Quantitative description of epimerization pathways using the carbodiimide method in the synthesis of peptides

Carola Griehl,* Alfred Kolbe and Susanne Merkel

Department of Chemistry, Martin-Luther-University, Kurt-Mothes-Straße 2, D-06120 Halle (Saale), Germany

The mechanism of epimerization in carbodiimide synthesis has been investigated by varying the side chain of the activated acids. A series of peptides *N*-benzyloxycarbonyl-Ala-Xaa-OH with 20 different residues Xaa were coupled with valine methyl ester in dichloromethane and dimethylformamide. The kinetic data and the extent of epimerization were determined for the peptide synthesis and the aminolysis of isolated oxazol-5(4*H*)-ones by means of IR spectroscopy, polarimetry and reversed-phase HPLC. Combining the results we succeeded for the first time in a quantitative description of the overlapping pathways of epimerization and their dependence on amino acid sequence during carbodiimide synthesis.

Introduction

The synthesis of peptides involves the carboxy activation of *N*-substituted amino acids or peptides, followed by the nucleophilic attack of an amino component. Because peptides are substances with chiral centres, the configuration of the compounds used during the synthesis is important. With the exception of the activation of urethane-protected amino acids, the activated residue of the carboxy component undergoes partial epimerization during the coupling process.[1] In spite of many investigations, until now there has been no success in finding reactions in which epimerization during peptide segment coupling is completely suppressed.

The knowledge of the mechanism of epimerization is a prerequisite to developing effective coupling procedures free of epimerization. Our main interest is directed to the frequently used carbodiimide method. The first step of the carbodiimide reaction proceeds *via* an addition of the carboxy group to the diimide, leading to an unstable *O*-acylisourea. Because of its high reactivity the outcome of the reaction is an unselective one, and the aminolysis forming the peptide is accompanied by the formation of oxazol-5(4*H*)-one, symmetrical anhydride and *N*-acylurea.[1,2] Whereas the intermediate anhydride and oxazolone are able to undergo aminolysis, the *N*-acylurea resulting from rearrangement of *O*-acylisourea does not react with the amino component. The contribution of each intermediate depends on the concentration of substrates, on the nature of the base present in the reaction mixture, on the structure of the carboxy/amino component, as well as on the nature of the solvent and carbodiimide.

The epimerization during carbodiimide synthesis may take place *via* two different overlapping mechanisms. The first one results from enolisation of *O*-acylisourea. According to Bodanszky the epimerization might be caused by the presence of a basic centre in the reactive *O*-acylisourea promoting an intramolecular proton abstraction from the chiral carbon atom *via* a cyclic transition state.[3] The second one results from the formation of a steady-state concentration of oxazolone which epimerizes much more quickly than the ring-opening reaction takes place to give peptide product.[4] Recently, Kolodziejczyk *et al.* described the chiral stability of *N*-acylureas[5] and 2-phenyl-4-benzyloxazol-5(4*H*)-one[6] and concluded that *O*-acylisoureas, contrary to widespread opinion, preserve their chiral integrity before conversion to oxazolones or *N*-acylureas. Systematic mechanistic studies of epimerization, including sequence dependence, to support theoretical investigations are sparse.[7]

It was the aim of our investigations to illuminate the dependence of the mechanism of epimerization during carbodiimide synthesis on variations of the side chains of the activated amino acid.

In particular we have examined the kinetics of the epimer formation in the carbodiimide coupling of 20 different Z-dipeptides with a variety of activated residues, as well as in the oxazolone aminolysis. In this work we succeeded for the first time in a quantitative description of two overlapping pathways of epimerization during carbodiimide synthesis.

Experimental

Materials and instruments

N-Methylmorpholine (NMM), *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimidemetho-*p*-tosylate(CMC)and*N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) were purchased from Fluka (Buchs, Switzerland) and HPLC solvents (methanol and water) from Merck (Darmstadt, Germany). Dichloromethane (DCM) was distilled from anhydrous sodium carbonate and kept over type 4A molecular sieves. Dimethylformamide (DMF) was fractionally distilled before use.

