THE ENZYMIC INCORPORATION OF THE β-CARBON OF SERINE INTO DIHYDROXYACETONE

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SUMMARY

Dihydroxyacetone is formed from the β-carbon of serine acting, *via* serine aldolase action, as the 1-carbon acceptor for transketolase with either hydroxypyruvate, fructose-6-phosphate or L-erythrulose as donors. The results obtained are considered in relation to the *in vivo* incorporation pathways of ^{14}C into glycogen from [3-^{14}C]hydroxypyruvate and [3-^{14}C]serine.

INTRODUCTION

In 1958, formaldehyde was shown, by DICKENS AND WILLIAMSON[1], to act effectively as an acceptor for transketolase with hydroxypyruvate giving dihydroxyacetone and carbon dioxide. A new route was suggested for carbohydrate synthesis from L-serine via serine aldolase giving formaldehyde (and glycine) which might react with transketolase and hydroxypyruvate formed from L-serine by a specific transaminase[2].

This communication describes conditions under which the β-carbon of serine becomes incorporated into triose in the presence of hydroxypyruvate and partially purified preparations of serine aldolase and transketolase.

MATERIALS AND METHODS

Transketolase was prepared from dried yeast (Distillers Co. Ltd.) by the procedure of DE LA HABA, LEDER AND RACKER[3] omitting the stages of treatment with protamine and alumina. The preparation (10,000 units/ml; 1450 units/mg protein) was free of carboxylase activity tested by measuring carbon dioxide production from hydroxypyruvate (10 μmoles) in the presence of thiamin pyrophosphate (0.05 mg), magnesium chloride (10 μmoles), phosphate buffer (pH 6.2; 53 μmoles) and enzyme preparation (0.2 ml) in a total volume of 1.2 ml.

Serine aldolase was prepared by the procedure of BLAKLEY[4] from an acetone powder of rabbit liver. The enzyme was assayed by determining the amount of serine produced in a system (2.3 ml) containing potassium phosphate buffer (pH 7.5; 40 μmoles), enzyme preparation (0.05 ml), formaldehyde (30 μmoles), glycine (30

Abbreviations: FH_4, tetrahydrofolic acid; BAL, 2,3-dimercaptopropanol; hFH_4, hydroxymethyl FH_4.

μmoles) and FH_4 (2 μmoles) incubated for 30 min at 37° in an atmosphere of N_2–CO_2 (95:5 %). The serine was estimated by heating the incubation systems for 2 min at 100° and measuring, in a Warburg manometer, the amount of CO_2 produced from a 1-ml aliquot *plus* potassium phosphate buffer (pH 5.8; 500 μmoles) treated with sodium metaperiodate (150 μmoles) in a total volume of 2.3 ml. The gas evolution was measured in air and was usually complete in 20 min. The serine aldolase preparations used in the coupling experiments for the synthesis of triose, showed only low serine synthesizing activity in the absence of added tetrahydrofolic acid.

Tetrahydrofolic acid was prepared from pteroylglutamic acid (20 μmoles) by catalytic hydrogenation at 37° in a Warburg manometer; the system (1.55 ml) contained potassium phosphate buffer (pH 6.2; 66 μmoles) and platinum oxide (5 mg) in an atmosphere of hydrogen. The catalyst was saturated with hydrogen before the solution of pteroylglutamic acid was tipped from a side-bulb. The hydrogenation was complete in 30 min, when 1.5 μmoles of BAL, were added. The mixture was quickly filtered and the filtrate stored in an evacuated Thunberg tube at 0°.

The dihydroxyacetone, formed in the incubation systems (3.0 ml) from the coupling experiments, was isolated with carrier dihydroxyacetone (100 μmoles) by the following procedure. At the end of the incubation time, trichloroacetic acid (0.4 ml; 50 %) was added and the supernatant from centrifuging made up to 10 ml with water. After treatment with Amberlite 1R 120 (H^+) to remove unchanged serine and Biodeminrolit G to remove other ions, the solution was concentrated under reduced pressure. The dihydroxyacetone was converted to the bis-2,4-dinitrophenylhydrazone of methylglyoxal by distilling the solution (containing approx. 100 μmoles triose) with water (5 ml) and sulphuric acid (1 ml; sp. gr., 1.84) into a saturated solution (15 ml) of 2,4-dinitrophenylhydrazine in 2 *N* HCl. The derivative was collected by centrifugation, plated and counted, if radioactive.

Radioactivity was measured at infinite thickness with correction, where necessary, for self-absorption on 1 cm^2 discs in polythene planchettes; details were identical with those described by DICKENS AND WILLIAMSON[5].

L-[3-^{14}C]serine was supplied by the Radiochemical Centre, Amersham, Bucks, England, and the radioactivity of the solutions used was determined by persulphate oxidation of an aliquot and subsequently counting the barium carbonate prepared from the CO_2 produced[6].

