# Enzyme Recognition by Polypyrrole Functionalized with Bioactive Peptides

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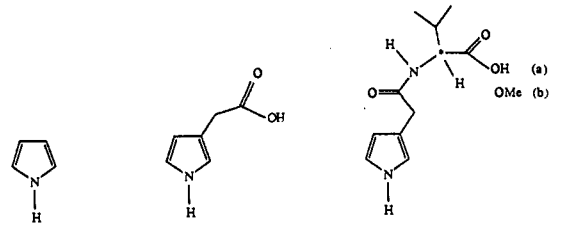
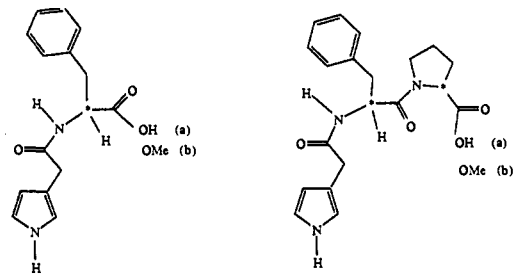
Functionalized conducting polymers constitute materials which specifically interact with external physical or chemical quantities, depending on the nature of the functionalized group.[1] Thus, the covalent binding of chiral substituents, e.g., amino acids, on pyrrole or thiophene monomers led to the development of functionalized electroactive polyheterocycles, showing molecular recognition properties toward optically active anions in solution.[2-5] A further step toward the recognition of biologically active species appears tempting, as small biologically active peptides are known to control a large number of biological functions, and to bear significant therapeutic potential, either as receptor agonist and antagonist[6] or as potent inhibitors which strongly bind to enzymes, as currently used in affinity chromatography.[7] We report here the development of new electroactive poly(dipeptide-pyrrole), in which the functionalization with bioactive dipeptides is designed for the specific recognition of proteolytic enzymes, as evidenced by the modification of the polymer electrochemical voltammogram which follows enzyme binding. Furthermore, the electrochemical control of the dipeptide pH allows monitoring of the release of the complexed enzyme, opening an interesting field of biospecific electroactive materials for the extraction or delivery of enzymes.

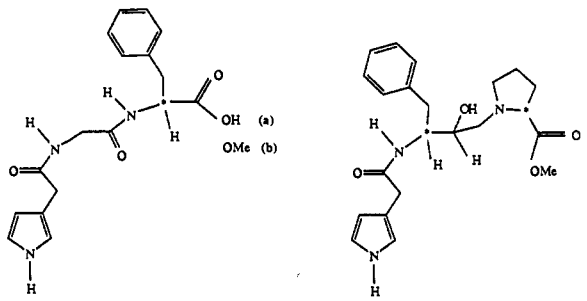
Polypyrrole was chosen as conjugated backbone, owing to its permeability and biocompatibility.[8,9] Various mono- and dipeptides, with an unprotected (**a**) or methyl ester protected (**b**) carboxylic end group, were covalently bonded on pyrrole, **1**, through an acetyl spacer, A, for preserving the conducting and electroactive properties of polypyrrole chains.[3] Series of functionalized pyrrole monomers, Py[A] (**2**), PyA[Val] (**3a,b**), PyA-[Phe] (**4a,b**), PyA[Phe-Pro] (**5a,b**), and PyA[Gly-D-Phe] (**6a,b**), have been synthesized, following already described procedures.[3,10] Glycyl-D-phenylalanine, [Gly-D-Phe] (Sigma), was selected for its specificity and binding capacity toward carboxypeptidase A (type II, Sigma) and trypsin (type II, Sigma).[11] Even larger dipeptide derivatives can be covalently bonded to pyrrole, such as phenylalanine-hydroxyethylamine-proline, which has been proposed as a potent and selective inhibitor of HIV-1 protease,[12] PyA[Phe[Hea]Pro] (**7b**).These monomers were purified and characterized by NMR, mass spectrometry and microelemental analysis.




