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TYROSINE HYDROXYLASE ACTIVITY IN TWO-DIMENSIONAL MONOMOLECULAR FILMS

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Tyrosine hydroxylase (TH) is a neurospecific regulatory enzyme of catecholamine biosynthesis, catalyzing oxidative conversion of L-tyrosine into L-dihydroxyphenylalanine (L-dopa). TH is an important component of the catecholaminergic systems of the brain. Agonists and antagonists [4] of presynaptic dopamine receptors are its specific allosteric modulators. It was shown previously that determination of the kinetic parameters of TH in the presence of various pharmacologically active substances in vitro can be effectively used to detect a catecholaminergic component in the molecular mechanism of action of these substances [4, 5]. It is accordingly interesting to use TH as a sensory element for the primary detection of compounds with a dopaminergic mechanism of action. With this aim we have therefore investigated the kinetic characteristics of TH in two-dimensional monomolecular films, formed on solid surfaces.

EXPERIMENTAL METHOD

Highly purified TH was isolated from the rat hypothalamus with the aid of biospecific chromatography, using as the adsorbent di-iodothyroninesepharose 4B [3]. A 10% homogenate of hypothalamic tissue in 0.32 M sucrose was centrifuged at 1000*g* (10 min) and the supernatant was centrifuged at 100,000*g* (60 min). The residue was suspended in 50 mM Tris-maleate buffer, pH 6.0, containing 0.1% Triton X-100. After centrifugation at 15,000*g* (30 min) the supernatant was applied to a column of Sephadex G 6-10, equilibrated with 50 mM Tris-maleate buffer, pH 6.0. The eluate from this column, containing a protein peak, was applied to a column of di-iodothyroninesepharose 4B, equilibrated with 10 mM K-phosphate, pH 6.0. After efflux of the ballast proteins and oligomeric form of TH, the column was eluted with 10 mM K-phosphate buffer, pH 8.5. After emergence of the peak at pH 8.5, elution was carried out with water. The aqueous eluate was lyophilized and used for the work. This eluate contained TH with molecular mass of 36 kDa.

Monomolecular films of TH were obtained by the Langmuir—Schafer technology, by means of which monomolecular layers of amphiphilic molecules can be formed on the surface of water and transferred to a solid-phase carrier with surface tension of 20 mN/m. TH also was immobilized by a simple sorption method, by placing solid surfaces in a solution of the enzyme (1 mg/ml) for 30 min. As solid-phase surfaces (slabs measuring 9 × 9 mm) we used surfaces of gold, polycor,

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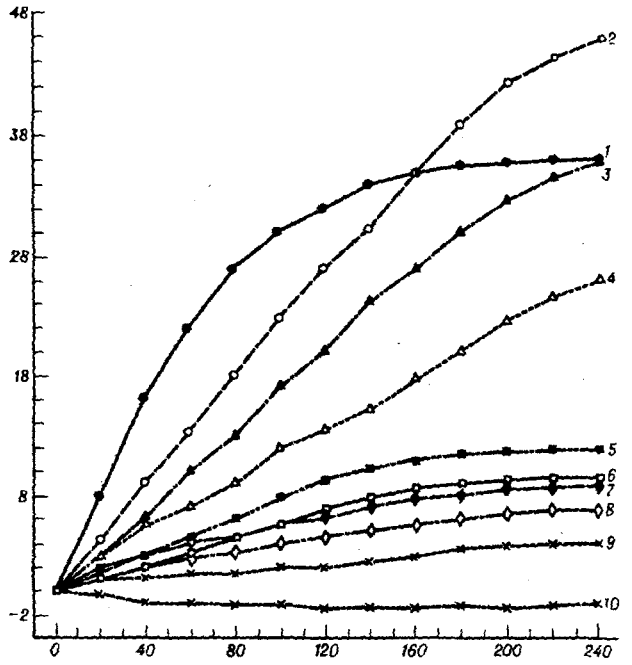

Fig. 1. TH activity in monomolecular films formed on different surfaces. Abscissa, time (in min); ordinate, absorbance at 334 nm (conventional units). 1) TH immobilized on a surface containing ionized carbon, by the sorption method. Monomolecular films on the following surfaces; 2) gold, 3) ionized carbon, 4) PEI monolayer on gold, 5) PEI monolayer on silicon, 6) PEI monolayer on PEI adsorbed on silicon; 7) silicon, 8) polycor, 9) adsorbed PEI on silicon; 10) PEI monolayer on polycor.

and silicon. The velocity of the tyrosine hydroxylase reaction was measured by a direct spectrophotometric method [2], the increase in absorbance at 335 nm being measured with continuous recording on an "Aminco DW-2" differential spectrophotometer (USA) at 37°C. The composition of the sample was: 3 ml of 50 mM Tris-maleate buffer, pH 6.15, 0.03-100 μM L-tyrosine, 100 μM 5,7-dimethyl-5,6,7,8-tetrahydropterine ($DMTH_4$), and the slabs with the enzyme. The comparison cuvette did not contain L-tyrosine. The velocity of the tyrosine hydroxylase reaction was calculated as the tangent of the angle of slope of the curve, using a molar extinction 4200 difference coefficient. The Michaelis' constant (K_m) was calculated by the method in [1].