Recrystallised lithium hydroxypyruvate monohydrate was prepared by the method of DICKENS AND WILLIAMSON[7]. L-erythrulose was prepared by the action of *Acetobacter suboxydans* NCIB 7069 on erythritol[8] and isolated as the *o*-nitrophenylhydrazone. Solutions of L-erythrulose were prepared by refluxing the hydrazone with benzaldehyde and a little benzoic acid followed by ether extraction of these substances from the cooled filtrate.

RESULTS

Comparison of formaldehyde with (a) hFH_4 + formaldehyde and (b) hFH_4 as acceptors for transketolase action

The carbon dioxide evolution from an incubation system identical to that described in METHODS for the assay of transketolase (hydroxypyruvate, 10 μmoles and formaldehyde, 10 μmoles) was compared manometrically with the gas evolution

from similar systems in which (a) 17.6 μmoles hFH_4 (prepared by mixing equimolar amounts of FH_4 and formaldehyde[9]) were added and (b) 17.6 μmoles hFH_4 replaced the formaldehyde. Carbon dioxide was readily released in the three systems as compared with the control containing hydroxypyruvate but no formaldehyde or hFH_4 (Fig. 1). In the case where hFH_4 was the only added acceptor, the lower rate

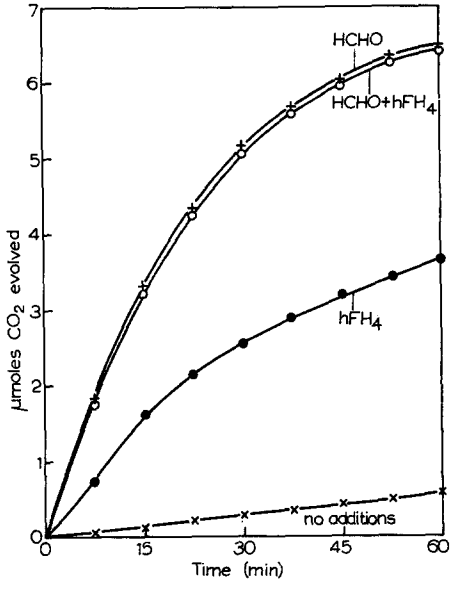

Fig. 1. Release of carbon dioxide from hydroxypyruvate (10 μmoles) in the presence of transketolase (0.2 ml), $MgCl_2$ (10 μmoles), thiaminpyrophosphate (0.05 mg), phosphate buffer (pH 6.2; 53 μmoles); total volume 1.2 ml. ×—×, no additions; +—+, with formaldehyde (10 μmoles); ●—●, with hFH_4 (17.6 μmoles); O—O, with HCHO (10 μmoles) *plus* h FH_4 (17.6 μmoles). Systems incubated in Warburg manometers at 37°. Gas phase, air.

of reaction was probably due to a slow rate of release of formaldehyde from reversible combination with tetrahydrofolic acid. The equilibrium constant for the reaction $HCHO + FH_4 \rightleftharpoons hFH_4$ has been reported[10] as $K = 1.3 \cdot 10^4\ M^{-1}$.

Triose synthesis from [3-^{14}C]serine (and serine aldolase) with hydroxypyruvate as donor for transketolase

The systems described in Table I were incubated aerobically and the unchanged serine removed by shaking with Amberlite IR 120 (H^+); other ions were removed

TABLE I

TRIOSE SYNTHESIS FROM [3-^{14}C]SERINE AND HYDROXYPYRUVATE

The reaction mixture (3.0 ml) contained hydroxypyruvate (15 μmoles), dihydroxyacetone (100 μmoles), thiamin pyrophosphate (0.02 mg), magnesium chloride (10 μmoles), glycylglycine buffer (pH 7.6; 250 μmoles), L-[3-^{14}C]serine (30 μmoles containing 1.48 μC ^{14}C), serine aldolase (0.2 ml) and transketolase (0.2 ml); incubation: 2 h at 37° in air. The total radioactivity in the dihydroxyacetone isolated from the systems at the end of the experiment was determined.

Additions (μmoles)	*Radioactivity in triose (μC)*	*Radioactivity in triose as % of amount in L-[3-^{14}C]serine*	*Ratio of amount incorporated into triose [0.06% ≡ 1.0]*
None	0.00093	0.06	1
FH_4 (5 μmoles)	0.103	7.0	114
FH_4 (5 μmoles) + pyridoxal phosphate (0.04 mg)	0.110	7.4	121

subsequently by mixing the systems with Biodeminrolit G. The solutions of dihydroxyacetone were concentrated *in vacuo* and the 2,4-dinitrophenylhydrazones of methylglyoxal prepared from the triose samples as described in METHODS.

