CHIMIA 52 (1998) Nr. 12 (Dezember)

NOTES

Chimia 52 (1998) 734–739

ISSN 0009–4293

Biological and Pharmacokinetic Studies with β-Peptides

Dieter Seebach[a]*, **Stefan Abele**[a][1], **Jürg V. Schreiber**[a][1], **Bruno Martinoni**[a]), **Alexander K. Nussbaum**[b]), **Hansjörg Schild**[b]), **Henk Schulz**[c]), **Hauke Hennecke**[c]), **Ralph Woessner**[d]), and **Francis Bitsch**[d])

Abstract. Interactions and cleavage reactions of β-amino acids and β-oligopeptides (up to nine residues, carrying the side chains of Ala, Val, Leu, Ile, Phe, Ser, Lys, and Hop) with biological systems, such as the most potent peptidases (pronase, proteinase K, 20S proteasome), microorganisms (*Pseudomonas aeruginosa* and *Pseudomonas putida*), and mammalian blood (intravenous application to rats) have been investigated and compared with α-peptides. The results are: *i*) the three peptidases do not cleave β-peptides at all (within 24 h), and they are not inhibited by a β-peptide; *ii*) except for certain 3-aminobutanoic-acid (β-HAla) derivatives, neither free, nor *N*-acetyl-β-amino acids, nor β-peptides (offered as sole *N* and *C* source) lead to growth of the two bacteria tested; *iii*) two water-soluble β-heptapeptides (with Lys side chains) were shown to have elimination half-lives $t_{1/2}(\beta)$ of 3 and 10 h at 100- and 30-ng/ml levels, respectively, in the rodent blood – *much* larger than those of α-peptides. Thus, the preliminary results described here confirm the much greater stability of β-peptides, as compared to α-peptides, towards metabolization processes, but they also suggest that there may be interactions (by hitherto unknown mechanisms) between the worlds of α- and β-peptides.

1. Introduction

Many β-amino acids have been identified as building blocks in peptides and antibiotics [2][3] (from plants and microorganisms [4–6]), few have been found in mammals [7]. Their metabolism has been examined in some cases [7][8]. Early studies by *Abderhalden* [9][10] suggested that peptide bonds involving β-amino acids are resistant to enzymatic hydrolysis. Thus, certain β-amino acids have been incorporated into naturally occurring peptides of important pharmacological properties [11–13].

Recently, surprisingly stable secondary structures of β-peptides (oligomers of β-amino acids) have been disclosed [14–20]. Potential applications of β-peptides as physiologically active agents will be favored by their resistance to enzymatic degradation. So far, we have studied the stability of β-peptides towards a variety of peptidases [21]. In the present study, we have subjected several water-soluble β-amino acids and β-peptides (*Fig. 1*, **1–23**) [22][23] to systems of increasing complexity: digestion with the most potent peptidases, bacterial growth on media of β-amino-acid derivatives, and *in vivo* metabolic behavior. The outcome of these experiments is relevant to the question as to whether the β-peptidic world is 'orthogonal' to the α-peptidic one [14].

2. Enzymatic Degradation Studies

We selected the β-hepta- (**14–19**) and β-nonapeptides (**20**) and the two α-peptides **22**, **23** (eight and 21 residues, respectively) [24] for an investigation with strong peptidases and the 20S proteasome. With the exception of **15** [19], the syntheses of these compounds have been described previously [22b][23][25]. The β-peptides **14–20** contain the β-amino-acid building blocks (*R*)- and (*S*)-β^3-HAla, (*R*)- and (*S*)-β^3-HPhe, (*S*)-β^3-HLys, (*S*)-β^3-HVal, (*S*)-β^3-HSer, (*S*)-β^3-HLeu, (*S*)-β^3-HHop, and (*S*)-β^2-HHop (for β-amino-acid abbreviations, see [14][17][23][25]).

The two commercially available peptidases pronase and proteinase K and the 20S proteasome were employed for the enzymatic degradation experiments. Pronase is a mixture of several endo- and exopeptidases. Proteinase K is an endoserinase. The 20S proteasome (isolated from human erythrocytes [26]) is an N-terminal threonine protease. Pronase and proteinase K were used in specific concentration (0.02 U/ml), such that α-peptide **23** is nearly totally degraded after 15 min. The 20S proteasome was used with a standard concentration of 7 ng/ml (in the presence of 0.01% sodium dodecyl sulfate [27]).

Solutions containing one of the peptides **14–23** in buffer were treated with the peptidases and with the 20S proteasome. For inhibition studies, solutions containing the α-peptide **23** and the β-peptide **14** were subjected to the peptide-cleaving

*Correspondence: Prof. Dr. D. Seebach[a])
[a]) Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule ETH-Zentrum Universitätstrasse 16 CH-8092 Zürich Tel.: +41 1 632 29 90 Fax: +41 1 632 11 44 E-Mail: seebach@org.chem.ethz.ch
[b]) Interfakultäres Institut für Zellbiologie der Universität Tübingen Abteilung für Immunologie Auf der Morgenstelle 15 D-72076 Tübingen
[c]) Institut für Mikrobiologie der Eidgenössischen Technischen Hochschule ETH-Zentrum Schmelzbergstrasse 7 CH-8092 Zürich
[d]) *Novartis Pharma AG* Drug Metabolism & Pharmacokinetics Compound & Formulation Selection Support CH-4002 Basel

