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α-CHYMOTRYPSIN-CATALYZED (3+7) SEGMENT SYNTHESIS OF THE LUTEINIZING HORMONE RELEASING HORMONE

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Abstract: The formation of the luteinizing hormone releasing hormone (LHRH) Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (**1**), from the segments Glp-His-Trp-OEt (**2**) and H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (**3**) is catalyzed by α-chymotrypsin . A yield of 71% was achieved when the reaction was performed at -15°C in a solution which contained 38 vol% DMSO and was saturated with KCl.

Recently, a procedure for the α-chymotrypsin-catalyzed 3+7 segment condensation of [D-Phe^6]-LHRH has been described.[1] The enzyme was shown to catalyze the acyl transfer[2] from the acyl donor **2** to the acyl acceptor H-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2 in high yield without racemization. The application of this approach to the synthesis of **1** was not successful due to some undesired side reactions based on enzyme labile peptide bonds, e.g. ...Tyr-Gly... and ...Leu-Arg... in the original sequence. In the D-Phe^6-analog the substrate character of these bonds is reduced.

We have examined the coupling of **2** with **3** under various reaction conditions with respect to the yield of **1** in the kinetic optimum[3] of the reaction. The results are compiled in Table 1. Obviously, a

Table 1. α-Chymotrypsin-catalyzed synthesis of LHRH (**1**)[a]

temperature (°C)	[KCl]	reaction time (min)	yield[b] of **1** (mole %)
35	saturated	no reaction	
24	saturated	10	48
12	saturated	15	54
-2	saturated	18	60
-15	saturated	60	71
-15	0	45	60

[a] conditions: **3** (62.5 mM), **2** (81.2 mM), KOH (81 mM), α-chymotrypsin (0.62 mg/ml), DMSO (38 vol %);

[b] yield in the kinetic optimum (related to the initial concentration of **3**).

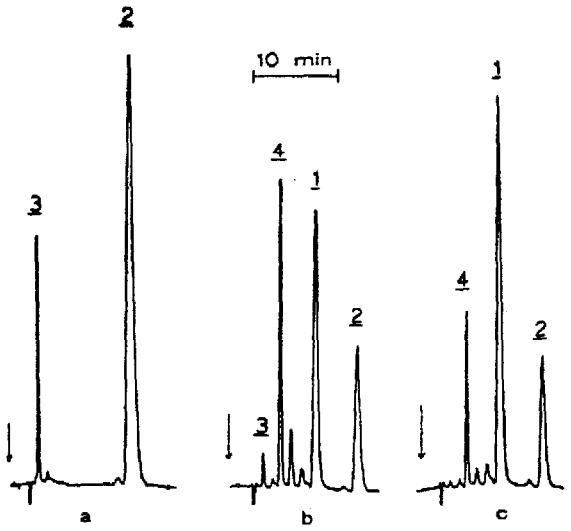

Fig. 1. HPLC-Analysis of the α-chymotrypsin-catalyzed synthesis of LHRH (1).
Conditions: 3 (62.5 mM), 2 (81.2 mM), KOH (81 mM), α-chymotrypsin (0.62 mg/ml), DMSO (38 vol %), reaction mixture saturated with KCl;
a - reaction mixture before enzyme addition ; b - reaction mixture after 10 min of incubation with the enzyme at 24°C; c - reaction mixture after 75 min of incubation with the enzyme at -15°C.

decrease of the reaction temperature caused an increased yield in the optimum. This trend is illustrated by Fig. 1 a and b, too. The formation of the hydrolysis product Glp-His-Trp-OH (4) is suppressed when the reaction is performed at a lower temperature. Due to the rapid inactivation of the enzyme no reaction was observed at 35°C. Saturation of the reaction mixture with KCl resulted in an increased product yield.

The time course of a synthesis reaction performed under optimized reaction conditions[4] is shown in Fig. 2. In the initial phase of the reaction 1 was formed much faster than the hydrolysis product 4. In the kinetic optimum a considerable amount of acyl donor ester was still present in the reaction mixture. This excess of the acyl donor was hydrolyzed giving 4 in the second phase of the reaction. Simultaneously, a slow degradation of 1 was observed. The formation of side-products was neglectable under the chosen conditions.