The IR spectra were recorded on a Zeiss (Jena) M 80 IR spectrometer at 22 °C (controlled by a water jacket device). The thickness of the NaCl cells was 0.22 mm. The oxazolone kinetics were measured by recording the increase and decrease of the strong CO absorption band at 1825 cm^{-1} as well as the decrease of the N=C=N absorption of CMC. The optical rotation was measured with a Polartronic D (Schmidt & Haensch) polarimeter at 589 nm and at 22 °C. HPLC experiments were performed with a Merck Hitachi instrument equipped with a L 6200 intelligent pump, a D 2500 integrator and a LiChrograph L-4000 UV detector. The chromatograph was fitted with a 250 mm × 4 mm column packed with 5 μm Lichrospher 100 RP-18 (Merck). Aqueous MeOH was used as mobile phase, the flow rate was 0.8 ml min^{-1} and the detection wavelength was 220 nm. All kinetic measurements were performed starting from equimolar amounts of the reaction partners in DCM (*c* 0.025 mol l^{-1}) at 22 °C. The quantitative evaluation results from calibration of reference substances.

Peptide synthesis

The synthesis of Z-protected dipeptides Z-Ala-Xaa-OH and Z-protected tripeptides Z-Ala-Xaa-Val-OMe (LLL and LDL) is described in earlier work.[8]

Synthesis of oxazol-5(4H)-ones

The oxazol-5(4*H*)-ones of Z-Ala-Xaa-OH were synthesized from Z-Ala-Xaa-OH and EDC or CMC in DCM at 0 °C[9] in yields of 60–90%, except for Z-Ala-Asn-OX, Z-Ala-Gln-OX and Z-Ala-Arg(Tos)-OX (10–15%). Most of the Z-Ala-Xaa-oxazolones are chirally stable for at least several hours in DCM solution. Oxazolones with side chain protected amino acids in Xaa-position like Ser(Bzl), Cys(Bzl), Asp(X), Tyr(Bzl), Lys(Z), Arg(Tos) and Z-Ala-Asn/Gln-oxazolone are optically unstable.

Coupling reactions, determination of epimerization

To a solution or suspension of Z-Ala-Xaa-OH (0.05 mmol) and freshly distilled H-Val-OMe (0.05 mmol) in 2 ml of DCM or DMF, CMC (0.05 mmol) was added. (For the measurements by IR spectroscopy about 0.1 ml was run into the NaCl cell.) The reaction mixture was left for 20 h at 22 °C. From DCM solutions an aliquot was diluted with eluent (*ca.* 10 μl reaction solution in 150–200 μl eluent) and used for direct determination of epimers by RP-HPLC. The DMF solutions were evaporated to dryness, the residue was dissolved in 2 ml of DCM and washed successively with dilute HCl, water, 10% aqueous $NaHCO_3$ and water again. The DCM extracts were dried and freed of solvent. The determination of epimerization during peptide synthesis is based on the separation of LLL/LDL-tripeptide diastereoisomers by RP-HPLC. The optimal chromatographic conditions are given in an earlier paper.[8] The value for epimerization of tripeptide synthesis %LDL was calculated from the formula %LDL = 100 × LDL/LLL + LDL.

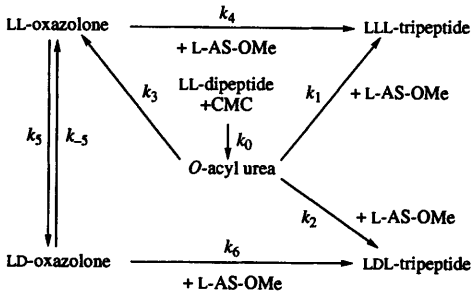
The aminolysis of oxazol-5(4H)-ones were carried out in an analogous manner, starting from Z-Ala-Xaa-oxazolone (0.05 mmol) and Val-OMe (0.05 mmol) in 2 ml of DCM. The degree of epimerization of tripeptides results from the aminolysis of LL-oxazolones and is named $\%LDL^{max}$.

