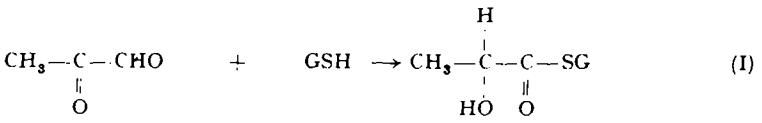
Mechanism of the action of glyoxalase I*

The enzyme glyoxalase I catalyzes the intramolecular oxidation-reduction and simultaneous thiolesterification shown in equation (I). The reaction is of considerable interest because of its formal resemblance to the Cannizzaro reaction which gives lactic acid from methyl glyoxal in alkali. It is known that the latter reaction occurs without exchange with the hydrogen of the water in the medium[1], and indeed it has been confirmed in this laboratory that the lactic acid thus formed in the presence of TOH is not radioactive.

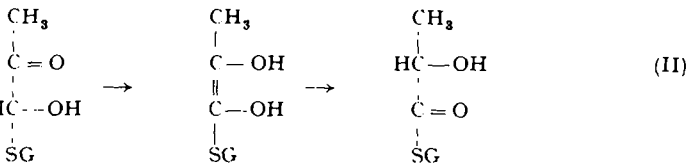
For the glyoxalase I reaction, RACKER[2] has proposed a mechanism the essential feature of which is the formation of an ene-diol of the thiohemiacetal formed between the two substrates (equation II). It would be predicted from this mechanism that, if the enzymic reaction were performed in the presence of TOH, the resulting S-lactylglutathione should acquire a tritium atom, since the hydrogen of the alpha carbon of the lactate must come either from the medium or from an atom readily exchanged with the medium. On the other hand, failure of the lactate to become labeled would indicate the operation of a mechanism similar to that of the nonenzymic Cannizzaro reaction.

To test these alternatives, yeast glyoxalase I was prepared up to the second heat step according to RACKER[2]. Its specific activity was about 20% of that reported for the crystalline enzyme. The preparation was shown to be free of thiolesterase activity upon S-lactylglutathione. Methyl glyoxal, assayed according to FRIEDEMANN[3], was prepared free of lactic acid by passing the freshly redistilled material through Dowex-1-Cl^-. Glutathione was freshly dissolved and neutralized before use.

The following mixture was incubated at 30° in 2.6 ml of water containing TOH, glutathione (200 μmoles), methyl glyoxal (175 μmoles), and enzyme preparation (33 mg protein). The pH was adjusted to 6.6 with alkali. The progress of the reaction was followed by observing the optical density at 240 mμ of an aliquot of the incubation mixture diluted in acid. The reaction proceeded with the formation of 160 μmoles of lactylglutathione (assuming E_M of 3,370) in 15 min. It was then stopped with trichloracetic acid, protein was removed by centrifugation, and the supernatant solution was extracted with ether to remove acid. The remaining solution was neutralized carefully and placed on Dowex-1-Cl^-. After extensive washing of the resin with water, the lactylglutathione was eluted with 0.01 *N* HCl with a recovery of 85% as determined spectroscopically. Radioactivity was determined in a scintillation counter, and a small amount of radioactivity, which did not follow the elution curve of the thiolester, was found. The maximum specific activity that could be attributed to the thiolester was 40 c.p.m./μmole, whereas that of the TOH in the incubation mixture was 1700 c.p.m./μatom.



(I)



(II)

It has been suggested that thiolesterification has the effect of labilizing hydrogen at the alpha carbon atom of the acyl group[4]. This idea was put forth to explain the "activation" of acetyl-coenzyme A for condensation at the methyl of the acetyl group. Such labilization might well be accompanied by a rapid exchange of such hydrogens, in which case the result obtained above would be meaningless. To test this possibility another procedure was followed. After completion of the reaction, performed exactly as before, the solution was made strongly alkaline by the addition of a small volume of conc. KOH. The hydrolysis of S-lactylglutathione is very rapid under these conditions as determined by the loss of absorption at 240 mμ of an acidified aliquot. Since the hydrolysis is done essentially *in situ*, any radioactivity in the lactyl group is "stabilized" in the free lactic acid. This was then isolated by ion-exchange or by extraction from diluted acid solution with ether, and lyophilization of the lithium salt. Since the latter method has been used[5] in the isolation of α-deuterolactic acid, it is clear that the α position of

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lactic acid is stable throughout the manipulation. In both cases, the lactic acid isolated was found to contain less than 4% of the specific activity of the water. These results contra-indicate the mechanism of the ene-diol intermediate (II) as well as the proposition that the alpha-hydrogen of this ester is chemically labile*, at least under the conditions of these experiments.