Py [1] PyA [2] PyA[VAL] [3a, b]




PyA[PHE] [4a, b] PyA[PHE-PRO] [5a, b]




PyA[GLY-D-PHE] [6a, b] PyA[PHE[HEA]PRO] [7b]

Monomers have been polymerized onto Pt foil electrode, 0.7 $cm^2$, and Pt gauze, 10 $cm^2$, in propylene carbonate–$LiClO_4$ 0.5 M, at a constant potential of +0.8 V/SCE. Polymer films with thicknesses up to 10 $\mu$m are obtained. The electroactivity of these functionalized polypyrrole films was confirmed by cyclic voltammetry in $H_2O$–NaCl 0.5 M solution, Table 1. Owing to the higher hydrophobicity of methyl ester protected peptides, polypyrroles of the **b** series show a lower electrochemical reversibility and a higher oxidation potential, $E_{pa}$, between +0.15 and +0.45 V/SCE, than the corresponding unprotected series, **a**, between –0.20 and –0.14 V/SCE.

The *specific recognition* properties of these polymers toward enzymes have been analyzed using carboxypeptidase A, with which [Gly-D-Phe] is known to form a stable enzyme–inhibitor complex at neutral pH.[11] Voltammograms have been recorded in the presence of increasing concentrations of carboxypeptidase A, 1–5 mg in 5 $cm^3$ aqueous electrolyte, with a waiting time of 10 min after immersion of electrode for reaching complexation equilibrium. Unsubstituted polypyrrole poly[**1**], or unspecific poly-[**2**, **3**, **4**, or **5**] show no variation of voltammogram. On the other hand, unprotected or protected [Gly-D-Phe], poly[**6a** or **6b**], leads to an increase of the oxidation potential $E_{pa}$ with enzyme concentration, from –0.14 V/SCE to +0. 1 V/SCE for poly-[**6a**], and +0.34 V/SCE to +0.50 V/SCE for poly[**6b**] (Figure 1). These modifications are attributed to the decrease of the interfacial electron-transfer rates between polymer and electrode, due to binding of the enzyme.[13,14] The formation of an enzyme–

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**Table 1.** Electrochemical Oxidation Potential of Dipeptide-Functionalized Polypyrroles[a]

| | poly- | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | [1] | [2] | [4a] | [6a] | [3b] | [4b] | [5b] | [6b] |
| $E_{pa}$ (V/SCE) | −0.20 | −0.30 | −0.21 | −0.14 | +0.15 | +0.40 | +0.45 | +0.34 |

[a] $H_2O$–NaCl 0.5 M, pH = 7. Sweep rate, 10 mV/s.

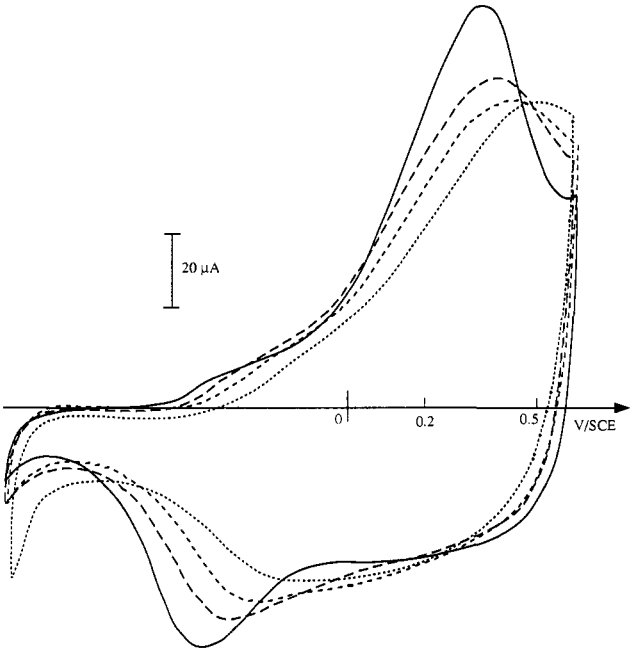


**Figure 1.** Electrochemical voltammograms of poly([Gly-D-Phe]pyrrole), poly[**6b**], in the presence of increasing carboxypeptidase A concentrations, (—) 0.0 mg, (– – –) 1.2 mg, (---) 2.4 mg, (•••) 5 mg, in 5 $cm^3$ buffered $H_2O$–NaCl 0.5 M, pH = 7. Sweep rate: 10 mV $s^{-1}$.

