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Determination of phenolic compounds using recombinant tyrosinase from *Streptomyces antibioticus*

Katrin Streffer[a], Erik Vijgenboom[b], Armand W.J.W. Tepper[b], Alexander Makower[a], Frieder W. Scheller[a], Gerard W. Canters[b], Ulla Wollenberger[a,*]

[a] *Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Golm, Germany*
[b] *Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

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Abstract

Properties of *Streptomyces antibioticus* tyrosinase and the implementation of the enzyme in a biosensor for the detection of phenolic compounds were investigated. The tyrosinase from *S. antibioticus* is a monomer and has a molecular weight of 30.6 kD. The specific activity is about 5 U/mg with catechol as substrate and 1225 U/mg with L-dopa as substrate. The activity of tyrosinase upon long-term storage is best maintained in buffer at temperatures of −80 or +4°C. Storage at −18°C, with or without glycerol, resulted in quick enzyme inactivation.

For the construction of the sensor bi-enzymatic substrate recycling was exploited. Quinoprotein glucose dehydrogenase (GDH) and tyrosinase were immobilised in polyvinyl alcohol and coupled to a Clark-type oxygen electrode that allowed for monitoring of the oxygen consumption during catechol conversion. This design of the sensor facilitates the determination of phenolic compounds in the nanomolar range. The lower limit of detection for L-dopa, dopamine, and adrenalin was 5 nM.

Keywords: Biosensor; Tyrosinase; Glucose dehydrogenase; Phenolic compounds; Substituted catechols

1. Introduction

Tyrosinase is a copper-containing monophenol mono-oxygenase that performs also the two-electron oxidation of catechols to *o*-quinones. It is present in microorganisms, plants and animals. The tyrosinase used here has been isolated from *Streptomyces antibioticus*, which excretes tyrosinase into the medium. A simple and quick procedure has been developed to isolate the tyrosinase in a pure form from the medium [1].

The substrates of tyrosinase include a large variety of phenolic compounds with relevant significance in clinical analysis and control of environmental pollutants. Levodopa (L-β(3,4-dihydroxy-phenyl)-alanine), for instance, is routinely used for the treatment of Parkinson's disease and chlorinated phenols are widespread as insecticides, pesticides and disinfectants. The complexity of the samples calls for selec-

* Corresponding author. Tel.: +49-331-977-5122; fax: +49-331-977-5051.
E-mail address: uwollen@rz.uni-potsdam.de (U. Wollenberger).

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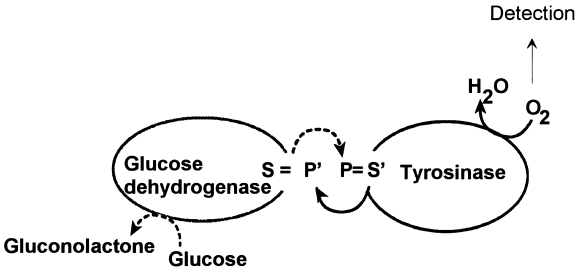

Fig. 1. Schematic representation of the enzymatic substrate recycling between tyrosinase and quinoprotein glucose dehydrogenase (GDH).

tive and sensitive detection systems to avoid matrix effects and sample preparation.

Several methods to detect phenols are available [2–4]. Among them are biosensors, where the biocomponent is tyrosinase, which is isolated from mushrooms and commercially available [5–7]. One major drawback of mushroom tyrosinase is that it is a large multi-subunit enzyme, which is relatively unstable and has a very short lifetime. To compensate for the short lifetime of the enzyme a surplus of tyrosinase is used in diffusion limited biosensors,.

Tyrosinase can be used in biosensors in different ways. For example, direct immobilisation of the enzyme in or on carbon based electrodes is possible, which can be used with and without mediators. Furthermore oxygen electrodes are covered with a membrane containing tyrosinase. They have a micromolar measuring range. However, if another enzyme is added to the membrane, which regenerates the substrate of tyrosinase a substrate recycling will be obtained.

Fig. 1 illustrates the substrate regeneration in which repeated oxidation of a diphenolic compound by tyrosinase and reduction of quinone by GDH takes place. The substrate S' of tyrosinase, is identical to the product P' of GDH and S of GDH equals P of tyrosinase. If a monophenolic compound is the substrate of tyrosinase, then the first reaction would be the *ortho*hydroxylation of it by tyrosinase. After that the substrate regeneration can start.

