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CATALYTIC PROPERTIES AND ELECTROSTATIC POTENTIAL OF CHARGED IMMOBILIZED ENZYME DERIVATIVES

PYRUVATE DECARBOXYLASE ATTACHED TO CATIONIC POLYSTYRENE BEADS OF DIFFERENT CHARGE DENSITIES

J. BEITZ[a], A. SCHELLENBERGER[a], J. LASCH[b] and J. FISCHER[c]

[a]*Sektion Biowissenschaften, Wissenschaftsbereich Biochemie,* [b]*Physiologisch-chemisches Institut der Martin-Luther-Universität, DDR-402 Halle, (G.D.R.) and* [c]*Institut für Enzymologie und technische Mikrobiologie, DDR-1017 Berlin (Germany)*

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Summary

Pyruvate decarboxylase has been covalently attached to positively charged macroporous polystyrene resins. The changes in the functional behaviour of the enzyme resulting from the electrostatic interaction of the cationic carrier polymer and the anionic substrate have been studied as function of the fixed charge concentration of the carrier. The findings confirm the connection of two widely used theoretical expressions relating electrostatic parameters with Michaelis constants.

Enzymes linked artificially to support materials which carry charged functional groups have received considerable attention in the last two decades [1—5]. The catalytic behaviour of an enzyme in a polyelectrolyte phase to a charged substrate may differ from that of the same enzyme in solution even if the intrinsic catalytic parameters are unchanged. The quantitative relationship between the $K_{m,app}$ of an enzyme attached to a polyelectrolytic carrier, the K'_m of the corresponding uncharged enzyme derivative, and the electrostatic potential, Ψ, of the charged support was derived by Goldstein et al. [3]:

$$K_{m,app} = K'_m \exp(z\, e\, \Psi / k\, T) \quad (1)$$

where z is a positive or negative integer; e, the electronic charge and k, the Boltzmann constant. Wharton et al. [5] used a different approach in which

the dependence of $K_{m,app}$ on the ionic strength, I, and the fixed charge concentration in the matrix volume, $Z_{m,c}$, is given explicitly:

$$I/K_{m,app} = I/\gamma K'_m + Z_{m,c}/2\,K'_m \quad (2)$$

where γ is the ratio of the mean ion activity coefficients of the matrix and the bulk phase.

The purpose of this work was to study the kinetic behaviour of a polyelectrolyte enzyme derivative as function of the concentration of fixed charges in order to find out whether the relationship between the two theories could be verified experimentally. The reaction studied was the decarboxylation of pyruvate by pyruvate decarboxylase [6]. Aminomethylpolystyrene was chosen as carrier, because under reaction conditions (pH 6.0) its cationic form can interact electrostatically with the negatively charged substrate.

Pyruvate decarboxylase (2-oxo-acid carboxy-lyase, EC 4.1.1.1) was isolated from *Saccharomyces carlsbergensis* [7]. The purified enzyme [8] had specific activities of 20—35 U/mg, where one unit of enzyme activity is defined as the activity converting 1 μmol pyruvate per min (50 mM maleate buffer, 20°C).

Five types of chloromethylpolystyrene AD 41 (crosslinked with 5.5% divinylbenzene, particle size 500—600 μm, inner surface 40 m^2/g) containing different amounts of -CH_2Cl (1—4 mmol Cl/g resin) were obtained from VEB Chemiekombinat Bitterfeld. The chloromethyl derivatives were converted to aminomethylpolystyrenes with hexamethylenetetramine according to Ref. 9. The resins treated in this way contain, besides aminomethyl, aromatic aldehyde groups (Sommelet reaction [10]). Aromatic aldehyde groups (0.30—0.7 mmol/g) were detected by infrared spectroscopy (υ_{CO} = 1705 cm^{-1}) and determined according to [11]. The resins were denoted P_1 to P_5 in order of increasing amino group content (Table I). A reference resin devoid of amino groups, P_0, was prepared by suspending 1 g of P_2 (0.95 mmol-NH_2/g, 0.3 mmol -CHO/g) in 10 ml of 15% acetic acid, adding 1 g $NaNO_2$ in 3 ml water and stirring for 4 h at 85°C. After washing with water no amino group could be detected. The content of -CHO groups was unchanged.

The immobilization was carried out as follows. 1—3 ml enzyme solution (10—40 mg enzyme/ml) in 50 mM maleate buffer, pH 6.0, were shaken with 50—500 mg of the different resins (P_0 to P_5) for 3 h at 4°C. Thereafter, the enzyme resins were washed with 50 mM maleate buffer, pH 6.0/1M NaCl and water with stirring. Pyruvate decarboxylase is bound covalently to the polystyrenes by formation of Schiff bases with the matrix aldehyde groups [16].

The K'_m value of P_0-pyruvate decarboxylase (Table I) is about 30 times higher than the K_m of the soluble enzyme which is 1.2 mM [17]. It was carefully checked that there was no diffusion limitation by varying the enzyme load of the carriers and by calculating the effectiveness factors (above 0.95) with the help of Engasser nomograms [18]. The high K'_m value of P_0-pyruvate decarboxylase can be explained by the chemical modification and/or noncovalent matrix-enzyme interactions. The substrate-activation behaviour of

TABLE I

CHARACTERISTIC PARAMETERS OF POLYSTYRENE-PYRUVATE DECARBOXYLASE DERIVATIVES

To estimate $Z_{m,c}$, 1 g of each resin was swollen in 50 mM maleate buffer, pH 6, in a measuring cylinder for 16 h and the volume of the settled particles was read off. The volume of the swollen matrix was calculated by subtracting the void volume (37%) [12]. The amino group content [13] of P_1 to P_5 amounted to 0.8—3.6 mMol/g dry resin. The amount of bound protein was calculated from the difference of protein added and pooled in the washings (protein determination, see Ref. 14). Enzyme activities were determined by the coupled optical test [15] in 50 mM maleate buffer, pH 6.0, at 20°C. Before the assays, the enzyme resins were activated with 67 mM pyruvate solution for 12 min under stirring and thereafter washed with buffer. During the reaction (15 min) the suspension was stirred intermittently in a cuvette and the absorption at 340 nm was read off in 3-min intervals. The mM). Ψ_1 was calculated from the Michaelis constants according to Eqn. 1 and Ψ_2 by Eqn. 4 with I = 0.1 M and $\gamma = 1$. The results presented are mean values of three experimental series. P_0—P_5 are polystyrene resins denoted 1—5 in order of increasing content of amino groups.

