THE USE OF BIOSENSORS IN BIOORGANIC SYNTHESIS:
PEPTIDE SYNTHESIS BY IMMobilIZED α-CHYMOTryPSIN ASSESSED WITH
AN ENZYME THERMISTOR

B. Stasinska*, B. Danielsson* and K. Mosbach
Pure and Applied Biochemistry, University of Lund,
Box 124, S-221 00 Lund, Sweden

SUMMARY
Biosensor analysis of peptide synthesis in organic solvents using immobilized α-chymotrypsin is described. It is shown that with the enzyme thermometer sensor used, a direct correlation exists between the negative ΔT-values registered and the amount of peptides formed (essentially various N-acetyldipeptide amides) allowing concentrations of 0.1 mM peptide and lower to be determined directly in the reaction medium.

INTRODUCTION
During the last few years the interest in using enzymes for bioorganic synthesis has increased considerably (for reviews see e.g., Klibanov, 1986; Kasche, 1986; Jakubke and Körncke, 1987; Morihara, 1987). Although studies and applications in this general field of interest have been performed using cells and enzymes in the free and immobilized form, it is only now that the potential of enzymes has been fully realized. This is in part due to the realization that enzymes can be used in more or less water-free systems and that they may accept "unusual" substrates. In the former case, hydrolytic enzymes may also be used for synthetic purposes. Parallel to these developments, the general area of biosensors has grown in importance (Turner et al., 1987).

In this communication we would like to focus attention to the fact that biosensors can be successfully applied to the monitoring of bioorganic syntheses performed in solvent-water mixtures. The example chosen is the

---

1 Present address:
Institute of Microbiology, Biochemistry and Food Analysis, University of Agriculture, Mazowiecka 48, 60-623 Poznan, Poland.
enzymic synthesis of dipeptide derivatives, such as N-acetyl-L-tyrosyl-L-
alanine amide, N-acetyl-L-tyrosyl-L-leucine amide, N-acetyl-L-tyrosyl-L-
valine amide, N-acetyl-L-phenylalanyl-L-glycine amide, and N-acetyl-L-phen-
nylalanyl-L-valine amide from N-acetylated esters and amino acid amide
using a normally proteolytic enzyme, α-chymotrypsin, but under conditions
allowing synthesis to occur as has been reported previously (Mori et al.,
1987; Nilsson and Mosbach, 1984; Mori et al., 1987). The biosensor used
was the enzyme thermistor (ET), developed and recently reviewed by Daniels-
son and Mosbach (1987) in which substrate determinations can be made based
on the heat associated with the reaction of an immobilized biocatalyst in a
small, well insulated column. In contrast to the normally studied exother-
mic reactions, enzymic peptide synthesis is endothermic. However, the
"negative" signals obtained from these reactions should likewise be of
value and in fact were found to correlate well with the amount of peptide
found.

MATERIALS AND METHODS

Abbreviations used:
CT = α-chymotrypsin
Ac-Tyr-OH = N-Acetyl-L-tyrosine
Ac-Phe-OH = N-Acetyl-L-phenylalanine
Ac-Tyr-OEt = N-Acetyl-L-tyrosine ethyl ester (ATEE)
Ac-Phe-OEt = N-Acetyl-L-phenylalanine ethyl ester (APEE)
Ac-Phe-OMe = N-Acetyl-L-phenylalanine methyl ester (APME)
Gly-NH₂ = L-Glycine amide-HCl
Ala-NH₂ = L-Alanine amide-HCl
Leu-NH₂ = L-Leucine amide-HCl
Val-NH₂ = L-Valine amide-HCl
Ser-NH₂ = L-Serine amide-HCl
Trp-NH₂ = L-Tryptophane amide-HCl.

All carboxyl group donors and amino acid derivatives, except Ac-Phe-OH,
Val-NH₂ and Ser-NH₂, were from Serva Fine Biochemicals (Heidelberg, FRG).
Ac-Phe-OH, Val-NH₂ and Ser-NH₂ were obtained from Sigma Chemical Co. (St.
Louis, MO, USA). Ac-Tyr-OH was prepared by saponification of Ac-Tyr-OEt.
Eupergit C was obtained from Röhm Pharma (Darmstadt, FRG) and CPG (Control-
led Pore Glass, mean pore diam. 13 nm, 40-80 mesh) from Corning Glass
Works (Corning, NY, USA). α-Chymotrypsin (E.C.3.4.21.1, bovine pancreas,
type II, 38 I.U./mg) was purchased from Sigma Chemical Co. Octadecyl-
silica for reversed phase chromatography (Speri-5, RP-18, 5 μ; column dim.
130 x 4.6 mm) was obtained from Brownlee Labs Inc., Santa Clara, CA, USA).
Analytical grade organic solvents were purchased from Merck (Darmstadt,
FRG). All other reagents used were of analytical grade.

Immobilization of α-chymotrypsin on CPG. About 1900 units (50.5 mg) of
denyme were added to 1.0 g of glutaraldehyde activated propylamino-CPG in
2 ml of 0.1 M sodium phosphate buffer, pH 7.8. Coupling proceeded over-
night at 4°C under gentle shaking. After washing, the preparation was
packed into small, plastic columns for use in the ET (0.8 ml, diam. 7 mm).

282