The enzymatic substrate regeneration principle is an effective method to increase the sensitivity of an enzymatic assay. With this method the detection limit of biosensors can be lowered by several orders of magnitude [8]. In addition, note that without a reduction step for the products P of tyrosinase, they would easily undergo non-enzymatic radical formations in aqueous solutions, resulting in polymeric products. Summarisation of this principle of enzymatic substrate regeneration leads not only to an increase in sensitivity but also to an increase in stability of the biosensor, because almost no polymeric products are accumulated. Enzymatic substrate recycling has also been used in combination with immunoassays [9] and it has been applied in inhibitor studies [10].

This paper reports on the characterisation of *S. antibioticus* tyrosinase and the application of the enzyme in a sensor.

2. Experimental

2.1. Reagents and solutions

L-proline, L-tyrosine, catechol, *p*-aminophenol, *p*-nitrophenol, *p*-nitrocatechol, hydroquinone, and tyrosinase (EC 1.14. 18. 1, from mushroom) were purchased from Sigma (Germany). The quinoprotein glucose dehydrogenase (GDH, EC 1.1.99.17, from *Acinetobacter calcoaceticus*) was a kind gift from Roche (USA). L-dopa, *p*-chlorophenol, *p*-cresol and 4-methylcatechol were obtained from Aldrich (Germany), Oxoid soya broth from Oxoid Ltd. (UK) and dopamine, adrenalin and sucrose from Fluka (Switzerland). Phenol was received from Merck (Germany). Thiostrepton was a kind gift from Bristol-Meyers Squibb (USA) to Gerard W. Canters and E. Vijgenboom.

All chemicals purchased were of analytical grade and used without further purification.

2.2. Tyrosinase isolation

The plasmid pIJ703 containing the *melC* operon encoding tyrosinase from *S. antibioticus* was a kind gift from Prof. Dr. E. Katz and coworkers [11]. The plasmid was transformed to *S. antibioticus* by standard methods [12] and tyrosinase was expressed in medium containing 30 g/l Oxoid soya broth, 10% (w/v) sucrose, 50 μM cupric sulfate and 5 μg/ml thiostrepton. After 28 h of growth, the medium which contained the enzyme was separated from the cell mass by centrifugation. Protein was purified according to a modified version of published procedures [1,13,14]. Tyrosinase

was stored in 40 mM phosphate buffer pH 7.2 with 20% glycerol at −80°C.

2.3. Activity assays

2.3.1. Tyrosinase

Method 1 used for the estimation of the tyrosinase activity in solution is based on the reaction of proline and quinones according to Rezepecki and Waite [15] (proline-assay). For the measurements a cuvette was filled with 1.44 ml phosphate buffer (100 mM; pH=6.5 containing 1 M sodium chloride), 30 μl proline (1 M), and 15 μl catechol solution (10 mM). The solutions were mixed in the cuvette for 1 min. To start the assay, 0.5–2.5 μg of tyrosinase was added and the solution was stirred again. After 10 s, the differential absorption at 525 nm was measured using a Beckman DU 640 spectrophotometer (Beckman, USA). The stock solutions were stored on ice in dark containers. Measurements were performed at 22°C. Calculation of activity was based on an extinction coefficient of 5400 M^{-1} cm^{-1} for 4-*N*-prolyl-*o*-quinone.

Method 2 was the standard dopachrome method according to Fling et al. [16]. For the measurements a cuvette was filled with 1 ml phosphate buffer (100 mM; pH=6.8) containing 5 mM L-dopa. To start the assay, 7.5–22.5 μg mushroom or 0.075–0.225 μg of *S. antibioticus* tyrosinase was added and the solution was mixed in the cuvette for 5 s. Then the increase in absorption at 475 nm due to the formation of dopachrome (ε_{475}=3600 M^{-1} cm^{-1}) was monitored as a function of time. The measurements were performed at 22°C. The activity was expressed as μmol of L-dopa oxidised per minute. For the determination of the pH-optimum of *S. antibioticus* tyrosinase a universal buffer (Davies-buffer) instead of phosphate buffer was used.

2.3.2. GDH

Activity was determined spectrophotometrically at room temperature by monitoring the reduction rate of 2,6-dichlorophenolindophenol (DCIP) at 610 nm. The activity was expressed as μmol of DCIP reduced per minute based on an extinction coefficient of 2100 M^{-1} cm^{-1} for DCIP [17]. The reaction mixture contained 0.066 M Na_2HPO_4/KH_2PO_4 pH=7.0, 0.1 mM $CaCl_2$, 1 mM DCIP, 20 mM glucose and enzyme 0.03–0.228 μg in a total volume of 1 ml.

