kinetics, but above pH 6 deviations from first-order kinetics were found. Buserelin and triptorelin especially, gave non-linear results, as demonstrated for triptorelin in Figure 2A. The main degradation product of the three analogues was found to be the D-serine[4] epimer. Since epimerisations in general do not obey first-order kinetics (6) the kinetics of epimerisation were analysed and a method was developed for calculating epimerisation and hydrolysis parameters from parallel epimerisation and hydrolysis reactions.

Degradation Products of Gonadorelin Analogues

There are three types of mechanisms involved in the degradation of the gonadorelin analogues (4). The degradation profile can be divided into three sections; in each section one type of mechanisms dominates. In Figure 3 three chromatograms of degraded buserelin samples are presented at different pH values

In the pH range 1–3 (Figure 3A), the proton-catalysed mechanisms dominate. Buserelin and goserelin degrade mainly through debutylation of the tertiary butylated D-serine[6] residue (4) while triptorelin undergoes mainly deamidation. This last mentioned mechanism is identical to that of gonadorelin (7).

At pH 4–6 (Figure 3B) solvent-catalysed mechanisms are dominating with a remarkable rate constant difference between buserelin and triptorelin, on one hand; and on the other hand, goserelin. Buserelin and triptorelin were found to undergo hydrolysis of the backbone on the N-terminal side of the serine[4] residue (4), as was also found for gonadorelin (7). In goserelin

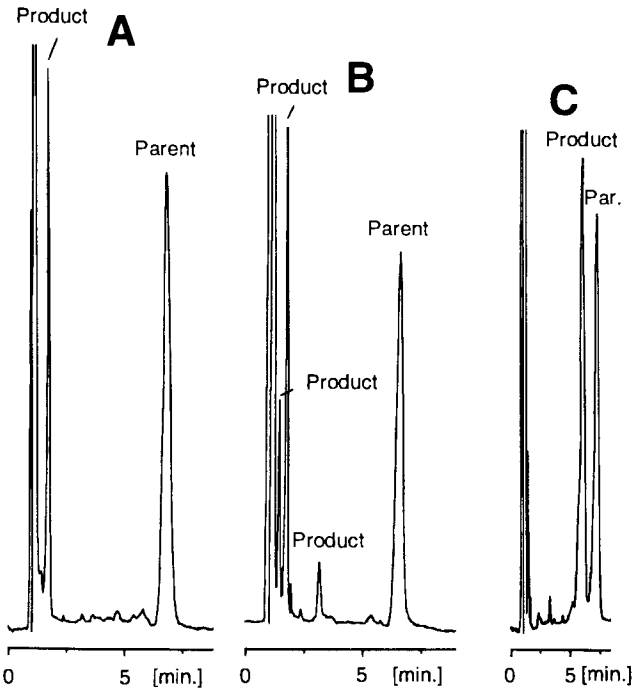

Fig. 3. Chromatograms of the degradation samples of buserelin at (A) pH 2, (B) pH 5 and (C) pH 9.

the C-terminal azaglycinamido group is the center of reaction at pH 4–6 (4).

At pH 9 (Figure 3C) serine epimerisation is the most important degradation reaction of all four analogues. In goserelin there is also some azaglycinamido degradation and triptorelin was found to deamidate (4).

Mathematics of the Kinetics of Epimerization

Equation (4) is the description of the epimerisation process using the conventional forward-and-reverse theory (6):

$$[L] = \frac{k_r}{k_f + k_r} + \frac{k_f}{k_f + k_r} e^{-(k_f + k_i)t} \quad (4)$$

in which k_f and k_r represent the rate constants of the forward and reverse reaction. However, since amino acid residue epimerisation involves the formation of a non-chiral intermediate, this equation does not describe fully the process.