CHIMIA 52 (1998) Nr. 12 (Dezember)

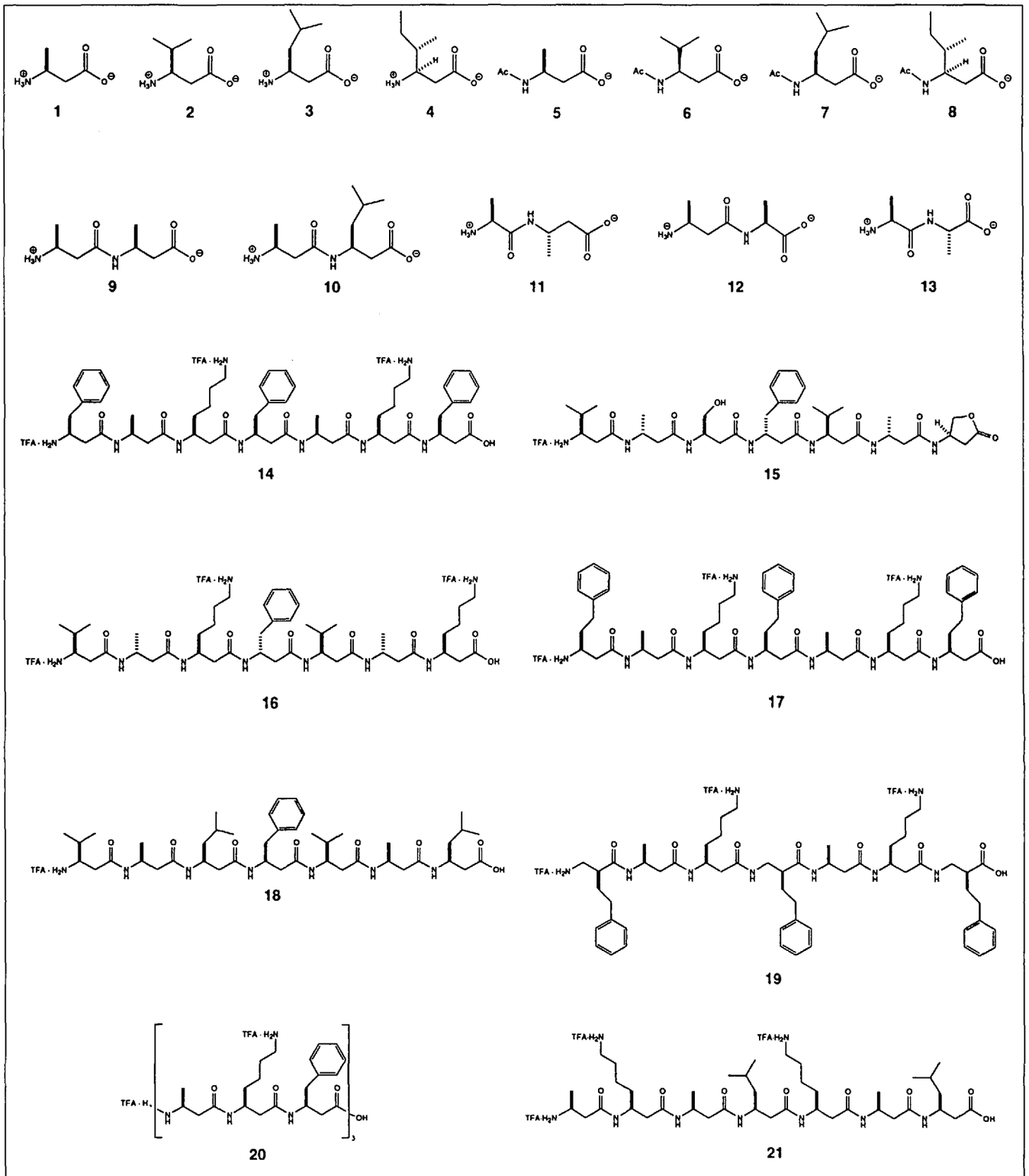

Fig. 1. *β-Amino acids and peptides used in the present investigation* (TFA = trifluoroacetic acid)

CHIMIA 52 (1998) Nr. 12 (Dezember)

enzymes. After various periods of time, aliquots were taken and analyzed by reversed-phase HPLC and MALDI mass spectrometry.

The result is unambiguous: None of the β-peptides **14–20** was digested, and the enzymes were not inhibited by β-peptide **14**. The β-peptides were stable for at least 23 h against the peptidases and for 62 h against the 20S proteasome, whereas the α-peptides **23** and **22** were digested within 15 min and 7 h, respectively. In *Fig. 2*, typical HPLC profiles before and after incubation with the 20S proteasome are shown.

3. Bacterial Growth Experiments

To test the ability of certain microorganisms to metabolize β-amino acids and to cleave β-peptides, we have done first experiments with *Pseudomonas aeruginosa* and *Pseudomonas putida*: free β-amino acids (**1–4**), *N*-acetyl-β-amino acids (**5–8**), β-dipeptides (**9**, **10**), 'mixed' dipeptides (*i.e.*, dipeptides consisting of a β- and an α-amino acid, **11** and **12**) [28], and, for comparison, an α-dipeptide (**13**) were offered as sole carbon and nitrogen source.