2.4. Protein concentration

The protein concentration of *S. antibioticus*tyrosinase was determined from the optical absorption at 280 nm in 10 mM sodium phosphate buffer, pH 6.8, by using a molar absorption coefficient of 82 000 M^{-1} cm^{-1} according to Jackman et al. [18]. Instead of the protein content of mushroom tyrosinase the total amount of lyophilisate of this tyrosinase was used for calculations, for example to determine the specific activity. Previous investigations in our group have demonstrated that only 10–15% of the lyophilisate actually consists of tyrosinase (Makower, pers. commun.). Also for GDH the total amount of lyophilised enzyme was used for the calculations.

2.5. Bi-enzyme membrane preparation and assembly of the biosensor

GDH and tyrosinase were immobilised within polyvinyl alcohol as described earlier [19]. The membranes contained approximately 1 U tyrosinase according to the proline-assay and 40 U GDH according to the standard assay with 2,6-dichlorophenolindophenol as electron-acceptor. They were stored at 4°C. The tyrosinase/GDH membrane was fixed onto a Clark-type oxygen electrode (Elbau GmbH, Germany) between a polypropylene (thickness 6 μm) and a regenerated cellulose (thickness 8 μm) membrane. In the following 'ST' will be used for the biosensor with *S. antibioticus* tyrosinase and 'MT' for the mushroom tyrosinase containing biosensor.

2.6. Apparatus and procedure

For the measurements of current–time curves a stirred measuring cell was used as described by Streffer et al. [20]. Before starting the measurements, the tyrosinase/GDH electrode was incubated for 2 h in 50 mM phosphate buffer (pH=6.5). The working platinum electrode (diameter of 0.5 mm) was polarised at a potential of −600 mV (vs. Ag/AgCl reference electrode) using a potentiostat GKM 01 (ZWG, Germany) and transient currents were allowed to decay to a steady-state value. The decrease in current at the electrode upon application of the analyte was amplified and recorded by a chart recorder (Kipp & Zonen,

The Netherlands). All electrochemical measurements were performed at room temperature.

3. Results and discussion

3.1. Characteristics of tyrosinase from S. antibioticus

The purity of tyrosinase was more than 98% as judged by SDS-PAGE. Preparations from different isolation experiments had a consistent high degree of purity. According to the MALDI mass-spectroscopy, the isolated protein has a size of 30.6 kD which is in good agreement with the molecular weight of 29.5 kD as estimated from SDS-PAGE and 30.6 kD predicted from the sequence [13].

Various spectrophotometric methods are available for the determination of tyrosinase activity [20]. To compare the activities of the two tyrosinases used in this study we have applied different assays because the substrate spectrum differs substantially.

Method 1 is very user-friendly and allows to determine the tyrosinase activity with a very good reproducibility. Catechol, one of the compounds which has to be analysed in the environmental application, is used as the substrate in this assay. Method 2 was chosen because L-dopa, a physiologically interesting substrate, is used.

In Fig. 2 the results of the two methods are illustrated. Estimation of the specific activities using Method 1 resulted in comparable values of 31 U/mg protein as the specific activity of *S. antibioticus* tyrosinase and 48 U/mg for the lyophilisate of mushroom tyrosinase (Fig. 2B). However, with L-dopa as substrate (Method 2) the activities differ significantly. An activity of 1225 U/mg protein was obtained for *S. antibioticus* tyrosinase, but the mushroom tyrosinase had only an activity of 5.2 U/mg lyophilisate (Fig. 2A).

Fig. 3 shows the enzyme activity (normalised with respect to the highest activity) as a function of pH when an *o*-diphenol (L-dopa) is used as a substrate (Method 2). The activity optimum around pH 7 is comparable to the pH-optimum of *Streptomyces glaucescens* tyrosinase [14].

Maintaining high activity levels even after long-term storage is an important feature for a successful sensor. Therefore, the activity of *S. antibioticus* tyrosinase was determined as a function of the time upon