The process of epimerisation of peptides can be described as follows:

$$L \underset{H_2O}{\overset{OH^-}{\rightleftarrows}} I \underset{OH}{\overset{H_2O}{\rightleftarrows}} D$$

in which L and D represent the L and D configuration, and I the non-chiral carbanion intermediate. The intermediate formation in water by abstraction of the α-hydrogen with OH^- is likely to be the rate-determining step whereas, the reaction of the intermediate with a water molecule is likely to be relatively fast.

When the reaction rate is determined by the stability of the intermediate, the formation rate constants of I from L and D are identical because both sources form the same intermediate. As a result the rate at which [L] decreases is given by equation (5):

$$-\frac{d[L]}{dt} = P_D \cdot k_i \cdot [L] - p_L \cdot k_i \cdot [D] \quad (5)$$

in which [L] and [D] are concentrations ranging from 0–1 (0–100%), k_i is the rate constant of the formation of the intermediate from both the L and the D configuration, p_L and p_D are the fractions of the L and the D configuration, respectively, resulting from the reaction of the intermediate with water, and t represents the reaction time in seconds. Since the sum of p_L and p_D is defined as 1, $(1\text{-}p_L)$ can be substituted for p_D. Similarly, the sum of [L] and [D] is equal to 1, so (1-[L]) is substituted for [D]. After simplification, this results in:

$$\frac{d[L]}{dt} = k_i \cdot (p_L - [L]) \quad (6)$$

Through rearrangement an integral can be formed:

$$\int_1^{[L]} \frac{d[L]}{p_L - [L]} = k_i \int_0^t dt \quad (7)$$

As stated before, intergration yields equation (1):

$$[L] = p_L + (1 - p_L) \cdot e^{-k_i t} \quad (1)$$

This simple equation can be used to fit plots of [L] versus time with non-linear regression. The equilibrium K_e is calculated from p_L using equation (2)

$$K_e = \frac{(1 - p_L)}{p_L} \quad (2)$$

The advantage of giving [L] and [D] in percentages is the hydrolysis of L and D does not influence the epimerisation parameters. If the hydrolysis rates of the L and D configuration are identical the first-order rate constant of the hydrolysis can be calculated from the slope of the semi-logarithmic plot of the sum of [L] and [D] against the degradation time. When the centers of epimerization and hydrolysis are a considerable distance from each other this condition is likely to be met.

An example of a degradation curve of triptorelin analyzed with this method is given in Figure 2B, 2C, and 2D together with the conventional semi-logarithmic plot 2A. The semi-logarithmic plot can not be fitted properly with a straight line. In Figure 2B the curves of triptorelin and its epimer clearly show an equilibrium, but hydrolysis of the two compounds prevent the curves from reaching horizontality. Hydrolysis has been excluded from the plot (Figure 2C) by plotting both the triptorelin and the epimer concentration as percentages of the total (triptorelin + epimer) concentration. The parameters k_i and p_L can now be calculated by fitting the triptorelin curve with equation (1). The rate constant of hydrolysis is provided by the slope of the plot of the logarithm of the sum of the triptorelin and the epimer concentration against the degradation time (Figure 2D).

In Table I an overview of the relative standard deviations (RSDs) determined in the degradation parameters of buserelin, goserelin, and triptorelin are given. All RSDs are acceptable for determination of kinetics with the exception of the RSDs in k_i, k_h and K_e for buserelin and goserelin at pH 8.3. Goserelin is excluded from further epimerisation evaluation because a number of products elute closely to the parent and epimer peak, making accurate area determination of both of these peaks impossible. These extra products are the result of degradation reactions located at the semicarbazido group.