The incorporation of radioactivity into the triose during incubation (Table I) was almost entirely dependent on the presence of added tetrahydrofolic acid; the addition of pyridoxal phosphate[4] did not greatly influence the transfer of ^{14}C from serine to glycine.

Triose synthesis from [3-^{14}C]serine (with serine aldolase) and either L-erythrulose or fructose-6-phosphate as donors for transketolase

The systems described in Table II were incubated aerobically and the incorporation of radioactivity from [3-^{14}C]serine into dihydroxyacetone was measured. The amount of radioactivity recovered in the triose with hydroxypyruvate as the transketolase donor was lower than in the previous experiment (Table I). This was probably due to the use, in this experiment, of a serine aldolase preparation which had been frozen and thawed twice since its preparation from an acetone powder of rabbit liver. Although L-erythrulose and fructose-6-phosphate were active as transketolase donors as measured by the percentage incorporation of ^{14}C from [3-^{14}C]serine into dihydroxyacetone, they were not as effective as hydroxypyruvate in this respect (Table II). The incorporations in all three cases were almost completely dependent on the presence of tetrahydrofolic acid.

TABLE II

TRIOSE SYNTHESIS FROM [3-^{14}C]SERINE AND HYDROXYPYRUVATE, FRUCTOSE-6-PHOSPHATE OR ERYTHRULOSE

The reaction mixture (3.0 ml) contained dihydroxyacetone (100 μmoles), thiamin pyrophosphate (0.02 mg), magnesium chloride (10 μmoles), glycylglycine buffer (pH 7.6; 250 μmoles), L-[3-^{14}C]-serine (30 μmoles containing 1.48 μC ^{14}C), serine aldolase (0.2 ml) and transketolase (0.2 ml); incubation: 2 h at 37°. The total radioactivity in the triose was determined at the end of the incubation period.

Additions (μmoles)	*Radioactivity in triose (μC)*	*Radioactivity in triose as % of amount in L-[3-^{14}C]serine*	*Ratio of amount incorporated into triose [0.035% ≡ 1.0]*
Hydroxypyruvate, 15	0.00065	0.044	1.3
Hydroxypyruvate, 15 + FH_4, 5	0.0118	0.8	22.8
Erythrulose, 15	0.00077	0.05	1.4
Erythrulose, 15 + FH_4, 5	0.0044	0.3	8.6
Fructose-6-phosphate, 15	0.00052	0.035	1.0
Fructose-6-phosphate, 15 + FH_4, 5	0.0049	0.33	9.4

DISCUSSION

The results obtained show that serine with serine aldolase (and tetrahydrofolic acid) may provide the 1-carbon acceptor for transketolase action. The dependence of the reactions on the presence of tetrahydrofolic acid indicated that the radioactivity present in the dihydroxyacetone was derived from serine via the serine aldolase reaction

$$\text{Serine} + FH_4 \underset{\text{Serine aldolase}}{\rightleftharpoons} \text{Glycine} + h\,FH_4$$

$$h\,FH_4 \rightleftharpoons FH_4 + HCHO$$

$$HCHO + \text{"Active glycolaldehyde"} \xrightarrow[\text{Transketolase}]{} \text{Dihydroxyacetone}$$

These findings support the possibility stated by DICKENS AND WILLIAMSON[1] that "the route from L-serine to hexose might proceed via serine aldolase (giving formaldehyde and glycine) in conjunction with the formation of hydroxypyruvate from L-serine by the specific transaminase of SALLACH[2]." However, the low percentage incorporations from serine into dihydroxyacetone found in these experiments suggest that, if the pathway occurs *in vivo* it is probably a minor one for serine. The incorporation of ^{14}C from [3-^{14}C]serine into liver glycogen *via* dihydroxyacetone would be expected to give glucose labeled equally in carbons 1, 3, 4 and 6, from the results described by BATT, DICKENS AND WILLIAMSON[11] showing that there is no appreciable enzymic discrimination between the two primary alcohol groups of dihydroxyacetone by the kinase which phosphorylates it. However, the distributions of ^{14}C in liver glycogen from both [3-^{14}C]hydroxypyruvate[5] and DL-[3-^{14}C]serine[12] are similar; only a small percentage (4–5 %) of the incorporated ^{14}C from either substrate was recovered in carbons 3 or 4 of the glucose. Most of the radioactivity occurred in carbons 1, 2, 5 and 6. Similar results have recently been reported for L-serine[13].

The incorporation values obtained from the coupling of the serine aldolase and transketolase reactions (Tables I and II) are of the order expected from the values reported for the equilibrium constant of the serine aldolase reaction, given as $K = 2.76 \cdot 10^3\ M^{-1}$ by ALEXANDER AND GREENBERG[14]. Although the transketolase reaction with formaldehyde as acceptor favours dihydroxyacetone synthesis (Fig. 1), the availability of formaldehyde from serine *via* the serine aldolase reaction presumably limits the rate at which the coupled systems interact.

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