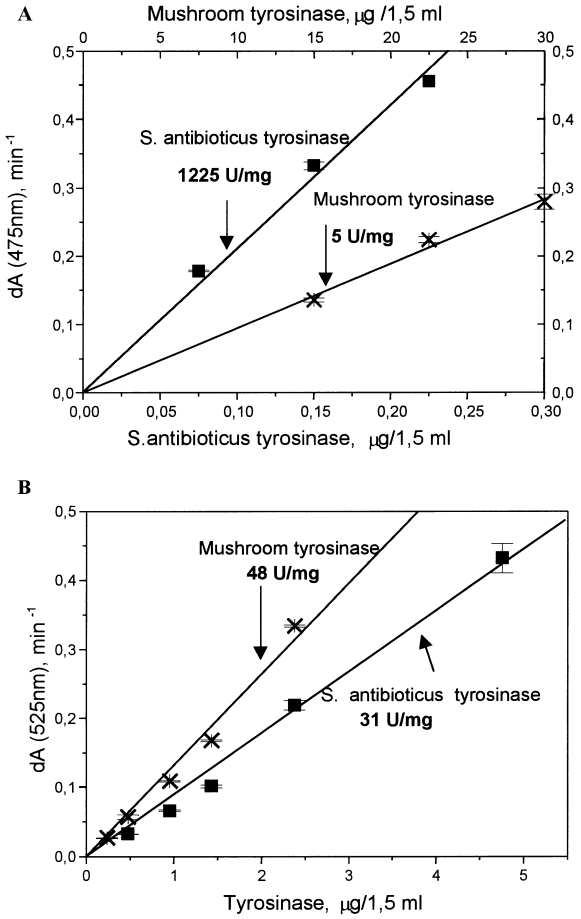

Fig. 2. Determination of the specific activity using L-dopa (A) or catechol (B) as a substrate for *S. antibioticus* tyrosinase (■) in comparison to mushroom tyrosinase (×).

storage in 40 mM phosphate buffer pH 7.2 at four different temperatures (−80, −18, +4 and +37°C). The enzyme activity was reduced by only 30% after 50 days storage at 4°C, while storage of the enzyme at −18 or +37°C led to an almost complete loss of activity within the first few days (see Fig. 4A).

The addition of glycerol resulted in a stabilisation. In the presence of 20% glycerol no loss of activity was observed after 170 days storage at 4 and −80°C. But even in the presence of 20% glycerol the activity drop already within the first days was significant at −18 and +37°C (Fig. 4B).

The loss of enzyme activity at −18°C is due to the storage conditions since enzyme solutions which

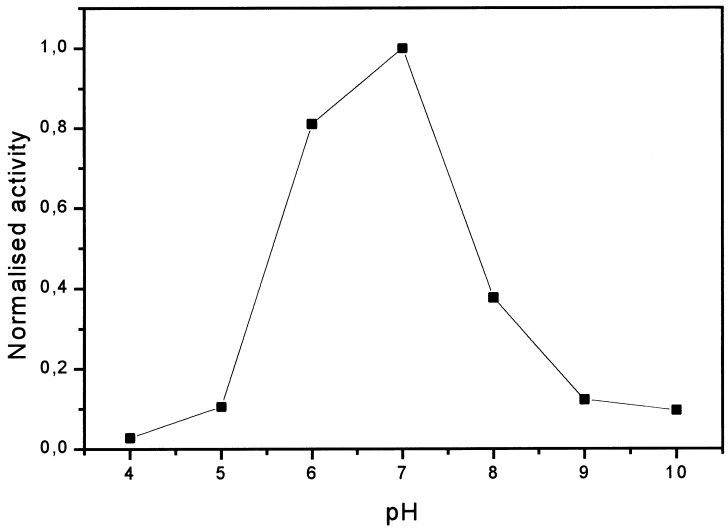

Fig. 3. Dependence of *S. antibioticus* tyrosinase activity on pH.

initially were stored at −80°C showed a loss of activity upon further storage at −18°C. Tyrosinase solution stored at −80°C followed by a period of storage at +4°C maintained full activity.

In addition, the storage of *Streptomyces* tyrosinase as a dried sample was investigated. In this case storage at temperatures below zero, −18 and −80°C, turned out to be the best condition. Samples have been stored for at least 6 months without any loss of activity at these temperatures (Fig. 4C).

Streptomyces glaucescens tyrosinase which is quite similar to tyrosinase from *S. antibioticus* has been reported to retain its activity after 2 months storage at −30°C in the presence of 20% glycerol [14]. This is in good agreement with our data for *S. antibioticus* tyrosinase.

3.2. S. antibioticus tyrosinase used in a sensor application

For the application of *S. antibioticus* tyrosinase in a sensor, the enzyme was co-immobilised with GDH as described for other phenol oxidising enzymes [19]. First the efficiency of tyrosinase/GDH substrate recycling as a function of the pH was determined. Fig. 5 illustrates the change in activity (normalised with respect to the highest activity) when either L-dopa or catechol were used as substrates for tyrosinase. For catechol a relatively broad pH-optimum, from 6.5 to 8.0 was observed. In contrast, the pH-optimum for L-dopa conversion extends from 6.5 to 7.0, which is similar to that of *S. antibioticus* tyrosinase in solution.

Consequently, the highest sensitivity for both substrates is obtained in the pH range from 6.5 to 7.0. Therefore, all further studies were carried out in a phosphate buffer pH 6.5.