Buserelin is not excluded since the RSD value in k_i is not unacceptably high. However, the K_e and k_h values of buserelin have to be evaluated conservatively. Although the epimerization

Table I. Standard Deviation in k_{obs}, k_i, k_h and K_e

Compound	pH	Average [$\times 10^{-6}$ s^{-1}]	RSD[a]	n
Buserelin	1.9	k_{obs}: 11.0	6.4%	6
Buserelin	8.3	k_{obs}: 4.9	8.2%	6
Buserelin	8.3	k_i: 9.0	10%	6
		k_h: 0.81	27%	
		K_e: 1.27[b]	14%	
Goserelin	2.1	k_{obs}: 8.3	6.1%	8
Goserelin	8.3	k_{obs}: 8.3	5.8%	8
Goserelin	8.3	k_i: 6.8	25%	6
		k_h: 3.9	10%	
		K_e: 20[b]	68%	
Triptorelin	2.0	k_{obs}: 3.8	3.9%	6
Triptorelin	8.3	k_{obs}: 6.6	1.5%	6
Triptorelin	8.3	k_i: 10.0	4.0%	6
		k_h: 2.9	3.4%	
		k_e: 1.28[b]	4.6%	

[a] Relative standard deviation.
[b] Dimensionless.

calculations in general lead to higher RSD values relatively to semi-logarithmic plots, theoretical correctness justifies their use.

Influence of the Buffer on k_{obs}, k_i, and k_h

The influence of buffer ions on the degradation parameters was studied to find out whether kinetic data obtained for the various pH-rate constant (pH-k_x) profiles have to be corrected for buffer influences. Acetate, phosphate, borate, and carbonate buffers were investigated. The results are given as the slope (linearly correlated with k_B), intercept, and correlation coefficient of the linear fit of the buffer concentration versus the degradation rate constant. Buffer ions that are involved in the reaction with the parent compound should give a positive slope and an acceptable correlation coefficient.

Data are presented in Table II. Data sets with correlation coefficients<0.9 or lacking a positive slope were excluded from further study. The combinations of buserelin and carbonate, and triptorelin with any of the three buffers, might involve buffer-induced degradation. No changes in the peak patterns are observed in either of the buffer experiments. It can be concluded that any buffer-induced degradation only promotes already present mechanisms.

Data acquired for the pH-k_x profiles at 25 mM are not corrected because possible deviations caused by buffer ions are small (<20%). Since corrections might introduce new errors in data, it should only be used in case of explicitly expected data improvement.

Influence of the pH on Degradation Parameters

Three different pH-log(k_x) plots were constructed: pH-log(k_{obs}), pH-log(k_i), and pH-log(k_h) plots. All plots were fitted with a model developed by Van der Houwen et al. (8), yielding macro constants M_0–M_4 that are sums of kinetically indistinguishable proton-, solvent-, and hydroxyl-catalysed reaction rate constants of the different ionised species. Examples of pH-log(k_{obs}) and pH-log(k_i) plots fitted with the van der Houwen model (8) are given in Figure 4A and 4B, respectively. There are three residues with ionisable groups in the gonadorelin analogues, namely histidine (pK_{a1}), tyrosine (pK_{a2}), and arginine (pK_{a3}). The influence of the arginine residue on the pH-k_x plots was found to be constant because it is not in the range of the constructed plots with its pK_a value of about 12. The pK_a

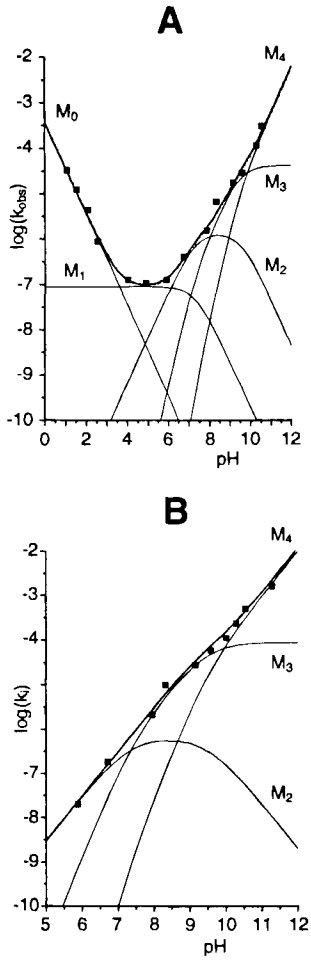

Fig. 4. (A) pH-log(k_{obs}) and (B) pH-log(k_i) profiles of triptorelin.