The amino acids and peptides were dissolved in buffer solution with $MgSO_4$, $CaCl_2$, and trace elements. The antibiotics spectinomycin and chloramphenicol were added to *P. aeruginosa* and *P. putida*, respectively. The bacteria were first grown on so-called full media (*Luria Bertani* media, LB media [32]). Samples of the cultures were washed with buffer solution and added to the media containing the β-amino acids **1–8** or dipeptides **9–13**. With one exception (**1**), the bacteria grew neither on the β-amino acids **2–4**, nor on the *N*-protected β-amino acids **5–8**, nor on the β-dipeptide **10**. As expected, *P. aeruginosa* and *P. putida* grew on the α-dipeptide **13**. At least in some cases, growth was observed on the media containing dipeptides **9**, **11**, and **12** (with a β-HAla component!) after the first incubation or after a second incubation with a small amount of a grown culture (for details see *Exper. Part*). In *Fig. 3*, the growth curves *of P. aeruginosa* and *P. putida* are presented; the curve obtained with *P. aeruginosa* on the 'mixed' dipeptide **11** displays an interesting pattern: It appears that the bacteria cut the dipeptide into two parts, grow on the first one (the α-amino acid ?), adapt to and then grow on the second one [33]. Only little but significant growth was observed with *P. aeruginosa* on the β-dipeptide **9** and with *P. putida* on the dipeptides **9** and **11**.

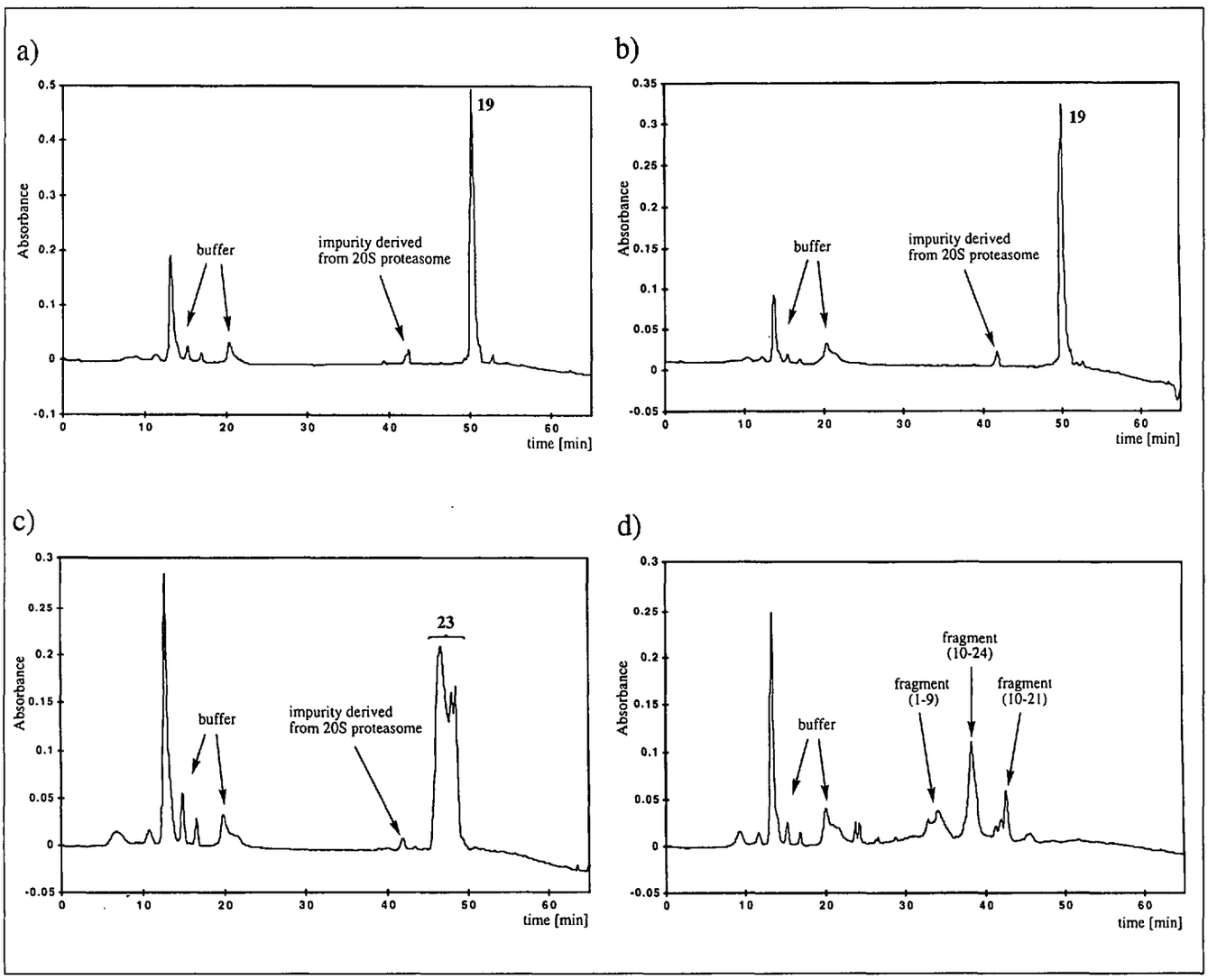

Fig. 2. *Comparison of the HPLC profiles of β-peptide* **19** *and α-peptide* **23** *with the 20S proteasome in buffer soln*: a) *β-peptide* **19** *before incubation*; b) *β-peptide* **19** *after 62 h incubation*; c) *α-peptide* **19** *before incubation*; d) *α-peptide* **23** *after 7 h incubation*. Absorbance was recorded at 214 nm. The α-peptide **23** is totally degraded under the same conditions after only 7 h (for details see *Exper. Part*).