The calibration lines obtained in the amplified (Fig. 6A) or non-amplified (Fig. 6B) mode, and L-dopa or catechol as substrate are shown. The system operates in the non-amplified mode when glucose is absent in the solution. Under these conditions the GDH is not active and the sensor response corresponds to the activity of tyrosinase. In the amplified mode when glucose is added to the buffer the full reaction cycle between tyrosinase and GDH takes place (see Fig. 1).

It is obvious from a comparison of the calibration graphs that the sensitivity for the two analytes, L-dopa and catechol, is opposite in one mode compared to the other. When the ST-sensor was used in the amplified mode a sensitivity of 16.5 nA/μM was found for L-dopa and a sensitivity of 6.6 nA/μM for catechol. The detection limit of L-dopa in the amplified mode, 5 nM, is half that of catechol. For measurements in the non-amplified mode with the same sensor we obtained a sensitivity of around 0.06 nA/μM for L-dopa and a sensitivity of 0.15 nA/μM for catechol.

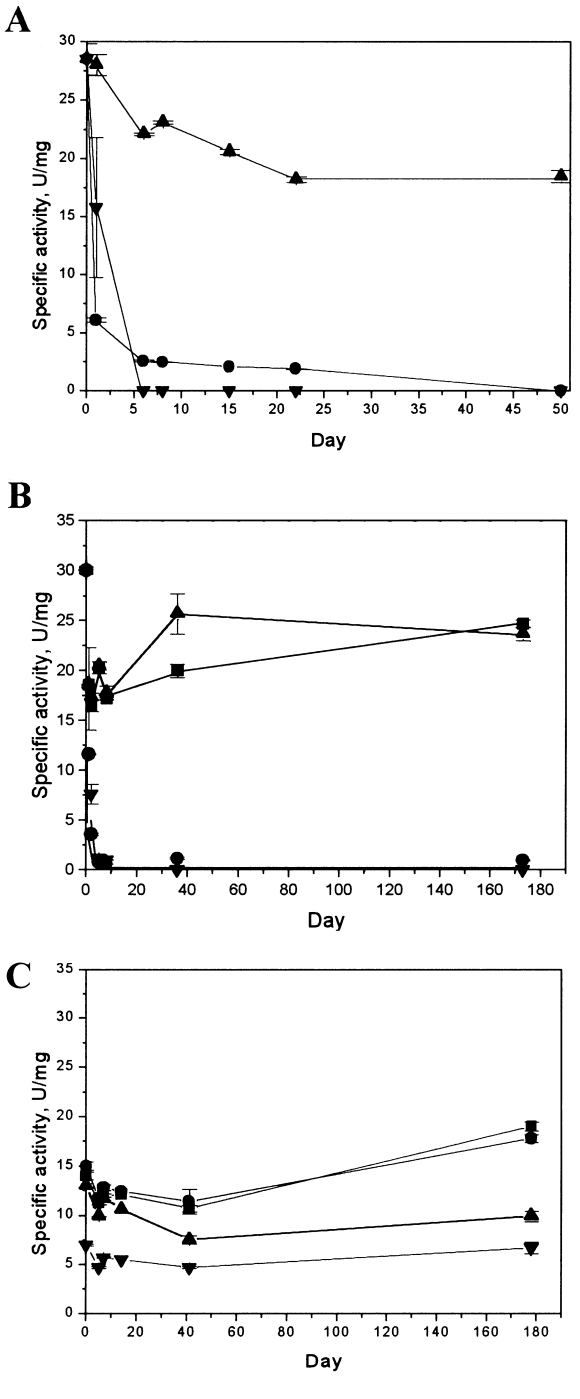

Fig. 4. Storage stability of *S. antibioticus* tyrosinase in A: phosphate buffer, 40 mM, pH=7.2; B: phosphate buffer, 40 mM, pH=7.2 containing 20% glycerol and C: the dried enzyme at different temperatures: −80°C: ■, −18°C: ●, +4°C: ▲, +37°C: ▼.

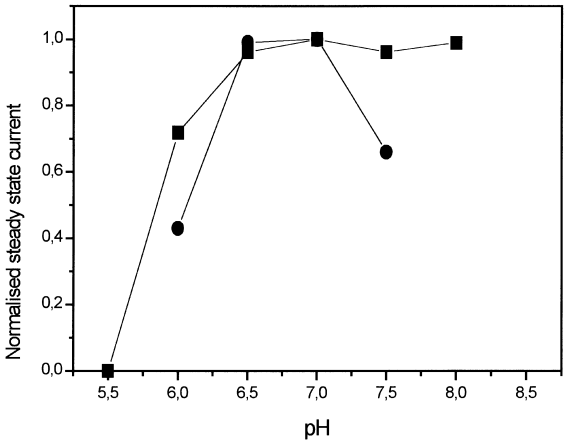

Fig. 5. pH dependence of the bi-enzyme electrode response for 100 nM L-dopa (●) and 250 nM catechol (■) in 50 mM phosphate buffer containing 10 mM glucose.