Polystyrene-pyruvate decarboxylase	Bound protein (mg/g)	Activity* (μmol·min^{-1}·g^{-1})	$Z_{m,c}$ (mol -NH_2/l swollen resin)	$K_{m,app}$ (mM)	Ψ_1 (mV)	Ψ_2 (mV)
P_0-PDC	16	17	0.00	33.2**	0.0	0.0
P_1-PDC	22	27	0.39	12.3	25.1	27.3
P_2-PDC	15	15	0.45	11.7	26.4	29.8
P_3-PDC	11	21	0.65	7.9	36.3	36.6
P_4-PDC	21	23	1.18	5.1	47.4	48.8
P_5-PDC	31	35	1.48	4.6	50.0	53.8

*Preserved activities, 6—8%.
**K'_m (see text).

the immobilized enzyme lends considerable support to this interpretation. Pyruvate decarboxylase (exhibiting a sigmoidal *v/s*-characteristic) is activated reversibly by its substrate [19]. Contrary to the soluble enzyme, P_0- to P_5-pyruvate decarboxylase remains in its active conformation even if the pyruvate is removed. In other words, the enzyme bound to modified polystyrenes, loses its regulatory properties (Hill coefficient $n = 1$) [16]. The soluble enzyme has a Hill coefficient of 2.

The electrostatic potentials Ψ_1 given in Table I were calculated from the $K_{m,app}/K'_m$ ratios, according to Eqn. 1. K'_m and not the K_m of the soluble enzyme was taken as reference.

The dependence of the $K_{m,app}$ values on the fixed charge concentration of the enzyme resins is illustrated by Fig.1 according to Eqn. 2. The condition $0 < (K_{m,app}\, Z_{m,c}/2\, K'_m\, I) < 1$ was checked and found to be fulfilled. The agreement between the K'_m of P_0-pyruvate decarboxylase as determined from the Lineweaver-Burk plot, K'_m = 33.2 mM (Table I), and the value obtained from the ordinate intercept of Fig.1, K'_m = 32.1 mM, is excellent.

From Eqns. 1 and 2 it follows [1] that

$$\exp(-z\, e\, \Psi/k\, T) = 1/\gamma + Z_{m,c}/2I \qquad (3)$$

Rearrangement of Eqn. 3 leaves us with

$$-\Psi = (k\, T/z\, e)\, \ln(1/\gamma + Z_{m,c}/2I) \qquad (4)$$

Knowing γ and I, Eqn. 4 was used to calculate Ψ as function of the fixed charge concentration (Ψ_2 in Table I). The activity coefficient ratio γ, which was determined by plotting $\exp(-ze\,\Psi_1/k\,T)$ vs. $Z_{m,c}/2I$ (Eqn. 3), is equal to unity. The potentials Ψ_1 computed from the $K_{m,app}/K'_m$ ratios are in good agreement with the potentials Ψ_2 which were calculated on the basis

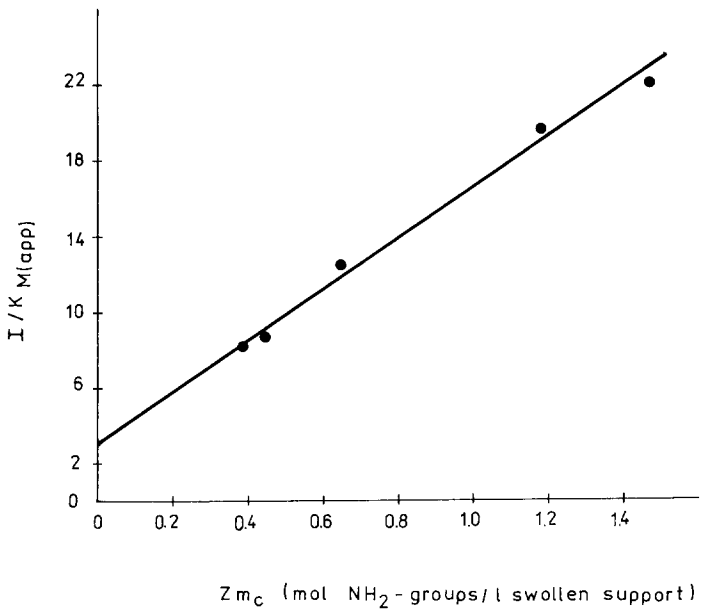

Fig.1. Plot of $I/K_{m,app}$ vs. $Z_{m,c}$ according to Eqn. 2. The values of $K_{m,app}$ and $Z_{m,c}$ are given in Table I; I = 0.1 M.

of the fixed charge concentrations via Eqn. 4.

We conclude that Eqn. 4 can be utilized to estimate the electrostatic potential if the charge density of synthetic charged enzyme carriers is known. On the other hand, the charge density might be derived from the potential which in turn can be computed from measured K_m shifts.

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