CHIMIA 52 (1998) Nr. 12 (Dezember)

4. Pharmacokinetic Studies

To learn about the *in vivo* stability of β-peptides, compounds **16** and **21** [34] were administered intravenously (*iv.*) to rats, and their concentrations in the blood followed by sampling and HPLC/MS analysis (for experimental details see *Exper. Part*). The elimination half-life values $t_{1/2}(\beta)$ were *ca.* 3 h for **16** and *ca.* 10 h for **21**; after a rapid decrease of peptide concentration in the first hour, the concentration remained almost constant at *ca.* 30 ng/ml and 100 ng/ml, respectively (*Fig. 4*).

As reviewed in the literature [35], determination of peptide metabolic stability *in vivo* is experimentally complex, and the cooperative analysis of half-lives of natural or synthetic peptides in serum, plasma, or blood is further complicated, because experimental methods often differ among investigators. Half-lives of α-peptidic drugs, administered intravenously, lie in the range of minutes [36] (Table 3 in [37]). β-Peptides **16** and **21** thus display a much greater stability towards metabolization processes.

5. Discussion

We have demonstrated that even strong peptidases such as pronase or proteinase K do not cleave β-peptides (*Fig. 2*). However, before β-peptides can be considered as active substances in medicinal chemistry, it was important to explore their interaction with natural enzymes (*Fig. 3*). Arbitrarily, we chose two *pseudomonas* species for the experiments. The next step will be to include other β-peptides and a larger variety of microorganisms in further investigations.

The animal experiments revealed that β-peptides **16** and **21** have an increased *in vivo* metabolic stability, as demonstrated by the elimination half-lives of *ca.* 3 and 10 h, respectively. These two β-peptides differ in their secondary structure: The configurational pattern of β-peptide **16** does not allow for the formation of a 3_1 helix [17][22b], whereas β-peptide **21** has a 3_1-helical structure, according to its CD spectrum [34]. Whether the higher level in blood of β-peptide **16**, as compared to **21**, is caused by the different structures can not be decided at this point. Considering the large variety of β-peptides, there is no way of telling whether the two – water-soluble – β-peptides chosen for the animal experiments are, in any way, characteristic examples. Achievement of metabolic stability of peptides is no guarantee of either oral activity or sustained biological activity, because absorption barriers (*e.g.*, intestinal, nasal, and buccal) and hepatobiliary excretion mechanisms may severely compromise the therapeutic potential of a peptide or peptidomimetic drug, thereby subjecting it to acute or chronic intravenous administration requirements. It will now be important to study the elimination pathways of β-peptides that account for the rapid decrease of their concentration in the first 30 min and to localize the β-peptides in the animals with radioactively labeled samples ('slow-release' effect?).