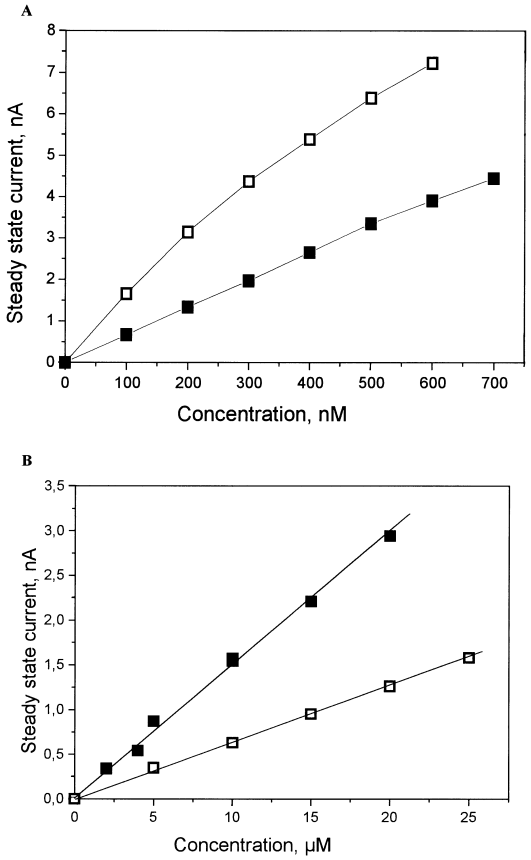

Fig. 6. The calibration lines for L-dopa (□) and catechol (■) measured in the amplified (A) and the non-amplified (B) mode with an *S. antibioticus* tyrosinase/GDH–membrane (ST-sensor).

Table 1
Sensor characteristics of the *S. antibioticus* tyrosinase/GDH-sensor (ST-sensor)

Parameter	ST-sensor characteristics
Lower detection limit (nM)	
L-Dopa	5
Catechol	10
Linear measuring range (nM)	
L-Dopa	5–300
Catechol	10–700
Response time (100 nM)	
L-Dopa	6 min
Catechol	2 min
Reproducibility (100 nM catechol)	3% (n=9)
Storage stability	Several months

Table 2
Relative response of two different biosensors, the ST-sensor, containing a *S. antibioticus* tyrosinase/GDH-membrane, and the MT-sensor, containing a mushroom tyrosinase/GDH-membrane, for 100 nM of monophenolic compounds[a]

Compound	Relative ST-sensor response (%)	Relative MT-sensor response (%)
Phenol	107	100
p-Aminophenol	250	12
Hydroquinone	28	8
p-Chlorophenol	190	60
p-Cresol	330	130
L-Tyrosine	430	5
p-Nitrophenol[b]	0.8	0.5

[a] The response is related to 100 nM catechol.
[b] The relative response of *p*-nitrophenol was calculated (simply by division through 10) from the signal measured with 1 μM of the compound and then related to 100 nM catechol.

An explanation for the change in selectivity may be found in the working principle of the sensor. For catechol measurement it has been shown that in the non-amplified mode the rate of substrate diffusion is limiting the sensor response, whereas in the amplified mode tyrosinase is limiting the recycling rate [19]. In the case of L-dopa conversion the situation is complex, both the regeneration rate by GDH and the restricted L-dopa permeation may lead to the observed shift of sensitivity ratio of 2.5:1 (ST sensor) and 40:1 (solution).

This hypothesis is underscored by the results of the MT-sensor, in which mushroom tyrosinase is used. Since mushroom tyrosinase in solution has its highest specific activity for catechol, also the highest response of the MT-sensor in the amplified mode was obtained with catechol. But the sensitivity for L-dopa and catechol in the non-amplified mode was the same as for the ST-sensor. Here a higher response for catechol was observed too. The assumption is furthermore supported by the prolonged response time, 6 min, which is significantly slower than the response time for catechol or other phenolic compounds.

The linear measuring range of the ST-sensor for catechol was up to 700 nM and for L-dopa from 5 to 300 nM, but the response for L-dopa was twice as high as for catechol (Table 1). The response time for L-dopa is higher than that for catechol. The reproducibility of the measurements of 3% for 100 nM catechol, and the storage stability of several months, makes this sensor a potentially interesting analytical device for the detection of phenolic compounds.