Table II. Influence of the Buffer Concentration on k_{obs}, k_i, and k_h

Compound	Buffer	pH	Concen. range [mM]	Slope[a]	Intercept[a]	R^{2a}
Buserelin	Acetate	4.9	25–100	-5.8×10^{-10}	1.7×10^{-7}	0.34
Buserelin	Phosphate	6.7	12.5–50	-1.4×10^{-9}	4.2×10^{-7}	0.078
Buserelin	Carbonate	9.2	25–100	k_i: 1.0×10^{-7}	k_i: 1.5×10^{-5}	k_i: 0.98
				k_h: -3.2×10^{-9}	k_h: 2.7×10^{-6}	k_h: 0.063
Goserelin	Acetate	4.4	10–100	-7.2×10^{-11}	5.1×10^{-7}	0.066
Goserelin	Phosphate	6.8	12.5–50	2.2×10^{-9}	8.6×10^{-7}	0.058
Goserelin	Borate	8.5	25–100	2.0×10^{-8}	8.2×10^{-6}	0.83
Goserelin	Carbonate	9.1	25–100	4.6×10^{-8}	2.5×10^{-5}	0.68
Triptorelin	Acetate	4.8	25–100	5.3×10^{-10}	9.0×10^{-8}	0.92
Triptorelin	Phosphate	6.3	50–200	k_i: 2.3×10^{-10}	k_i: 5.9×10^{-8}	k_i: 0.96
				k_h: 9.3×10^{-10}	k_h: 2.6×10^{-7}	k_h: 0.998
Triptorelin	Carbonate	9.1	25–100	k_i: 1.0×10^{-7}	k_i: 1.9×10^{-5}	k_i: 0.98
				k_h: 2.2×10^{-8}	k_h: 1.2×10^{-5}	k_h: 0.063

[a] Parameters obtained from the linear fit of the buffer concentration versus the degradation rate constant.

of the phenolic moiety of tyrosine was spectrofotometrically determined in a gonadorelin study and a value of 9.5 was measured at 70°C. This value was therefore fixed in the fitting procedure. The pK_{a1} of histidine is known to be 7 at 20°C, but its value at 70°C has to be determined through fitting the pH-log k_x profiles.

In Table III the results of the fits are given. The values for pK_{a1} can be obtained from the pH-log(k_{obs}) fits of buserelin, goserelin, and triptorelin, giving a reproducible value of around 7.2; although only a weak inflexion is present in the plots (Figure 4A). The pH ranges of the pH-log(k_i) and pH-log(k_h) profiles are too small to determine pK_{a1}, so, when fitting these profiles, pK_{a1} is fixed with the values found in the pH-log (k_{obs}) plots.

At pH < 4, buserelin and goserelin both undergo proton-catalysed debutylation (4) and, taking into account their structural similarity, their degradation rate constant should be identical. The M_0 values are equal within the experimental error. Under identical conditions triptorelin is involved in a slower proton-catalysed deamidation. Its M_0 value is significantly lower indicating a lower reaction rate constant.

In the horizontal part of the plots between pH 4–6, M_1 determines mainly the degradation rate constant. Buserelin and triptorelin were found to undergo serine-catalysed backbone degradation (4) and, indeed, the conventional fits give identical values. Goserelin undergoes degradation reactions at the azaglycinamido (=semi-carbazido) group, a group not present in the other analogues. The macro constant is considerably higher than for the other two indicating a 5 times higher degradation rate.

