CXLVII. MALIC DEHYDROGENASE[1]

By KÁLMÁN LAKI

From the Institute of Medical Chemistry, University of Szeged

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The dehydrogenation of malic acid by malic dehydrogenase has already been extensively studied [*v.* Harrison, 1935], but the question as to whether it is malic or fumaric acid which is dehydrogenated by this enzyme has not yet been settled. Tissues contain the very powerful catalyst, fumarase, which converts fumaric or malic acid into the equilibrium mixture of both substances. Fumarase, as compared with malic dehydrogenase is so active that it is immaterial which of the two substances is added for, owing to the activity of fumarase, both substances will behave in the same way as their equilibrium mixture.

Working with malic dehydrogenase I observed that in the Thunberg experiments the decoloration of dyes occurred slightly faster if fumaric acid was added instead of malic, an observation which made me conclude that it was fumaric acid which was dehydrogenated by the enzyme and that the somewhat longer time in the case of malic acid was due to the necessity of this substance being converted into fumaric acid by fumarase.

Green [1936], however, presented experiments which suggested that malic acid is dehydrogenated as such and not as fumarate. In the present paper further evidence, of a more decisive kind, has been obtained by experiments with a dehydrogenase preparation poor in fumarase. Such an enzyme can be prepared from horse meat by Lehman's [1920] method. Experiments with such preparations clearly show that, if the activity of fumarase is strongly reduced, malic acid is dehydrogenated more rapidly than fumaric acid, thus proving the correctness of Green's views. Muscle tissue thus seems to contain malic dehydrogenase but no fumaric acid dehydrogenase, and the catalytic transfer of H, according to Szent-Györgyi's theory, must occur via the reaction malic-oxaloacetic acid instead of via the reaction fumaric-oxaloacetic acid, as originally believed.

Experimental

Preparation of enzyme. 50 g. of minced horse meat (from young animals) were suspended in 50 ml. cold 0·9 % NaCl. This was pressed out through a cloth and the juice centrifuged. The deposit was then suspended in 100 ml. distilled water and kept for 4 min. in the water-bath at 50° with constant mixing. The warm suspension was centrifuged. (The centrifuge tubes and their holders were warmed beforehand to 50°.) The precipitate was suspended in 10 ml. 0·9 % NaCl. 1 ml. of this suspension was used for every experiment in which fumarase-poor enzyme was required.

Fumarase-rich enzyme was prepared in the same way, water at 0° being used instead of at 50°.

Dehydrogenation experiments were carried out in the modified Thunberg apparatus with six tubes [Szent-Györgyi, 1935].

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Solutions. 134 mg. *l*-malic acid were neutralized with 2 ml. *N* NaOH and diluted to 10 ml. with 2·7 *M*/15 phosphate *p*H 7·4. Fumaric acid solutions were prepared in the same way, 116 mg. of this substance being used. The coenzyme solution contained 0·1 mg. cozymase per ml.

Dye. 0·25 mg. sodium indo-2: 6-dichlorophenol-1-naphthol-2-sulphonate per ml. ($E_{h_0} = +0{\cdot}119$ V. at *p*H 7 [Gibbs *et al.* 1928].)

Estimation of fumarase activity

1 ml. enzyme and 3 ml. water were pipetted into 50 ml. Erlenmeyer flasks and incubated in the water-bath at 37·5°. After allowing 10 min. for temperature equilibrium to be reached, 1 ml. of the fumarate solution was added, the solution being warmed to 37·5° before addition. After 0, 5, 10, 20 or 40 min. 1 ml. 20 % trichloroacetic acid solution was added. After filtration the fumarate present was estimated by Straub's [1935] method. Table I gives the fumarate found in mg. calculated for the original volume of 4 ml.

Table I

Time min.	50° enzyme	0° enzyme
0	11·0	11·0
5	—	4·8
10	10·8	3·0
20	8·5	2·5
40	7·5	—

Thunberg experiment

1 ml. enzyme, 1 ml. fumarate or malate, 1 ml. coenzyme, 1 ml. dye; temp. 37·5°. The fumarate or malate was placed in the side-tube and added after evacuation.

Results obtained with enzymes poor in fumarate (50°) are summed up in Table II. The numbers give the time of decoloration in minutes. Most experiments were done in duplicate.

Table II

	Time of decoloration in min.	
No. of exp.	Malate substrate	Fumarate substrate
I	13, 12	21, 19
II	20, 22	40, 37
III	12	20
IV	18, 19	23, 24

In all experiments the control tubes (to which no malate or fumarate had been added) showed no decoloration in 120 min.

Table II shows that in all the experiments in which the fumarase concentration was strongly reduced the decoloration in presence of malate proceeded distinctly faster than in presence of fumarate.

In experiments carried out with the enzyme, washed at 0°, decoloration proceeded at the same rate in the case of malic and fumaric acids, apart from slight irregular variations.

SUMMARY

Muscle dehydrogenase enzyme preparations, in which the activity of fumarase is strongly reduced, dehydrogenate malate faster than fumarate. It is presumably, therefore, malic acid rather than fumaric acid that is dehydrogenated by the enzyme.

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