The determination of the epimerization rates of oxazol-5(4H)-ones results from 0.05 mmol Z-Ala-Xaa-oxazolone and 0.05 mmol NMM in 2 ml of DCM.

Measurements

We have tried to represent the integral carbodiimide reaction starting from *N*-substituted peptides based on the simplified Scheme 1, which describes seven partial reactions.

In the mathematical description this means a set of seven differential equations, which can be solved to give all rate constants. Because we cannot experimentally realize all partial reactions, we limited ourselves to four different measurements, namely:



$$dc_{DP}/dt = -k_0 c_{DP}^2 \quad (\text{if: } c_{CDP} = c_{CMC})$$
$$dc_{OAH}/dt = k_0 c_{DP}^2 - k_3 c_{OAH} - k_1 c_{OAH} c_L - k_2 c_{OAH} c_L$$
$$dc_{LLL}/dt = k_1 c_{OAH} c_L + k_4 c_{LL\text{-}Ox} c_L$$
$$dc_{LDL}/dt = k_2 c_{OAH} c_L + k_6 c_{LD\text{-}Ox} c_L$$
$$dc_{LL\text{-}Ox}/dt = k_3 c_{OAH} - k_4 c_{LL\text{-}Ox} c_L + k_{-5} c_{LD\text{-}Ox} - k_5 c_{LL\text{-}Ox}$$
$$dc_{LD\text{-}Ox}/dt = k_5 c_{LL\text{-}Ox} - k_5 c_{LD\text{-}Ox} - k_6 c_{LD\text{-}Ox} c_L$$
$$dc_L/dt = -k_1 c_{OAH} c_L - k_2 c_{OAH} c_L - k_4 c_{LL\text{-}Ox} c_L - k_6 c_{LD\text{-}Ox} c_L$$

Scheme 1 The carbodiimide method (simplified). The concentration c_i of seven participants of reaction are connected as may be described by seven kinetics equations.

(*i*) Monitoring formation of oxazolones from Z-Ala-Xaa-OH and CMC by IR spectroscopy using the C=O absorption at 1825 cm^{-1} (increase of the concentration of oxazolone) and the N=C=N absorption 2132 cm^{-1} (decrease of the concentration of CMC). The rate constant of oxazolone formation k_{OF} encloses k_0 and k_3 according to Scheme 1.

(*ii*) Monitoring the aminolysis of isolated oxazolones with Val-OMe by IR spectroscopy using the 1825 cm^{-1} absorption (decrease of the concentration of oxazolone) and by RP-HPLC (determination of $\%LDL^{max}$). The rate constant of oxazolone aminolysis corresponds to k_4 according to Scheme 1. Due to the basic character of the amino component the epimerization takes place during aminolysis. Therefore the measured rate constant of aminolysis k_{OA} describes both processes k_4 and k_6, which are not accessible to separate analysis.

(*iii*) Investigations on the configurational stability of isolated oxazolones by polarimetric measurements.

(*iv*) Monitoring the formation of peptides during CMC-synthesis from Z-Ala-Xaa-OH and Val-OMe by IR spectroscopy using the bands at 1825 cm^{-1} (increase and decrease of the concentration of oxazolone) and at 2132 cm^{-1} (decrease of the concentration of CMC) and RP-HPLC (increase of the concentration of LLL-/LDL-peptide, determination of %LDL).

In this approach, the increase of the concentration of oxazolone and the decrease of the concentration of carbodiimide are the main keys to a quantitative description of the kinetics of the reaction. Both components possess non-overlapping bands in the IR and therefore we used the bands at *ca.* 1825 and at 2132 cm^{-1} as analytical sensors, respectively.