EXPERIMENTAL RESULTS

Dependence of the velocity of the tyrosine hydroxylase reaction on time is illustrated in Fig. 1. Clearly all the curves have linear regions. Linearity of the reaction in the case of TH immobilized by the sorption method (Fig. 1, 1) is of short duration (about 60 sec), whereas in monolayers of the enzyme on a surface of gold and on a surface treated with ionized carbon (Fig. 1, 4) the linear regions have a longer period (200-240 sec). Activity of TH in monomolecular films on a polycor (Al_2O_3) or silicon (Si) surface is reduced (Fig. 1: 8, 7). Preliminary treatment of the surfaces with polyethylenimine (PEI) either led to partial inactivation of the TH (Fig. 1:4) or did not change TH activity (Fig. 1: 5, 6, 9, 10).

It was necessary to establish whether the kinetic parameters and and allosteric regulation of TH are modified in the superficial monomolecular films of the enzyme. The velocity of the reaction depending on L-tyrosine concentration is shown in Fig. 2. Dependence of velocity of the tyrosine hydroxylase reaction on substrate concentration is described by a curve with a maximum, and the descending branch of the curve indicates inhibition of the enzyme by high concentrations of L-tyrosine, just as in the case with the soluble enzyme [8]. Meanwhile an appreciable decrease in affinity for the substrate with K_m up to 180 μM compared with the enzyme in solution (K_m = 4 μM), possibly due to partial inactivation of

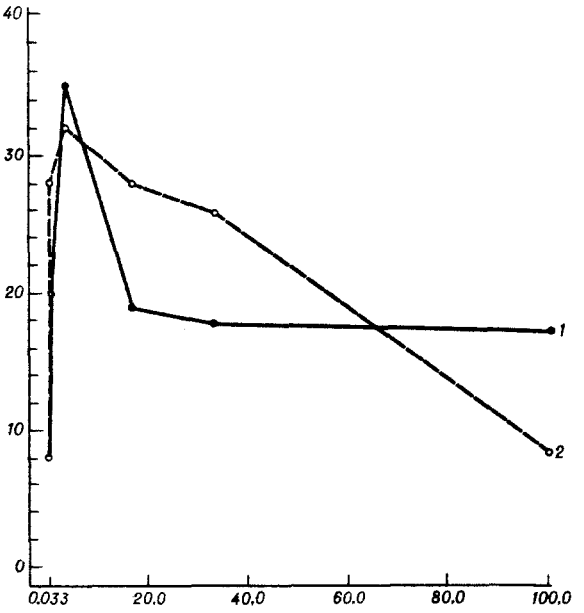

Fig. 2. Velocity of tyrosine hydroxylase reaction depending on L-tyrosine concentration. Abscissa, L-tyrosine concentration (in μM); ordinate, absorbance at 335 nm (conventional units). 1) TH on a gold surface; 2) TH treated beforehand with heparin, on a gold surface.

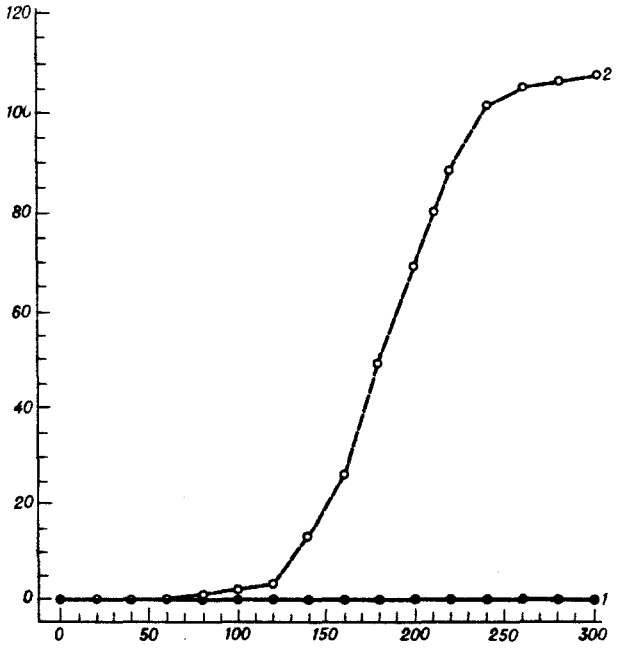

Fig. 3. Velocity of tyrosine hydroxylase reaction in the absence (1) and presence (2) of 10 M trifluperazine. Abscissa, time (in min); ordinate, absorbance at 335 nm (in conventional units).

TH on the water/air phase boundary. However, preliminary treatment of the enzyme with a polyanion (heparin, 1 μM) before formation of the monomolecular film led to a decrease in K_m to 20 μM.

To test the site of the allosterically regulatory region responsible for substrate inhibition of TH, we used a known typical neuroleptic, namely trifluperazine. It was shown previously that neuroleptics can act as specific allosteric ligands of TH, eliminating substrate inhibition [5]. The results of these experiments are given in Fig. 3. In the presence of trifluperazine (Fig. 3:2) a sufficiently high velocity of the tyrosine hydroxylase reaction was observed, i.e., inhibition of the enzyme by tyrosine (Fig. 3:1) is abolished.

Data in the literature [7] and the results of our own experiments indicate that an oxygen electrode can be used to analyze the tyrosine hydroxylase reaction (data not shown).

The investigation thus showed that TH in monomolecular films formed on negatively charged surfaces preserves its kinetic characteristics. The presence of specific allosteric regulation of the enzyme activity by a neuroleptic, in this case trifluperazine, means that monomolecular films of the enzyme can be used to create a biosensor for primary screening of compounds with neuroleptic structure by the method in [6].

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