Because of differences in reaction rates and diffusion velocities, the sensor measures various phenolic compounds with different sensitivity. To assess the analytical potential of our biosensor, different mono- and diphenolic compounds were investigated. The relative response of the electrode to monophenolic compounds in relation to catechols is shown in Table 2. The sensitivity for L-tyrosine, is highest, followed by *p*-cresol and *p*-aminophenol, for which the detection is 4, 3, and 2.5 times more sensitive than for catechol. Phenol is detected with a sensitivity in the same order of magnitude as catechol. The response for *p*-nitrophenol is low.

As can be seen from Table 3 among the diphenols studied 4-methylcatechol is detected with the highest sensitivity. The detection limit of 5 and 10 nM was obtained for adrenalin and dopamine, respectively, which is in the range of other sensors [21,22].

When we compared the electrode response for a monophenolic compound with the corresponding diphenolic compound, we always obtained similar sensitivities, e.g. *p*-cresol and 4-methylcatechol or L-tyrosine and L-dopa are determined with comparable sensitivities. Only the sensitivity for *p*-nitrophenol and *p*-nitrocatechol is remarkably different. The electrode response for *p*-nitrocatechol is almost the same as for catechol, while it is less than 1% for *p*-nitrophenol. This result may be explained by

Table 3
Relative response of two different biosensors, the ST-sensor, containing a *S. antibioticus* tyrosinase/GDH-membrane, and the MT-sensor, containing a mushroom tyrosinase/GDH-membrane, for 100 nM of diphenolic compounds[a]

Compound	Relative ST-sensor response (%)	Relative MT-sensor response (%)[b]
Catechol	100	100
L-Dopa	228	3*
Dopamine	65	8
Noradrenalin	11	0.55*
Adrenalin	170	1.15*
4-Methylcatechol	300	134
4-Nitrocatechol	130	0.95*

[a] The response is related to 100 nM catechol.
[b] The relative response marked with * was calculated (simply by division through 10) from the signal measured with 1 μM of the compound and then related to 100 nM catechol.

the fact that *p*-nitrophenol is a strong competitive inhibitor for catechol oxidation by tyrosinase [14].

3.3. Comparison of tyrosinase/ GDH sensors using S. antibioticus tyrosinase (ST-sensor) and mushroom tyrosinase (MT-sensor)

Parameters such as response time, reproducibility and storage stability of the ST-sensor are comparable with the MT-sensor. However, significant differences were obtained in sensitivity and selectivity.

In Fig. 7 the calibration graphs for the ST-sensor and the MT-sensor in the amplified mode for L-dopa and catechol are shown. Using the sensor prepared with mushroom tyrosinase a higher sensitivity for catechol is observed. The detection limit is rather different: 0.6 nM with the MT-sensor [19] and 10 nM with the ST-sensor.

The substrate which is faster oxidized by *S. antibioticus* tyrosinase, L-dopa, can also be detected in the lower nanomolar range. The sensitivity for the MT-sensor is significantly lower (note that the concentration range depicted in Fig. 7A for MT-sensor is 10-fold higher than for ST-sensor). A linear measuring range is obtained up to 3 μM.

The sensitivity of the two sensors for monophenolic compounds is listed in Table 2. The substrate spectrum differs substantially. With the MT-sensor, phenol and *p*-cresol can be determined with sensitivities in the same order of magnitude, but for all other monophenols the device was less sensitive than