Since pyridine nucleotide-coupled oxidation-reduction reactions are known to occur by direct "hydride shift"[6], it is of interest to examine the question of their involvement in the present reaction. The following evidence would seem to weigh against this: (1) the preparation of the partially purified enzyme has a 280 mμ: 260 mμ ratio of 1.55; (2) the small amount of material lost by treatment of the enzyme with charcoal of with Dowex-1-acetate likewise has a high ratio; (3) neither of these treatments of the enzyme caused any diminution of the glyoxalase activity.

The present data indicate therefore that a hydride shift occurs in the oxidation-reduction reaction catalyzed by glyoxalase I. In this respect the enzymic reaction is analogous to the alkaline-catalyzed Cannizzaro reaction in which methyl glyoxal is converted to lactic acid.

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After this paper had been submitted to the journal, the author learned of the prior publication by FRANZEN[7] of a study in which lactate formed from methyl glyoxal in D_2O by an acetone powder of yeast extract was found to be free of deuterium. We have thus confirmed this result and interpreted it in like manner.

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[1] H. FREDENHAGEN AND K. F. BONHOEFFER, *Z. physik. Chem., A*, 181 (1938) 379.
[2] E. RACKER, *J. Biol. Chem.*, 190 (1951) 685.
[3] T. E. FRIEDEMANN, *J. Biol. Chem.*, 73 (1927) 331.
[4] F. LYNEN, *Federation Proc.*, 12 (1953) 683.
[5] F. A. LOEWUS, P. OFNER, H. F. FISHER, F. H. WESTHEIMER AND B. VENNESLAND, *J. Biol. Chem.*, 202 (1953) 699.
[6] B. VENNESLAND AND F. H. WESTHEIMER, in W. D. MCELROY AND B. GLASS, *A Symposium on the Mechanism of Enzyme Action*, Johns Hopkins Press, Baltimore, 1954, p. 357.
[7] V. FRANZEN, *Chem. Ber.*, 89 (1956) 1020.

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* In an experiment in which transacetylase from *E. coli* was used to catalyse the coenzyme A-dependent arsenolysis of acetyl-phosphate in TOH, the acetate formed was without radioactivity. From this it may be concluded that the α-hydrogens in acetyl-coenzyme A are also stable to spontaneous exchange at neutral pH.

Instability of ketopentose-5-phosphates in buffer solutions of varying pH*

The interconversion of glucose-6-phosphate and ribose-5-phosphate in living cells requires the presence in the cell of a mixture of ribose-5-phosphate and its two ketopentose-5-phosphate isomers. COHEN[1] reported in 1953 that ribulose-5-phosphate is destroyed in tris buffer of pH 8.3 or higher. As the stability of the ketopentose-5-phosphates, which can be assumed to be, if only in small concentration, permanently present in the cells, should be of importance for the cell economy, we investigated the effect of incubation of the two ketopentose-5-phosphates in various buffers of more physiological nature in the range between pH 5.3 and 9.5 and compared it with that in tris buffer. The results are presented in the table. The experiments were carried out with preparations of ribulose- and xylulose-5-phosphate, both of which were free of any significant contamination with the other ketopentose-5-phosphate. In addition, a series of experiments was carried out on an equilibrium mixture of ribose- and the two ketopentose-5-phosphates obtained by incubation of ribose-5-phosphate in a hemolysate for 10 min at 33°, coagulation of proteins at 100°, and separation of the esters from the coagulum by dialysis. The solutions were incubated for 2½ h at 33°, and the reaction was then stopped by adding trichloroacetic acid to a concentration of 2%. The disappearance of ketopentose was measured by comparing its

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