Above pH 6, hydroxyl-catalysed mechanisms dominate the degradation of the analogues. Macro constants M_2-M_4 are involved in the reaction rate in this pH area. Three types of profiles were constructed and fitted: pH-log(k_{obs}), pH-log(k_i), and pH-log(k_h). In the pH-log(k_{obs}) plots it can be observed that all three analogues give almost identical M_{2-4}. The three analogues undergo serine epimerisation, but different hydrolysis mechanisms. Apparently the serine epimerisation determines mainly the value of these macro constants. In the pH-log(k_i) plots M_{2-4} are again in the same range and the standard deviation in the macro constants M_{3-4} has decreased significantly. This method therefore gives more accurate results. There is, however, a difference between the epimerisation of buserelin and triptorelin if K_e values are considered, 1.6 ± 0.2 and 1.2 ± 0.2, respectively. The K_e value of buserelin tends to be somewhat higher than that of triptorelin. This may be due to the tertiary butyl serine residue.

For translation of our results to the degradation kinetics of peptides with similar structural elements, the individual micro constants have to be calculated from the macro constants (Table III). Without any restriction, $k^{H^+}_{AH_{2+}}$ can be derived from M_0 since it is the only micro constant in M_0. To calculate other micro constants it has to be assumed that one type of mechanism is dominating, for example, only a solvent or hydroxyl-catalysed mechanism. From M_1, $k^{S}_{AH_3^{2+}}$ values for buserelin and triptorelin of 7.9×10^{-8} s^{-1} and 8.9×10^{-8} s^{-1} can be calculated which represent comparable results for the serine-catalysed backbone cleavage (4). Goserelin gives a value of 4.4×10^{-7} s^{-1} for the sum of the solvent-catalysed azaglycinamido degradation and the serine-catalysed backbone cleavage (4). This value can be corrected for the backbone cleavage with the average of buserelin and triptorelin yielding 3.6×10^{-7} s^{-1} for the azaglycinamido degradation rate constant.

Above pH 6, three micro constants for every reaction must be calculated for each analogue because three ionisation phases are involved: AH_3^{2+}, AH_2^{+} and AH. The values of the micro constants are influenced by the charge of the species in combination with the distance between the site of charge and the center of reaction. The restriction in using the data presented for predicting other peptide degradation rates is the charges and their distances to the center of reaction primarily differ from these gonadorelin analogues. At this moment no general procedure is available to correct for these charge differences, so the values for the micro constants can only be used if the influence of these charges is relatively small.

What can be derived from the pH-k_i plots of buserelin and triptorelin, is removing the positive charge from the histidine residue leads to a 1.2–1.6 times lower micro constant, whereas

Table III. Macro Constants and pK_a Values of the Fitted pH-log(k_x) Plots

Compound Type of fit	log(M_0) $k^{H^+}_{AH_3^{2+}}$	log(M_1) $k^{S}_{AH_3^{2+}}$	log(M_2) $k^{OH^-}_{AH_3^{2+}}$	log(M_3) $k^{OH^-}_{AH}$	log(M_4) $k^{OH^-}_{AH}$	pK_{a1}[a]
Buserelin	−2.99 ± 0.05	−7.1 ± 0.1	−13.3 ± 0.3	−21.2 ± 0.5	−30.8 ± 0.4	7.1 ± 0.4
pH-log(k_{obs})	1.03×10^{-3}	7.9×10^{-8}				
Goserelin	−3.06 ± 0.03	−6.36 ± 0.04	−12.9 ± 0.2	−20.8 ± 0.3	−31.2 ± 0.3	7.3 ± 0.3
pH-log(k_{obs})	8.7×10^{-4}	4.4×10^{-7}				
Triptorelin	−3.44 ± 0.06	−7.05 ± 0.09	−13.2 ± 0.2	−21.2 ± 0.4	−31.1 ± 0.4	7.3 ± 0.4
pH-log(k_{obs})	3.6×10^{-4}	8.9×10^{-8}				
Buserelin	—	—	−13.4 ± 0.2	−20.72 ± 0.05	−30.75 ± 0.05	7.1[b]
pH-log(k_i)			0.26	0.16	0.047	
Triptorelin	—	—	−13.52 ± 0.08	−20.88 ± 0.07	−30.84 ± 0.07	7.3[b]
pH-log(k_i)			0.20	0.17	0.060	
Buserelin	—	—	−13.69 ± 0.07	−21.78 ± 0.04	−32.00 ± 0.04	7.1[b]
pH-log(k_h)				0.014	0.0026	
Triptorelin	—	—	−13.4 ± 0.2	−21.5 ± 0.1	−31.6 ± 0.1	7.3[b]
pH-log(k_h)				0.042	0.011	

[a] pk_a Value of the side chain of the histidine residue.
[b] Fixed.