For the mathematical considerations which we have used, our starting point was always the concentration at the beginning of the reaction. We consider the direct conversion from *O*-acylurea to LLL-peptide, described by k_1, as the main reaction pathway. If there is an inversion of the configuration, the reaction pathway is denoted by k_2. In dependence on structural and reaction conditions, the formation of oxazolone from *O*-acylurea k_3 competes with k_1/k_2. After the formation of LL-oxazolone there is rather fast epimerization of this compound k_5. The epimerization of the oxazolone influences the absorbance of the carbonyl band, but this effect has not been taken into account. LL-Oxazolone was synthesized separately in order to carry out polarimetric measurements and obtain the rate constant k_5. To determine the rate of the epimerization we used the base NMM, which shows a similar pK_a value to Val-OMe, but it cannot couple with oxazolone. We learn from polarimetric measurements that the epimerization (pathway 5) is much faster than the aminolysis. Further it follows, from theoretical considerations, that the equilibrium constant between both epimers is approaching unity. Additionally, tentative measurements indicate that there is no significant difference between k_4 and k_6. The whole yield of LLL-peptide in our procedure may be deduced from HPLC measurements, summing the products of pathways 1 and 4. The formation of LDL-peptide may be also observed by HPLC as the sum of the reaction pathways 2 and 6.

We have to admit that the exactness and the reliability of our calculations may be influenced by the fact that all reaction products discussed here may be formed by at least two different ways, the starting reaction being an exception. The treatment given will only be useful if we can assume that we are considering those reaction pathways which are strongly in excess of the others.

Results

Formation of Z-Ala-Xaa-oxazolone

The rate of oxazolone formation v_{OF} follows second-order kinetics: $v_{OF} = d[\text{Z-Ala-Xaa-OX}]/dt = k_{OF}[\text{Z-Ala-Xaa-OH}][\text{CMC}]$; with [Z-Ala-Xaa-OH] = [CMC] = c, $v_{OF} = k_{OF} c^2$.

The oxazolone formation of Z-dipeptides with aromatic amino acids in the activation position shows the highest rate

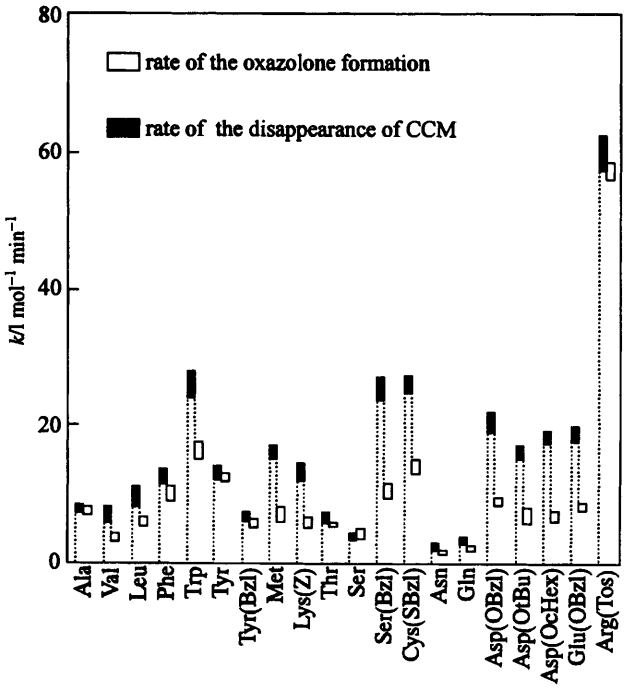


Fig. 1 Comparison of the rate constants of the oxazolone formation and of the disappearance of CMC

constants at *ca.* $k_{OF} = 32–36$ l mol^{-1} min^{-1}. For most Z-dipeptides exhibiting longer, protected side chains, values have been found in the region of k_{OF} between 10 and 25 l mol^{-1} min^{-1}. Comparatively slow cyclization rates with $k_{OF} = 2–6$ l mol^{-1} min^{-1} appear in Xaa like Ser, Thr, Asn and Gln possibly as a result of side reactions. Assuming that oxazolone will be formed quantitatively from Z-Ala-Xaa-OH and CMC, the velocities of oxazolone formation k_{OF} and the decrease of CMC k_{CMC} are equal. Mainly in the case of bulky substitutents CMC decomposes more quickly than the formation of oxazolone takes place, that means $k_{CMC} > k_{OF}$ (Fig. 1). Perhaps we have to expect the formation of *N*-acyl urea.[5] This effect may be due to sterical hindrance.