dipeptide complex on poly[**6**] electrodes has been further confirmed by the release of the enzyme, realized by immersing this enzyme-bound electrode in an aqueous solution at pH = 3, as classically performed in affinity chromatography,[7] the released enzyme being characterized by a protein-dye binding test.[15] When using a thin poly[**6a**] film containing $1.3 \times 10^{-6}$ Py[Gly-D-Phe] mole units, corresponding to 0.3 C electropolymerization charge, a significant quantity of 800 μg of carboxypeptidase A has been recovered after one cycle of complexation–release. Taking account for the size of carboxypeptidase A, 307 amino acid units,[16] this quantity of released enzyme shows that about one enzyme molecule has been bound for every 200 Py[Gly-D-Phe] monomers in the polymer, which appears in reasonable agreement with the comparative geometric sizes of enzyme and monomer unit. Experiments performed on protected poly[**6b**] led to comparable results on its binding capacity toward carboxypeptidase A. The absence of recognition, on the one hand, of poly[**2** to **5**] toward carboxypeptidase A and, on the other hand, of poly[**6**] toward albumin (bovine serum albumin, Sigma) confirms the selectivity of recognition. Similar increases of oxidation potential have been observed for the poly[**6a,b**]/trypsin system, evidencing the bioselective recognition properties of these poly[dipeptide-pyrrole] electrodes.

A further interesting feature concerns the *electrochemically controlled release* of the enzyme. It has been shown that polypyrroles bearing a free carboxylic side group, such as **2**, are in fact polyacids, which undergo a first proton dissociation when immersed in a neutral unbuffered solution, leading to a decrease of the solution pH from 7 to a $pK_a$ controlled value of about 6. When submitted to electro-oxidation, these polymers undergo instantaneously a further large dissociation of their carboxylic groups, triggered by the injection of positive charges in the polymer chains.[17-19] In a small volume electrolyte, 5 $cm^3$, pH decrease to values of 3.5 and 4 has been achieved with poly[**2**] and poly[**6a**], respectively, which gives evidence of an even larger decrease of local pH at the immediate vicinity of the polymer electrode. This property can be used for an electrochemically controlled release of the enzyme. Thus, a poly[**6a**] electrode, 0.2 C electropolymerization charge, was first immersed in a buffered neutral aqueous solution containing 5 mg of carboxypeptidase A, leading to the binding of the enzyme on the specific dipeptide **6a**. This electrode was then removed, rinsed, and immersed in an electrochemical cell containing 5 $cm^3$ of unbuffered solution $H_2O$–NaCl 0.5 M. Under no potential step, no release of enzyme is observed. However, when applying to poly[**6a**] an anodic step $\Delta E_{ox}$ to +0.1 V/SCE, the large decrease of pH at the electrode vicinity induces the simultaneous release of the enzyme, as confirmed by its quantitative determination.[15] The instantaneously released carboxypeptidase A, about 500 μg, retained its entire activity, as determined by the kinetics of *N*-[3-(2-furyl)-acryloyl]-Phe-Phe hydrolysis.[20] This electrochemically driven release of enzyme also has been achieved with methyl ester protected poly[**6b**], using a copolymer electrode, poly[**2,6b**], realized by electropolymerization of a 5:1 mixture of **2** and **6b**. The free carboxylic units **2** in this copolymer undergo a dissociation when submitted to anodic oxidation, the release of protons leading to that of the enzyme previously bound to the **6b** peptide units. We verified that, under the same experimental conditions, unspecific polymers, e.g., poly[**2**] or poly[**3**], do not lead to any transfer of enzyme.

These results underline the remarkable properties of these functionalized polymers, bearing (i) recognition sites, peptides, for selective enzyme complexation; (ii) release agents, carboxylic protons, which can be electrochemically triggered; and (iii) molecular wires, polypyrrole, for transduction of information to and from the electrode, following an already developed material concept.[1] These polymers act as bioselective electroactive materials and offer attractive potential for (maybe *in-vivo*) enzyme extraction or delivery. Finally, the here described functionalization of electroactive polymers can be generalized to other small peptides, and, taking account for the very large scope of their biological activities, interesting perspectives are opened for various biological or medical applications.

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