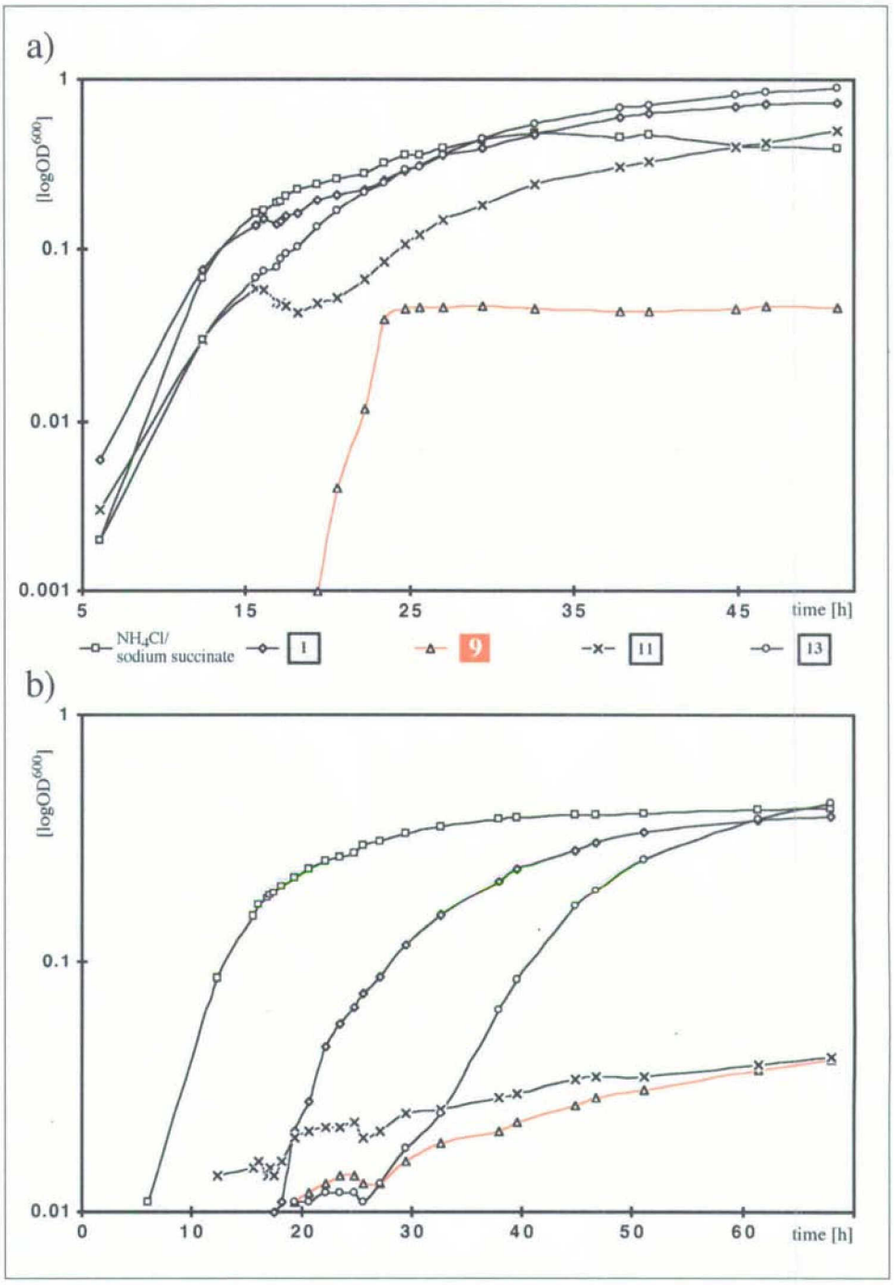

Fig. 3. *Growth curves of* Pseudomonas aeruginosa (*a*) *and* Pseudomonas putida (*b*) *on the indicated substrates as sole carbon and nitrogen source* (OD^{600} = optical density at 600 nm, for details see *Exper. Part*). The growth curve on β-dipeptide **9** is printed in red color.

6. Experimental Part

Degradation Experiments

The peptidase concentrations of the stock solns. were selected such that the α-peptide **23** (same concentration as the tested substrates) was nearly totally degraded after 15 min. The 20S proteasome was used in a standard concentration of 7 ng/μl in TEAD (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM NaN_3, 1 mM DTT).

General Procedure: 10 μl (0.01 μmol) of a stock soln. of the substrate in H_2O (1.0 mM) [38], 10 μl of the stock soln. of the peptidase or 15 μl (2.1 μg) of the stock soln. of the 20S proteasome with 3 μl of a SDS soln. (1% in H_2O) were added to 280 μl (peptidases) or 272 μl (20S proteasome)