Aminolysis and epimerization of isolated Z-Ala-Xaa-oxazolones

The epimerization rates of Z-Ala-Xaa-oxazolones with NMM are much higher than the ring-opening rates in the reaction with Val-OMe. Oxazolones with Xaa = Ala, Leu, Trp, Phe, Tyr, Tyr(Bzl), Ser, Lys(Z), Thr epimerize in less than 20 min. Oxazolones with side chain protected amino acids in the activation position, like Ser(Bzl), Cys(Bzl), Asp(X), Glu(Bzl) even lose their optical activity in less than 5 min. Z-Ala-Val-oxazolone with the bulky isopropyl group exhibits an epimerization time of 400 min, being thus the chirally most stable oxazolone of our investigations.

The aminolysis of oxazolones also follows second-order kinetics. The rate constants *k* are summarized in Table 1. Depending on the side chain structure, we observed differences in the ring-opening rates. We can establish a correlation between the rates of aminolysis and epimerization. Due to the fast epimerization of oxazolones there results from oxazolone aminolysis nearly 50% LDL-tripeptide (%LDL^{max}). Considering the correlation of the epimerization and ring-opening rates, a levelling in the degrees of epimerization after ring-opening reactions with Val-OMe (Table 1) is observed.

Peptide synthesis

The main feature of the carbodiimide peptide synthesis is the simultaneous overlapping of the formation of oxazolone and of its decomposition. Both reactions have been considered as isolated reactions before. As a consequence of this overlapping,

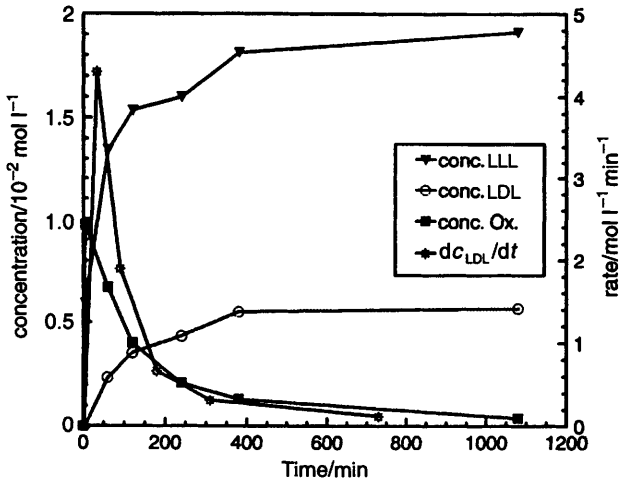


Fig. 2 HPLC-course of the CMC-synthesis of Z-Ala-L/D-Tyr-Val-OMe

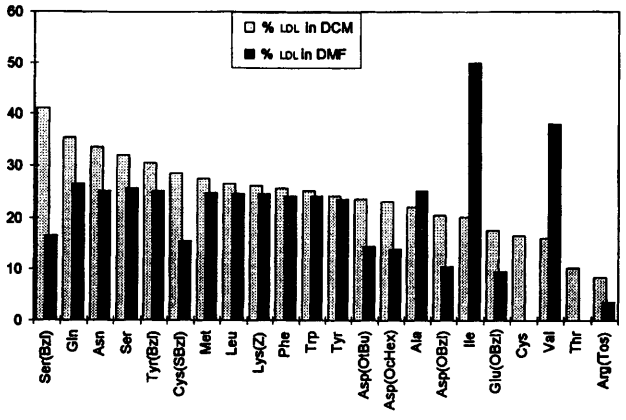


Fig. 3 Epimerization during CMC-coupling of Z-Ala-Xaa-OH and Val-OMe in DCM and DMF

there exists at any time less oxazolone to undergo epimerization. Further, the quantity of oxazolone will be smaller, because the *O*-acylurea will directly form the tripeptide in a procedure not involving the oxazolone state. Further, there may be an additional decrease of oxazolone concentration due to side reactions, which may be expected on activation of bifunctional amino acids in Xaa position. The results of the kinetic investigations, of the oxazolone aminolysis in the peptide coupling obtained by IR spectroscopy are given in Table 1. IR spectroscopy unfortunately does not give a description of the LLL/LDL peptide synthesis as a time function of the oxazolone concentration.

