Derivation of the Rate Equation for the Aspartate Aminotransferase Mechanism from the Michaelis-Menten Assumptions

The rate equation for the enzymatic mechanism exemplified by aspartate aminotransferase has been derived from steady-state theory (ALBERTY[1], VELICK and VAVRA[2]), but it has not been reported before that the derivation can be made using the Michaelis-Menten conditions.

Symbols are those recommended by the Enzyme Commission of the International Union of Biochemistry, modified in that the 2 half-reactions are treated separately, the symbols referring to the second enzyme form being distinguished where necessary by the prime ('), and in that certain additions are required, namely:

$$e_t = e + e' \text{ (total enzyme concentration)},$$

K_a, K_b are the 'true K_m' for the substrates A and B, as defined by VELICK and VAVRA[2].

The 2 half-reactions are:

$$E + A \rightleftharpoons E\,A \xrightarrow{k} E' + \text{product 1},$$

$$K_A \equiv (e - p)a/p\,,$$

$$E' + B \rightleftharpoons E'\,B \xrightarrow{k'} E + \text{product 2},$$

$$K_B \equiv (e' - p')b/p'\,.$$

For any constant overall reaction rate, the rates of the 2 half-reactions must be equal, for if they were not, the enzyme form produced by the faster would accumulate, and the enzyme form consumed by it would be depleted until the 2 rates became equal, i.e. $v = k\,p = k'\,p'$. Therefore $(p/p') = (k'/k)$ $(= R$, say). Thus the concentrations of the 2 enzyme-substrate complexes are in a fixed ratio during any period of constant reaction rate, irrespective of substrate concentrations. The value of R could be altered if k and k' themselves were altered but not otherwise.

Note that,

$$e = p\,(1 + K_A/a), \qquad e' = p\,(1 + K_B/b)/R\,.$$

Therefore,

$$e = \frac{e_t\,R\,(1 + K_A/a)}{1 + K_B/b + R\,(1 + K_A/a)}\,,$$

$$v = k\,p = k'\,e/R\,(1 + K_A/a) = \frac{k'\,e_t}{1 + K_B/b + R\,(1 + K_A/a)}\,.$$

The rate equation derived from steady-state theory may be written,

$$V_{max}/v = 1 + K_a/a + K_b/b\,,$$

and the following correspondences are readily obtained:

$$V_{max} = k'\,e_t/(1 + R)\,, \qquad K_a = R\,K_A/(1 + R)\,,$$

$$K_b = K_B/(1 + R)\,.$$

The new equation predicts half-maximal velocity when half the total enzyme exists as total enzyme-substrate complex. This may be achieved in an infinite variety of combinations of substrate concentrations, but fixing the concentration of one, fixes the other.

The factors $1/(1 + R)$ and $R/(1 + R)$ express the concentration of $E'B$ and EA respectively as proportions of the total enzyme-substrate complex. It should be noted that R cannot be equated with the ratio of pyridoxal to pyridoxamine forms of aspartate aminotransferase. Indeed it has no simple physical interpretation, since there are, in reality, more than 2 forms of enzyme-substrate complex.

Résumé. On peut déduire l'équation de vitesse de la réaction catalysée par la transaminase glutamique – oxalacetique (EC 2.6. 1.1) en utilisant les postulats de Michaelis et Menten, ainsi que l'hypothèse de «Steady-State».

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Effect of Hormones on Acid Mucopolysaccharide Synthesis in Mouse Skin. An Enzyme Study

It has been found that uridine diphosphate-D-glucuronic acid (UDPGA) and glucosamine-6-phosphate (Gm-6-P) are important intermediates as monosaccharide units for the synthesis of acid mucopolysaccharides (AMPS)[1]. UDPGA can be converted enzymatically from uridine diphosphate-D-glucose (UDPG) by a dehydrogenase[2], and hexose-6-phosphate and L-glutamine (L-glut.) form Gm-6-P and glutamic acid by the L-glut.-D-hexose-6-phosphate transamidase[3]. The metabolism of AMPS is known to be influenced by various hormones[4]. However, some of the data are still contradictory[5,6].

In this investigation, UDPG dehydrogenase (UDPG-DH), L-glut.-D-fructose-6-phosphate(F6P)-transamidase and L-glut.-D-glucose-6-phosphate(G6P)-transamidase activities were studied in the normal skin of mice, as were the effects of various hormones on these enzymes comparatively.

Materials and methods. Thirty-six female and 12 male Swiss albino mice, weighing 16–23 g, were kept on an optimal laboratory diet and were given water ad libitum. They were divided into 7 groups, i.e. normal and treated with 4 different hormones. In the sex hormone-treated

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UDPG dehydrogenase, L-glut.-F6P-transamidase and L-glut.-G6P-transamidase activities in the skin of normal and hormone-treated mice