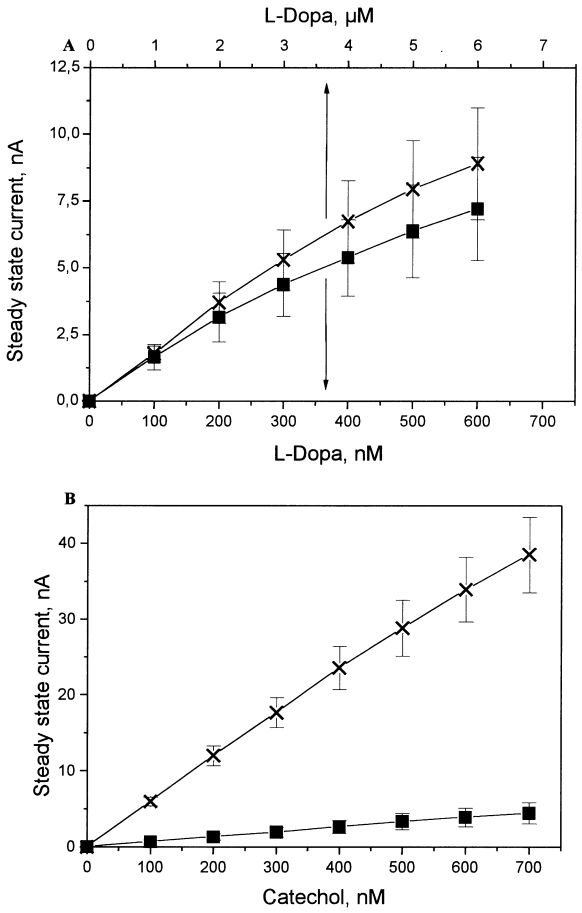

Fig. 7. The calibration lines obtained for L-dopa (A) and catechol (B) with the *S. antibioticus*tyrosinase/GDH-membrane (ST-sensor, ■) and the mushroom/GDH-membrane (MT-sensor, ×). Note different concentration ranges.

for catechol. Phenol is followed by *p*-chlorophenol and *p*-aminophenol. In contrast to that, L-tyrosine is the best analyte for the ST-sensor, followed by *p*-cresol and *p*-aminophenol. The substrate spectrum of the ST-sensor for monophenolic compounds is very broad with only slight differences in sensitivity (see above).The relative response of the two different sensors for diphenolic compounds related to catechol is shown in Table 3. In contrast to the mushroom tyrosinase containing sensor, the ST-sensor has shown the highest response for L-dopa, the physiological substrate for this tyrosinase. For the MT-sensor the sensitivity to dopamine is the highest (detection limit of 25 nM, [23]), followed by adrenalin, with one-fifth

of the signal of dopamine. The response of noradrenalin is even lower than the signal of dopamine and adrenalin in both sensors. Also here, except for noradrenalin, the sensitivity of the ST-sensor for the best analyte 4-methylcatechol is only five times higher than for the worst analyte dopamine. This is completely different for the MT-sensor. Here the difference between the sensitivity of the best and the worst analyte is around 100.

For both sensors comparable sensitivities for monophenolic and their related diphenolic compounds were obtained. The only exception was the response of the ST-sensor to *p*-nitrophenol, which was much less than *p*-nitrocatechol.

Parameters such as response time, reproducibility and storage stability of the ST-sensor are comparable with the MT-sensor. However, significant differences were obtained in sensitivity and selectivity.

4. Conclusions

S. antibioticus tyrosinase has been isolated, and used for the first time in a sensor device. Because of the almost exclusive use of mushroom tyrosinase in biosensors we have compared our results with mushroom tyrosinase.

Tyrosinase from mushroom (M) and *S. antibioticus* (S) show a number of differences in their characteristics. *S. antibioticus* tyrosinase is the smaller (M: 138 kD, S: 30.6 kD, subunits: M: 4, S: 1) and more purified (M: 10–15%, S: 98%) enzyme and has with respect to L-dopa a significantly higher specific activity of 1225 U/mg protein than mushroom tyrosinase (5.2 U/mg lyophilisate) This higher specific activity for L-dopa in solution was also obtained with the *S. antibioticus* tyrosinase based biosensor. L-dopa can be determined with the ST-sensor in the lower nanomolar range, which is impossible using the MT-sensor. Furthermore, it is possible to detect with the ST-sensor catecholamines in the lower nanomolar range, which is important in clinical analysis. But also other phenolic compounds, like *p*-cresol, *p*-aminophenol or hydroquinone can be determined with a higher sensitivity using the ST-sensor instead of the MT-sensor. Moreover, the ST-sensor has for most of the phenolic compounds a very similar sensitivity, whereas the MT-sensor has a broad sensitivity profile for these compounds. Thus, the new developed sensor could be used as detector for the sum of phenols.

A very important advantage of *S. antibioticus* tyrosinase is that information about the structure, the active centre and kinetic behaviour are available, while this is not for mushroom tyrosinase. The purification protocol and the good reproducibility also make tyrosinase from *S. antibioticus* an interesting enzyme for analytical applications. The purification procedure is very fast and results in an enzyme with a high purity. It is possible to obtain, with every new purification, the same quality of the enzyme preparation [1]. This is a great advantage in comparison with the commercially available mushroom tyrosinase, because it is known that the sensitivity and the selectivity of tyrosinase-modified electrodes are highly dependent on the purity of the tyrosinase preparation [24].

Our future research will focus on new techniques of enzyme immobilisation, including the development of new material for ultrathin coating of the electrode surface. This is of particular importance in the miniaturisation of biosensors. Furthermore, studies will be carried out to manipulate the enzymatic substrate selectivity, for instance by protein engineering. For future developments of tyrosinase-based biosensors it is imperative that an expression system is available combined with an excellent and fast purification procedure, which is the case for *S. antibioticus* tyrosinase.

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