The HPLC-course of tripeptide synthesis (Fig. 2) reveals that the first step during carbodiimide activation is the quick formation of LL-oxazolones. Passing the maximum concentration of oxazolone, the formation of LDL-peptide increases very strongly to a maximum. With decreasing oxazolone concentration the rate of formation of LDL-peptide slows. From this we may conclude: $dc^{Ox}_{LDL}/dt > dc^{DP}_{LDL}/dt$.

The extent of epimerization during CMC synthesis of Z-Ala-Xaa-OH and Val-OMe in DCM and DMF is given in Fig. 3.

Discussion

Now we try to combine our measurements in DCM to give a general description of the epimerization pathways. During the reaction of oxazolone to tripeptide, an equilibrium between LL- and LD-oxazolone is formed. The oxazolone formation and epimerization, in general, is a much faster process than the

Table 1 Kinetic data and epimerization values during tripeptide synthesis in DCM

Xaa	Ox.-formation[a] k_{OF}/l mol^{-1} min^{-1}	Ox.-aminolysis[b] k_{0A}/l mol^{-1} min^{-1}	LDL$^{max\,b}$ (%)	LDL[c] (%)	$c_{Ox}^{max}/c_0 \times 100\%$[d]	$t_{50\%}$[c]/min
Ala	6.5	0.22	43	22	35	65
Val	3	0.06	16.2	16	58	240
Leu	5	0.2	46	26.5	43	75
Phe	8.5	0.23	50	25.5	40	60
Trp	14.5	0.21	50	25	50	70
Tyr	12.5	0.24	50	24	40	55
Tyr(Bzl)	5.5	0.19	50	30.5	52	85
Met	5.8	0.21	50	27.5	50	75
Lys(Z)	4.5	0.16	49	26	52	110
Thr[e]	5.5	—	—	10	30	
Ser	3.5	1.6	48	32	10	30
Ser(Bzl)	9.5	0.8	50	41	28	45
Cys(SBzl)	13.5	1.5	48	28.5	22	30
Asn[e]	1.6	—	50	33.5	5	
Gln	1.8	0.5	50	35.5	30	50
Asp(OBzl)	8.5	0.9	50	20.5	25	45
Asp(OtBu)	5	0.6	50	23.5	35	50
Asp(OcHex)	5.6	0.65	50	23	33	50
Glu(OBzl)	7.5	0.85	50	17.5	25	45
Arg(Tos)	55	—	50	8.4	10	5

[a] Following from Z-Ala-Xaa-OH + CMC, $k = 1/t(1/c\text{-}1/c_0)$. [b] Following from Z-Ala-Xaa-oxazolone + Val-OMe. [c] Following from Z-Ala-Val-OH + Val-OMe + CMC. [d] Amount of oxazolone during tripeptide synthesis c. [e] Side reaction.

aminolysis of the oxazolone. Therefore, we may assume during the whole process the validity of the following eqn. (1):

$$c_{LD\text{-}Ox} \approx c_{LD\text{-}Ox}^{Equ} \quad (1)$$

The LDL-concentration formed *via* LD-oxazolone is proportional to the maximum amount of oxazolone and is expressed by eqn. (2), where c_{LDL}^{Ox} is the concentration of LDL

$$c_{LDL}^{Ox} \cong \frac{\%LDL^{max}}{100\%} c_{Ox}^{total} \quad (2)$$

peptide formed *via* LD-oxazolone and c_{Ox}^{total} is the concentration of all oxazolone formed in the synthesis. The last quantity may be given approximately by the highest value which may be deduced from the IR band near 1826 cm^{-1} according to eqn. (3). Both equations may be combined to eqn. (4).