Table IV. Arrhenius Parameters of k_{obs}, k_i, and k_h

Compound	pH	Temperature range	E_a [kJ/mole]	ln (A)
Buserelin	2.0	60–90°C	122	31.3
Goserelin	2.1	50–80°C	118	29.8
Triptorelin	2.0	60–90°C	92	19.7
Buserelin	4.9	70–90°C	79	11.7
Goserelin	4.9	70–90°C	95	18.8
Triptorelin	4.9	70–90°C	79	11.8
Buserelin	8.3	60–80°C	121	29.9
Goserelin	8.6	25–80°C	110	27.3
Triptorelin	8.3	60–80°C	106	25.1
Buserelin	8.3	60–80°C	k_i: 115	k_i: 28.8
			k_h: 135	k_h: 33.2
Triptorelin	8.3	60–80°C	k_i: 114	k_i: 28.4
			k_h: 102	k_h: 23.1

negative ionisation of the tyrosine residue results in a 2.8–3.4 times lower micro constant. This difference in influence on the epimerisation rate of the serine residue nicely correlates with the distance between the serine residue on one hand, and histidine and tyrosine on the other; namely 1 and 0 residues, respectively. The changes in micro constants due to ionisation is indeed correlated to the center of charge and the center of reaction.

If the values of the epimerisation rate constants $k_{AH_2}^{OH^-}$ and $k_{AH}^{OH^-}$ for buserelin and triptorelin are compared, it is clear that structural differences do not necessarily lead to (important) kinetic differences. The values of $k_{AH_3^+}^{OH^-}$ are not equal, but the error in M_2 is relatively large since only two data points determine this value.

Influence of the Temperature on the Degradation Parameters

The energies of activation and the natural logarithm of the Arrhenius constant of the degradation of the three compounds were determined at pH 2, 5, and 8.5 (Table IV). If the degradation mechanisms are identical for different compounds, both the energies of activation (E_a) as well as the natural logarithm of the Arrhenius constants (ln(A)) should be equal. At pH 2 the degradation mechanism of buserelin and goserelin is identical, and indeed, the E_a and ln(A) are equal within the experimental error. Triptorelin degrades differently at pH 2 with a considerably lower E_a and ln(A). At pH 5, buserelin and triptorelin degrade similarly, giving almost identical E_a and ln(A) values. A good example of the usefulness of the epimerisation calculation method is given by the Arrhenius plots at pH 8.3. When the k_{obs} is fitted with the Arrhenius equation, all E_a and ln(A) values are in the same order of magnitude. Theoretically it is not possible to construct an Arrhenius plot using k_{obs} because k_{obs} is the sum of an epimerisation and a hydrolysis reaction with different energies of activation.

When k_i is used for the degradation of buserelin and triptorelin, E_a and ln(A) are almost equal, giving the Arrhenius parameters for the serine epimerisation. The hydrolysis mechanisms are fundamentally different, resulting in different E_a and ln(A) values.

No correlation of the temperature and the K_e of buserelin and triptorelin was found. The RSD in the determination of K_e is probably higher than the changes caused by the temperature.

CONCLUSIONS

Our main conclusion is the degradation of gonadorelin analogues is located at a relatively small number of chemical residues. Prediction of the kinetics of other oligopeptides with similar structural elements appears to be possible, however not all mathematical methods have yet been developed. The presented mathematical method for calculation of epimerisation parameters is especially useful if, parallel to the epimerisation, hydrolysis reactions occur. Under these conditions both epimerisation and hydrolysis constants can be calculated independently.

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