General discussion

In contrast to chemical procedures the enzyme-directed synthesis[5] requires an optimization of the reaction conditions depending on the peptide to be synthesized. Due to the bad solubility of the acyl donor 2 in water it was necessary to perform the reaction in the presence of 38 % DMSO. It was shown that

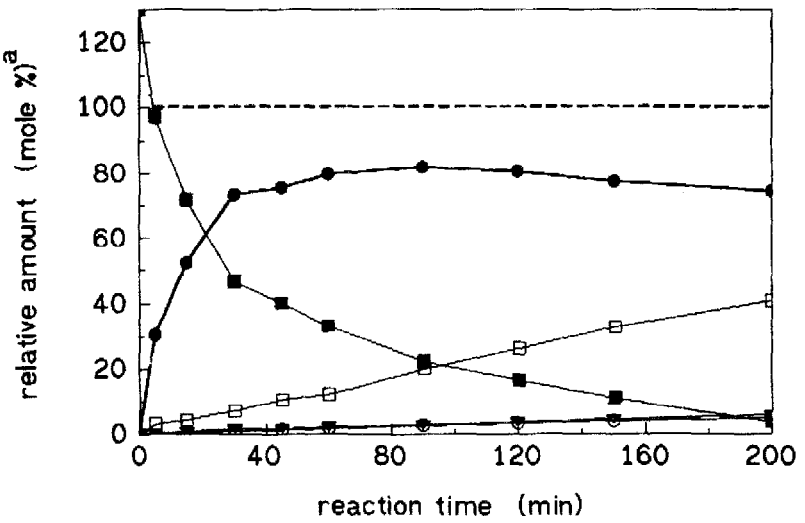

Fig. 2. Time course of the α-chymotrypsin-catalyzed synthesis of LHRH (1) at -15°C. Conditions: 3 (62.5 mM), 2 (81.2 mM), KOH (81 mM), [α-chymotrypsin] = 0.62 mg/ml, DMSO (38 vol %), reaction mixture saturated with KCl;
-■- = 2; -●- = 1; -□- = 4; -▽- = not identified.
[a] related to the initial concentration of 3.

the product yield depends on the reaction temperature. This dependence can be attributed to an improved aminolysis/hydrolysis ratio at low temperatures. The increase of the product yield caused by saturation of the reaction mixture with KCl can be explained by salting out of the amino component[6]. The competition of the excess of acyl donor with the reaction product 1 for α-chymotrypsin is assumed to prevent 1 from enzymatic degradation in the course of the reaction.

We suppose that the strategy of performing enzyme-catalyzed syntheses in the presence of organic cosolvents at low temperatures could be succesfully applied to other systems, too. The avoidance of enzyme denaturation under such conditions, probably due to the high dielectric constant of the medium at subzero temperatures, is a basic principle of cryoenzymology[7,8].

Acknowledgment

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References and Notes

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4. The standard reaction mixture (optimized with regard to pH and amount of cosolvent) contained 62.3 mg (0.13 mmol) **2**, 74.8 mg (0.1 mmol) **3**, 64.8 µl 2 M KOH, 611 µl DMSO and 890 µl water (or a saturated solution of KCl in water, respectively). Reactions were started by addition of 10 µl of a stock solution of α-chymotrypsin (100 mg in 1 ml 10^{-3} M HCl) after equilibration at the reaction temperature. Reactions were followed by RPHPLC. Isocratic elution was performed using a mixture of 0.1% TFA and methanol (v/v = 60/40) at a flow rate of 1 ml/min in combination with a Pep-S column (LKB-Pharmacia, Sweden). Calculating product yields it was assumed that $\varepsilon_{\underline{1}} = \varepsilon_{\underline{2}} + \varepsilon_{\underline{3}}$ and that $\varepsilon_{\underline{2}} = \varepsilon_{\underline{4}}$ (λ = 275 nm). The identity of **1** was proved by amino acid analysis (relative concentrations: 1.31 Ser, 1.06 Glu, 2.00 Gly, 0.99 Leu, 1.16 Tyr, 1.24 His, 1.04 Arg) and FAB-MS ($M + H^+ + H$: 1183.3) of the reaction product separated by HPLC.

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