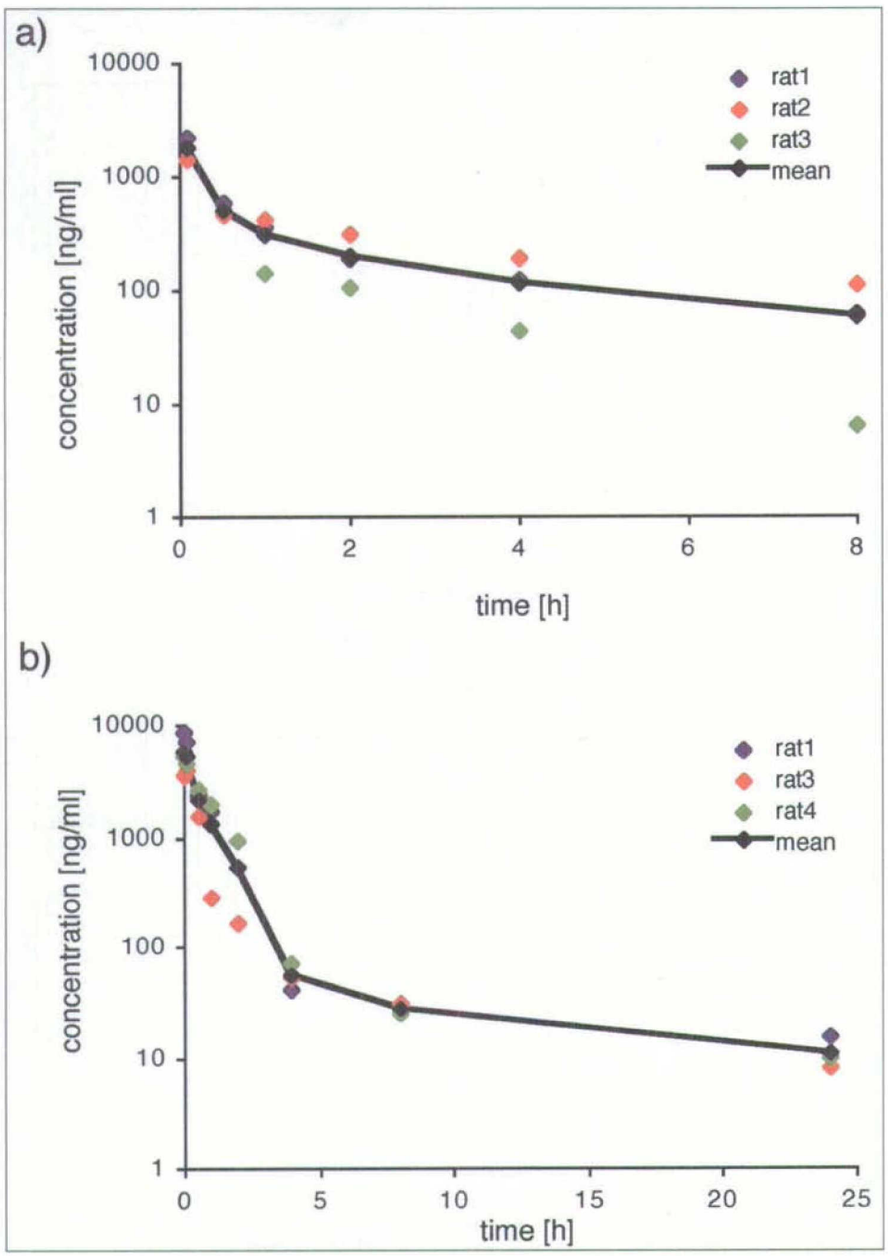

Fig. 4. *Concentrations of β-peptide* **16** *(a) and* **21** *(b) in blood, measured after 4.0-mg/kg* iv. *administration to rats* (for experimental details see *Exper. Part*)

buffer soln. (20 mM hepes/KOH, 2 mM $MgAc_2$, pH 7.6). The mixtures were incubated at 37°. Samples of 100 or 150 µl were taken, stopped with HOAc and analyzed by RP-HPLC and MALDI mass spectrometry.

HPLC-Analysis: For the analysis of degradation products, unfractionated peptide digests were injected onto *µRP SC 2.1/10*-columns (*Pharmacia*), *Microbore* HPLC system (*SMART system, Pharmacia*). Soln. A: 0.1% TFA in H_2O; soln. B: 0.081% TFA, 80% acetonitril in H_2O. Detection by measurement of the UV absorption at 214 nm. Gradients were 0% B for 5 min, in 40 min to 40% B, in 15 min to 75% B and up to 85% in another 5 min. The flow rate for all separations was 150 µl/min.

MALDI mass spectrometry: Matrix-associated laser desorption ionization (MALDI). Routinely, 0.8 µl of DHAP-matrix·(20 mg 2,5-dihydroxy-acetophenon, 5 mg ammonium citrate in 1 ml of 80% propan-2-ol) was mixed with 1 µl of concentrated HPLC-peak fraction on a gold target. Measurements were performed using a LD-TOF (laser desorption/time of flight)-system (*Hewlett-Packard G2025A*) at a vacuum of 10^{-6} Torr. For signal generation *ca.* 50 laser shots were added up in the single-shot mode.

Syntheses of the α-peptides: Peptides were synthesized in an automated peptide synthesizer *432A* (*Applied Biosystems*, Weiterstadt, Germany) following the Fmoc/*t*-Bu strategy and analyzed by *HPLC (system gold, Beckman Instruments*, München, Germany) and MALDI-TOF mass spectrometry (*G2025A, Hewlett-Packard*, Waldbronn, Germany). Peptides showing a purity of less than 80% were purified by preparative HPLC.

Enzymes: Pronase (EC 3.4.24.4) from *Streptomyces griseus* (*Fluka*). Lyophilized powder. Stock soln. in buffer. Proteinase K from *Tritirachium album* (*Boehringer*, Mannheim). Buffer soln.: 10 mM Tris-HCl, pH 7.5, with calcium acetate as stabilizer. Stock soln. in buffer. The 20S proteasome was purified from outdated blood as described in [26].

Bacterial Growth Experiments

The optical density (OD^{600}) was measured at 600 nm on a spectrophotometer *Novaspec II* (*Pharmacia Biotech*). The substrates (12.5 mM) were dissolved in buffer (42 mM Na_2HPO_4, 17 mM KH_2PO_4, 8.6 mM NaCl, pH 7.3) with $MgSO_4$ (1 mM), $CaCl_2$ (0.1 mM), and trace elements. Spectinomycin and chloramphenicol (both antibiotics) were added to *P. aeruginosa* (PAO1S) [39] and *P. putida* (S313, DSM 6884) [40], respectively. The microorganisms were first grown on full media (LB media). Samples of the cultures were taken ($1.5 < OD^{600} < 2.5$) and washed with buffer solution. A small amount of these washed cells (normally 0.4–1.0% of the incubating soln. volumina) were added to the media containing the substrates or to a blank soln. and incubated at 37° (*P. aeruginosa*) and 30° (*P. putida*), respectively. *P. aeruginosa* grew on **1**, **12** (OD^{600} = 0.40), and **13** and *P. putida* grew on **1** and **13**. No growth was observed on the other substrates. The media containing **9–11** and **9–12** were incubated for a second time with a small amount of a grown culture on **12** (*P. aeruginosa*) and on *N*-Ac-Val-OH (*P. putida*), respectively. After the second inoculation, growth was observed on **9** and **11** (*P. aeruginosa*) and on **11** (*P. putida*). To reproduce the growth experiments, a little amount (normally 0.4–1.0% of the incubating soln. volumina) of every grown culture was added to a new media containing the same substrate. This procedure was repeated for a second time.

Bacterial growth curves: The microorganisms were first grown on the substrates. A sample of the grown culture was taken and diluted with dist. H_2O (OD^{600} = 0.01). A small amount (2% of the incubating soln. volumina) of this soln. was added to a media containing the same substrates or to a blank soln. OD^{600} was measured after various periods of time.

Pharmacokinetic Studies

a) *Dosage and Administration*:

Animals: Fasted (20 h) male Wistar rats weighing 240–260 g, surgically prepared by implantation of a permanent cannula into the right femoral artery. Five animals per administration route.