Treatment		UDPG-DH	L-glut.-F6P-transamidase	L-glut.-G6P-transamidase
Normal		0.33 ± 0.02 (12)	23.1 ± 1.4 (13)	0.5 ± 0.1 (15)
Cortisone acetate		0.23 ± 0.02 (5)	13.2 ± 0.5 (5)	0 (5)
DOCA		0.20 ± 0.04 (5)	11.6 ± 0.7 (5)	0 (5)
Estradiol benzoate	female	0.42 ± 0.05 (5)	36.3 ± 3.3 (6)	0 (5)
	male	0.83 ± 0.09 (6)	57.3 ± 5.4 (6)	0 (5)
Testosterone propionate	female	0.22 ± 0.01 (5)	10.9 ± 1.7 (5)	0 (5)
	male	0.63 ± 0.03 (6)	43.8 ± 2.9 (6)	0 (5)

Data for enzyme activities are in the following units: UDPG-DH, μmoles of NAD reduced/min/mg protein; L-glut.-F6P-transamidase and L-glut.-G6P-transamidase, μmoles glucosamine synthesized/h/mg protein. Each value represents the mean ± standard error of the mean. The number of determinations are shown within brackets.

groups, animals of both sexes were used to reveal a possible sex difference. Each mouse received a s.c. injection in the lateral femoral region twice a week for 4 weeks. The names and doses of hormones at each time were as follows: 2.5% cortisone acetate suspension 0.1 ml (2.5 mg) (Cortison-acetat Ciba®, Ciba, Basle, Switzerland), 1% desoxycorticosterone acetate suspension 0.1 ml (1.0 mg) (Percorten®, Ciba, Basle, Switzerland) (DOCA), 0.1% estradiol benzoate oleosa 0.1 ml (0.1 mg) (Ph. Nord. 63), and 5% testosterone propionate oleosa 0.05 ml (2.5 mg) (Ph. Nord. 63). The animals were killed 3–6 days after the last injection. The abdominal skin was shaved, excised, freed of s.c. fatty tissue, and immediately minced with a razor blade. A 20% homogenate in 0.154 *M* KCl was made using a motor-driven glass homogenizer at 4 °C.

The supernatant centrifuged at 13,000 *g* for 60 min was used for the assay. The assay procedure of UDPG-DH was used with a few modifications of the method of STROMINGER et al.[2], as published previously by SASAKI[7]. L-glut.-F6P-transamidase and L-glut.-G6P-transamidase were determined by the method of POGELL and GRYDER[8]. Hexosamine and protein determinations were carried out by the methods of KIRK and DYRBYE[9], and WEICHSELBAUM[10], respectively.

Results. The enzyme activities of each group are illustrated in the Table. The activities of UDPG-DH and L-glut.-F6P-transamidase were parallel. Compared with enzyme activities of the untreated animals, significantly lower values were demonstrated in the skin of mice treated with cortisone acetate and DOCA ($P < 0.001$). The UDPG-DH and L-glut.-F6P-transamidase activities in the skin of estradiol-treated mice of both sexes were highly increased beyond the level of normals ($P < 0.001$), and male mouse skin showed higher values than female mouse skin ($P < 0.001$). These 2 enzyme activities in the skin of testosterone-treated mice were found to be higher in males ($P < 0.001$), and in females even lower than in the untreated. A slight activity was demonstrated by determining L-glut.-G6P-transamidase in the skin of untreated mice. However, no enzyme activity was observed in the skin of mice treated with various hormones.

Discussion. The reduced activity observed in the skin of cortisone-treated mice confirms several publications on the inhibitory effects of corticosteroids on the synthesis of AMPS[4,11]. Some histological studies and ^{35}S-uptake data have indicated increased accumulation and turnover of AMPS in connective tissue by administration of DOCA[12,13]. On the other hand, it was found that DOCA showed its inhibitory effect on fibroblasts in tissue culture[14]. It could be presumed that DOCA inhibits biosynthesis of AMPS, as the enzyme activities determined in this study were found lowered. Increased activities of UDPG-DH and L-glut.-F6P-transamidase in the skin of estradiol-treated mice may indicate that estradiol benzoate stimulates accumulation of AMPS in the skin as reported by SINOHARA and SKY-PECK[5], in spite of the opposite report by PRIEST et al.[6]. However, WARREN and FAGAN[15] demonstrated that among various rodents only Swiss albino mice responded with accumulation of AMPS by estradiol. It is of interest that after testosterone opposite data were seen between the sexes in this study. However, there is no satisfactory explanation for these results at present.

A rather low activity of L-glut.-G6P-transamidase, compared with L-glut.-F6P-transamidase activity, was observed in the normal skin. However, it seems remarkable that no activity was found in skin under the influence of 4 different hormones, even in the skin of mice treated with estradiol, of which the UDPG-DH and L-glut.-F6P-transamidase activities were very high. Two different metabolic pathways may exist for the synthesis of Gm-6-P, either from F6P or G6P. Although POGELL and GRYDER[8] and SASAKI[7] reported that the more active precursor was G6P, BOLLET and SHUSTER[16] found both G6P and F6P, and GHOSH et al.[17] demonstrated F6P. In this study, F6P seems to be the more active substrate for the formation of Gm-6-P.

Zusammenfassung. Die Aktivität dreier mucopolysaccharidsynthetisierender Enzyme in der Haut normaler Mäuse wurde von Cortison und DOCA gehemmt, von Östradiol und Testosteron hingegen stimuliert. Eine Ausnahme bildet die Gruppe testosteronbehandelter weiblicher Mäuse.

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