$$c_{Ox}^{total} \geqslant c_{Ox}^{max} \quad (3)$$

$$c_{LDL}^{Ox} \geqslant \frac{\%LDL^{max}}{100\%} c_{Ox}^{max} \quad (4)$$

The concentration of the LDL peptide is the sum of the LDL-concentration from the dipeptide c_{LDL}^{DP} (*via* *O*-acylurea) and the LDL-concentration *via* oxazolone c_{LDL}^{Ox} during synthesis expressed by the eqns. (5) and (6). According to eqn. (7) it is possible to determine c_{LDL}^{DP}.

$$c_{LDL} = c_{LDL}^{DP} + c_{LDL}^{Ox} \quad (5)$$

$$c_{LDL} \geqslant c_{LDL}^{DP} + \frac{\%LDL^{max}}{100\%} c_{Ox}^{max} \quad (6)$$

$$c_{LDL}^{DP} \leqslant c_{LDL} - \frac{\%LDL^{max}}{100\%} c_{Ox}^{max} \quad (7)$$

There is no experimental access to this value.

The following example illuminates our considerations. In the case of Z-Ala-Tyr-Val-OMe the following values are obtained: $\%LDL^{max} = 50\%$, $c_{Ox}^{max} = 0.01$ mol l^{-1}, $c_{LDL} = 0.006$ mol l^{-1}. Then, as the upper limit, $c_{LDL}^{DP} \leqslant 0.001$ mol l^{-1} can be deduced, corresponding to approximately one sixth (4%) of the whole LDL peptide (24%).

Considering that in our investigations the %LDL-values are always expressed in relation to the final concentration of the tripeptide c_{TP}, eqn. (6) is multiplied by a factor of $100\%/c_{TP}$ to give eqns. (8) and (9).

$$\frac{c_{LDL}}{c_{TP}} 100\% \geqslant \frac{c_{LDL}^{DP}}{c_{TP}} 100\% + \frac{c_{Ox}^{max}}{c_{TP}} \%LDL^{max} \quad (8)$$

$$\%LDL \geqslant \%LDL^{DP} + \frac{c_{Ox}^{max}}{c_{TP}} \%LDL^{max} \quad (9)$$

In order to obtain a relation which is independent of a specific peptide, we introduce normalized LDL-values χ_l by defining eqns. (10) and (11).

$$\chi_{LDL} = \frac{\%LDL}{\%LDL^{max}} \quad (10)$$

$$\chi_{LDL}^{DP} = \frac{\%LDL^{DP}}{\%LDL^{max}} \quad (11)$$

Using this and assuming that the total amount of dipeptide reacts ($c_{TP} = c_0$), we get eqn. (12) which, independent of the

$$\chi_{LDL} \geqslant \chi_{LDL}^{DP} + c_{OX}^{max}/c_0 \quad (12)$$

sequence of the amino acids, describes the dependence of the LDL formation on the concentration of oxazolone.

In the plot of χ_{LDL} *vs.* c_{Ox}^{max}/c_0, deviations from the linear slope present the quantity in this diagram of the directly formed LDL peptide χ_{LDL}^{DP} for all systems investigated (Fig. 4).

c_{Ox}^{max}/c_0 is determined directly by the relation of the maximum extinction value of the Z-Ala-Xaa-oxazolone during peptide synthesis (corresponding to c_{Ox}^{max}) to the reference extinction value of the synthesized reference oxazolones (corresponding to c_0).

Fig. 5 represents the deviations of the degree of epimerization from that which should be formed exclusively by oxazolone in dependence on the activated amino acid Xaa during CMC synthesis of Z-Ala-Xaa-Val-OMe. The results from the peptide synthesis with Xaa = Thr are not given in Fig. 5 because we have been unable to determine $\%LDL^{max}$ due to side reactions. Evidently, most of the peptides epimerize to a higher degree, than as may be assumed by considering the quantity of oxazolone intermediate. This means the direct formation of LDL peptide *via* *O*-acylurea should not be neglected. In the cases