Dosage: For the intravenous reference, a dosage of 4 mg/kg was used, which was injected into the left femoral vein.

Dosage vehicle: The drug substance was dissolved in physiol. saline. The concentration in the intravenous vehicle was 4 mg/ml (administered volume 1 ml/kg).

Exp. Design: After administration, blood (500 ml/time point) was withdrawn from the cannulated femoral vein into heparinized containers at the following time points: 0.083, 0.5, 1, 2, 4, 8, and 24 h. The amount of sampled blood was replaced regularly with fresh blood, prepared from donor rats. The analysis has been done from whole blood samples after extraction. The samples will be stored at –20° until analysis.

b) *Extraction Method, Protein Precipitation*:

30 µl of internal standard (Leu-enkephalin, MW 555.6, 50 µg/ml in H_2O) were added to 300

µl of blood (Vortex 5 s). Protein were precipitated with 200 µl of MeOH (Vortex 30 s, ultrasonication 1 min). Samples were centrifuged at 18 kRPM for 20 min. 300 µl of supernatant were collected. Volume was adjusted to 800 µl with H_2O, 0.2% HOAc (500 µl). 800 µl of sample were loaded on a Trace Enrichment Cartridge (TEC; *Gilson*), packed with *Hypersil* ODS stationary phase (70 mg, 10 µm particle size), and injected onto the HPLC system (see below).

c) *Calibration*:

Samples for calibration (1–2000 ng/ml) were obtained by 10fold dilution of aqueous solutions of β-peptides **16** and **21** in fresh blood.

d) *HPLC Separation* (**16**):

Separation was achieved using a *Dupont Zorbax SB C-18* (2.0 × 150 mm) packed with *C-18* reverse phase and fitted with an Optimize Technologies *C-18* precolumn. Flow was typically 300 µl/min and temperature of the column was kept at 60°. A gradient was run from 2% to 65% acetonitrile in H_2O in 9 min. Under these conditions, **16** and Leu-enkephaline had a retention time of 6.7 min and 7.5 min, respectively. Flow was split 1:3 prior to ionization allowing a flow of approximatively 100 µl/min into the ESI source.

e) *HPLC Separation* (**21**):

Separation was achieved using a *Nucleosil 100-5C18AB* (3.0 × 150 mm) packed with *C-18* reverse phase and fitted with a *Nucleosil 100-5C-18AB* precolumn. Flow was typically 500 µl/min and temperature of the column was kept at 70°. A gradient was run from 2% to 50% acetonitrile in H_2O in 15 minutes. Under these conditions, **21** and Leu-enkephaline had a retention time of 8.5 min and 10 min, respectively. Flow was split 1:5 prior to ionization allowing a flow of approximatively 100 µl/min into the ESI source.

f) *MS Method* (**16**):

Molecules were ionized by electrospray (ESI). Heated capillary temperature was kept at 200°. Under these conditions, **16** mainly yielded the MH_2^{++} and MH_3^{+++} ions (*m/z* 431 and 288, respectively). Leu-enkephalin mainly yielded the MH^+ ion at *m/z* 556. Multiplier was set at 1700 V. CID-MS/MS was performed with Ar pressure of 2.0 mTorr and –16 eV collision energy. Selected Reaction Monitoring (SRM) corresponding to the fragmentation pattern 431 (MH_2^{++}) → 397 (fragment) and 556 → 395 (for the internal standard) was used as detection method (SRM 431 → 397, 556 → 395, span 0.2, 1 s tot.).

Quantitation was based on integration of the peaks corresponding to the ion current from *m/z* 973 (**16**) and 395 (Leu-enkephalin). Ratio between **16** and the internal standard was used for quantitation.

g) *MS Method* (**21**):

Molecules were ionized by electrospray (ESI). Heated capillary temperature was kept at 220°. Under these conditions, **21** mainly yielded the MH_2^{++} and MH_3^{+++} ions (*m/z* 407 and 272, respectively). Leu-enkephalin mainly yielded the MH^+ ion at *m/z* 556. Multiplier was set at 2400 V. CID-MS/MS was performed with Ar pressure of 2.5 mTorr and –10 eV collision energy. Selected Reaction Monitoring (SRM) corresponding to the fragmentation pattern 271 (MH_3^{+++}) → 333 (fragment, 2+) and 556 → 556 (for the internal standard) was used as detection method (SRM 271 → 333, 556 → 556, span 0.3, 1 s tot.).

Quantitation was based on integration of the peaks corresponding to the ion current from *m/z* 333 (**21**) and 556 (Leu-enkephalin). Ratio between **21** and the internal standard was used for quantitation.

We thank Dr. *G. Guichard*, Dr. *S. Stevanovic*, and *D. Rigo* for supplying us with samples of some β- and α-peptides.

Received: October 21, 1998

[1] Part of the projected Ph.D. theses of *S.A.* and *J.V.S*, ETH-Zürich.

[2] C.N. Drey, in 'The Chemistry and Biochemistry of Amino Acids', Ed. B. Weinstein, Dekker, New York, 1976, Chapt. 4, pp. 241–299.

[3] C.N. Drey, in 'The Chemistry and Biochemistry of Amino Acids', Ed. G.C. Barrett, Chapman & Hall, London, 1984, pp. 25–54.

[4] G. Cardillo, C. Tomasini, *Chem. Soc. Rev.* **1996**, 117.

[5] C.A. Bewley, D.J. Faulkner, *Angew. Chem.* **1998**, *110*, 2280.

[6] T. Yasumoto, M. Satake, *Chimia* **1998**, *52*, 63.

[7] O.W. Griffith, *Ann. Rev. Biochem.* **1986**, *55*, 855.

[8] C.H. Chang, M.D. Ballinger, G.H. Reed, P.A. Frey, *Biochemistry* **1996**, *35*, 11084; K.D. Walker, H.G. Floss, *J. Am. Chem. Soc.* **1998**, *120*, 5333.

[9] E. Abderhalden, F. Reich, *Fermentforschung* **1928**, *10*, 173.

[10] E. Abderhalden, R. Fleischmann, *Fermentforschung* **1928**, *10*, 195.

[11] M. Rodriguez, P. Fulcrand, J. Laur, A. Aumelas, J.P. Bali, J. Martinez, *J. Med. Chem.* **1989**, *32*, 522.

[12] M. Llinares, C. Devin, J. Azay, G. Berge, J.A. Fehrentz, J. Martinez, *Eur. J. Med. Chem.* **1997**, *32*, 767.

[13] Y. Hayashi, J. Katada, T. Harada, A. Tachiki, K. Iijima, Y. Takiguchi, M. Muramatsu, H. Miyazaki, T. Asari, T. Okazaki, Y. Sato, E. Yasuda, M. Yano, I. Uno, I. Ojima, *J. Med. Chem.* **1998**, *41*, 2345.

[14] D. Seebach, M. Overhand, F.N.M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.

[15] D. Seebach, P.E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 2043.

[16] D. Seebach, J.L. Matthews, *J. Chem. Soc., Chem. Commun.* **1997**, *21*, 2015.

[17] D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J.L. Matthews, J.V. Schreiber, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1998**, *81*, 932.

[18] a) D.H. Appella, L.A. Christianson, I.L. Karle, D.R. Powell, S.H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071; b) D. H. Appella, L. A. Christianson, D.A. Klein, D.R. Powell, X. Huang, J.J. Barchi, Jr., S.H. Gellman, *Nature (London)* **1997**, *387*, 381.

[19] D. Seebach, S. Abele, hitherto unpublished results, ETH-Zürich, 1997.

[20] S. Krauthäuser, L.A. Christianson, D.R. Powell, S.H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719.

[21] T. Hintermann, D. Seebach, *Chimia* **1997**, *51*, 244.

[22] a) G. Guichard, D. Seebach, *Chimia* **1997**, *51*, 315; b) G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 187.

[23] S. Abele, G. Guichard, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, in press.

[24] For the syntheses of the α-peptides, see *Exper. Part.*

[25] T. Hintermann, D. Seebach, *Synlett* **1997**, 437.

[26] L. Kuehn, B. Dahlmann, F. Gauthier, H.P. Neubauer, *Biochim. Biophys. Acta* **1989**, *991*, 263.

[27] K. Tanaka, T. Yoshimura, A. Ichihara, *J. Biochem. (Tokyo)* **1989**, *106*, 495.

[28] The β-amino acids were synthesized by our standard method (using the *Arndt-Eistert* procedure [14][29][30]) and acetylated with acetic anhydride. The β-peptides **9** and **10** and the 'mixed' peptides **11** and **12** were prepared by coupling in solution [31].

[29] J. Podlech, D. Seebach, *Liebigs Ann.* **1995**, 1217.

[30] J.L. Matthews, C. Braun, C. Guibourdenche, M. Overhand, D. Seebach, in 'Enantioselective Synthesis of β-Amino Acids', Ed. E. Juaristi, Wiley-VCH, Weinheim and New York, 1997, Chapt. 5, pp. 105–126.

[31] D. Seebach, J. Schreiber, hitherto unpublished results, ETH-Zürich, 1998.

[32] J. Sambrook, E.F. Fritsch, T. Maniatis, in 'Molecular cloning: a laboratory manual', 2nd edn., Cold Spring Harbor Laboratory Press, 1989, Cold Spring Harbor NY.

[33] This is reminescent of the enzymatic cleavage experiments [21]: a β-tripeptide with an α-amino acid at the N-terminus was cleaved by leucine aminopeptidase.

[34] D. Seebach, D. Rigo, unpublished results, ETH-Zürich, 1996.

[35] M.F. Powell, *Ann. Rep. Med. Chem.* **1993**, *28*, 285.

[36] Thus, angiotensin II, an eight-residue peptide, has a half-life of less than 1 min (*iv.* administration).

[37] J.-L. Fauchère, C. Thurieau, *Adv. Drug Res.* **1992**, *23*, 127.

[38] A DMSO/H_2O mixture (162:50) was necessary for H_2O-insoluble **18**.

[39] S. Beil, H. Kehrli, P. James, W. Staudenmann, A.M. Cook, T. Leisinger, M.A. Kertesz, *Eur. J. Biochem.* **1995**, *229*, 385.

[40] a) H.-J. Busse, T. El-Banna, G. Auling, *Appl. Environ. Microbiol.* **1989**, *55*, 1578; b) D. Zürrer, A.M. Cook, T. Leisinger, *ibid.* **1987**, *53*, 1459.