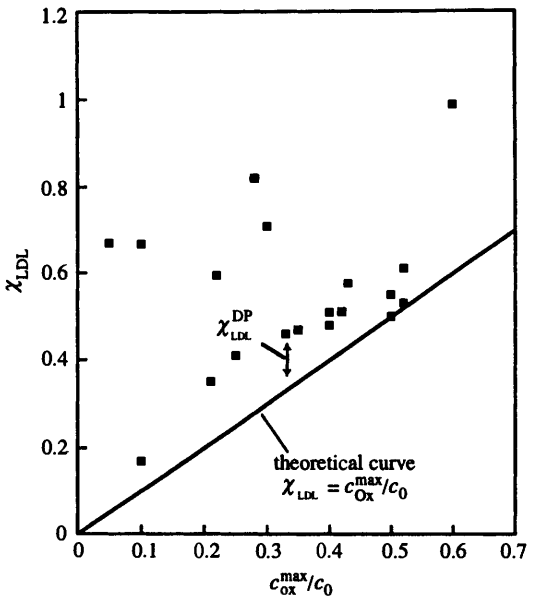


Fig. 4 Relation between the formation of oxazolone and epimerization

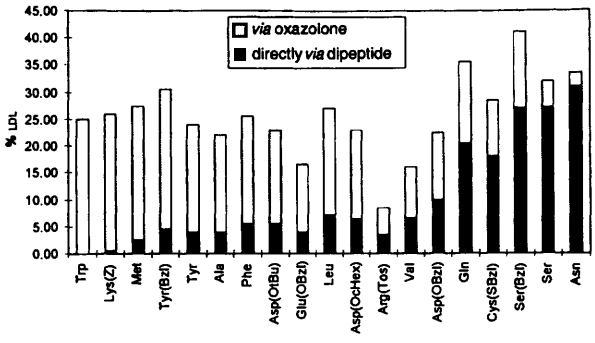


Fig. 5 Sum of LDL-peptide formed *via* the dipeptide directly and *via* the oxazolone

of Xaa = Trp, Lys(Z) and Met in the activation position, the LDL peptide is formed mainly *via* the oxazolone. Similarly for Xaa = Ala, Leu, Phe, Tyr, Tyr(Bzl), Asp(OBzl), Asp(OBu^t), Asp(cHex), Glu(OBzl) and Arg(Tos) the major part of LDL peptide, namely 55–85%, is produced in this way. But in the case of Xaa = Cys(Bzl), Ser(Bzl), Ser, Asn and Gln most (55–95%) of the LDL peptide is not produced *via* the oxazolone intermediate.

Acknowledgements

We are indebted to Dr John Shorter for making some suggestions concerning the manuscript. This work was supported by the Fonds der Chemischen Industrie.

References

1 L. Benoiton, in *The Peptides*, ed. E. Gross and J. Meienhofer, Academic Press, New York, 1983, vol. 1, pp. 217–284.
2 H. Rich and J. Singh, in *The Peptides*, ed. R. Walter and J. Meienhofer, Academic Press, New York, 1979, vol. 1, pp. 242–261.
3 M. Bodanszky, *Int. J. Peptide Protein Res.*, 1985, **25**, 449.
4 M. Goodman and W. J. McGahren, *Tetrahedron*, 1967, **23**, 2031.
5 M. Slebioda, Z. Wodecki and A. M. Kolodziejczyk, *Int. J. Peptide Protein Res.*, 1990, **35**, 539.
6 M. Slebioda, Z. Wodecki and A. M. Kolodziejczyk, in *Peptides 1994, Proc. 23rd Eur. Peptide Symp.*, Braga 1994, ed. H. L. S. Maia, ESCOM, Leiden, 1995, pp. 193–194.
7 L. Benoiton, Y. C. Lee, R. Steinaur and F. M. F. Chen, *Int. J. Peptide Protein Res.*, 1992, **40**, 559.
8 C. Griehl and S. Merkel, *Int. J. Peptide Protein Res.*, 1995, **45**, 217.
9 M. F. Chen, K. Kuroda and N. L. Benoiton, *Synthesis*, 1979, **230**.

Paper 6/00273K
Received 12*th January* 1996
Accepted 12*th June* 1996