ON THE FORMATION OF ACETYL-CoA FROM GLUTAMINE AND THE MUTUAL INTERCONVERSION BETWEEN OXALOACETATE AND PYRUVATE IN RABBIT RETICULOCYTES

Marianne MÜLLER, R. THAMM and S. RAPOPORT

Institut für Physiologie und Biologische Chemie, Bereich Medizin (charité) der Humboldt-Universität zu Berlin, DDR-104 Berlin, German Democratic Republic

Received 26 March 1974

1. Introduction

In a previous communication it was concluded that in reticulocytes oxaloacetate which arises from amino acids, in particular glutamate and aspartate, may also be the source of pyruvate and acetyl-CoA [1]. The evidence was indirect; it consisted in dilution experiments, which showed a diminution of $^{14}CO_2$ formation from variously labelled ^{14}C pyruvate from ^{14}C acetate, as well as ^{14}C-labelled fatty acids by glutamine and oxaloacetate. In the present communication we report on the isolation and identification of acetyl-CoA and of lactate from ^{14}C-glutamine, as well as on the demonstration of the functional reversibility of the decarboxyation of oxaloacetate to pyruvate.

2. Material and methods

The production of an anemia with high reticulocytosis in rabbits, the preparation of leucocyte-free suspensions of red cells, the measurement of oxygen consumption in the Warburg apparatus and that of radioactivity by means of a fluid scintillation spectrometer (3375 Tri-Carb, Packard) have been described before [2–5]. The radioactivity of thin layer chromatograms was evaluated by means of a scanner (II LB 2722 Berthold, Wildbad, BRD).

The isolation of lactate was carried out from a perchloric acid extract by chromatography on a column of Dowex 1 × 10 after removal of phosphate esters, nucleotides and amino acids [6]. The concentration of lactate was determined enzymatically [7]. Acyl-CoA derivatives were separated from the perchloric extract on a carbon column, which had been treated with stearic acid. Elution was performed with 5% pyridine after careful washing with water [8]. The eluate was lyophilized. The identification of the acyl-CoA derivatives was carried out by thin layer chromatography in cellulose MN 300 as hydroxamic acids with $FeCl_3$. The solvent was the upper layer of a mixture of butanol, glacial acetic acid and water (4:1:5) [10].

The amino acids were fractionated from a trichloroacetic acid extract on a analyzer (BC 200, Biocal Instruments, München, GFR), according to Moore and Stein [11]. The aspartate containing fractions were located by means of the ninhydrin reaction, carried out on small aliquots on a thin layer plate. They were pooled and lyophilized and their radioactivity was determined. Thereafter the quantitative determination of aspartate was carried out by means of the ninhydrin reaction. Incubations were carried out in isotonic Tris-buffer at pH 7.6 and 37° for 60 min. [U-^{14}C] glutamine was obtained from the Radiochemical Centre (Amersham, G.B.), [1-^{14}C] pyruvate was prepared from DL- [1-^{14}C] alanine (Rossendorf, GDR) by transamination with α-ketoglutarate catalyzed by means of alanine aminotransferase.

Table 1
The formation of lactate from [U-^{14}C]glutamine in the reticulocyte (all values in mM)

ΔO_2 hr	Δ [U-^{14}C] Glutamine	Lactate	Δ [U-^{14}C] Lactate	%Δ [U-^{14}C] Lactate / Lactate	%Δ [U-^{14}C] Lactate / Δ [U-^{14}C] Glutamine
13.9	1.94	1.47	0.055	3.7	2.8

Experimental conditions: as detailed in 'Materials and methods'.
The concentration of glutamine was 18 mM. The incubation was carried out in the presence of 0.07 mM 2,4-dinitrophenol in order to maximize lactate formation. The utilisation of glutamine was calculated from the value of $^{14}CO_2$ formation under the assumption of complete oxidation of glutamine.

3. Results and discussion

In table 1 are shown the results of one experiment of the formation of [^{14}C]lactate from [U-^{14}C]glutamine in the absence of external glucose. The experiment was carried out in the presence of 2,4 DNP in order to maximize lactate formation. The result is clear-out in showing a labelling of the lactate. However the extent of lactate formation appears to be low presumably owing to a sizeable pool of substances which may equilibrate with the pyruvate formed, which include among others alanine and pyruvate originating from the breakdown of ribose which arises from the degradation of the adenine nucleotides in the presence of 2,4 DNP [12].

In fig. 1 is shown the reproduction of a scan of a thin-layer chromatogram, which indicates the formation of radioactive acetyl-CoA as well as succinyl CoA. The amount of acetyl-CoA estimated appears quite high. It is more than twice the value determined for liver [13].

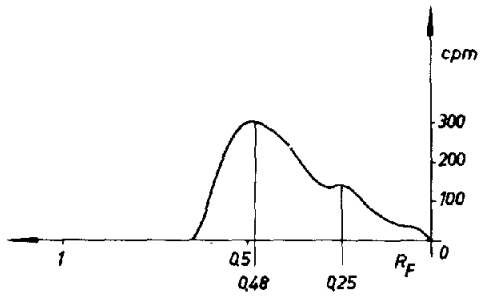

Fig. 1. Thin layer chromatography of [^{14}C]acyl-hydroxamic acids. The R_f values of the acyl-hydroxamic acids in the sample agreed well with the standard substances (acetyl hydroxamicacid R_f = 0.48; succinyl hydroxamic acid R_f = 0.25). The total amount of acyl-CoA compounds amounted to 0.25 μmoles per ml cells. The amount of acetyl-CoA was about three times that of succinyl-Coa.

In table 2 are shown 3 experiments on the formation of [^{14}C]aspartate from [1-^{14}C]pyruvate. The results are clear cut indicating a sizeable carboxylation of the pyruvate. Again it is difficult to assess quantitatively the importance of the conversion in view of the various possibilities of mixing with pools.

One may conclude from the data together with the evidence previously presented, that in the reticulocyte there is a ready functional reversibility of the intraconversions between oxaloacetate and pyruvate. The enzymes which may be instrumental in catalyzing this shuttle have sofar not been sufficiently characterized.

These data explain the ready utilization of glutamine, oxaloacetate and pyruvate, each of which may serve as the main substrate.

Table 2
The formation of aspartate from [1-^{14}C]pyruvate in the reticulocyte (all values in mM)

Exp. No	ΔO_2 hr	Aspartate	[^{14}C] Aspartate	%[^{14}C] Aspartate / Aspartate
1	12.0	1.4	0.058	4.2
2	8.2	1.4	0.062	4.4
3	6.7	2.6	0.113	4.4

Experimental conditions as described under Materials and methods. The concentration of pyruvate was 15 mM.

Acknowledgements

We should like to thank Mrs R. Dumdey for her careful technical assistance.

References

[1] Rost, J. and Rapoport, S. M. (1972) Eur. J. Biochem. 26, 106–111.
[2] Goetze, E. and Rapoport, S. (1954) Biochem. Z. 326, 53–61.
[3] Schultze, M. and Rapoport, S. (1964) Acta Biol. Med. Germ. 13, 310–316.
[4] Schultze, M., Rost, J., Augustin, W., Gellerich, F. and Rapoport, S. (1972) Eur. J. Biochem. 27, 43–47.
[5] Busch, D., and Pelz, K. (1966) Klin. Wochenschr. 44, 983–984.
[6] Albrecht, V., Roigas, H., Schultze, M., Jacobasch, G. and Rapoport, S. (1971) Eur. J. Biochem. 20, 44–50.
[7] Noll, F. (1966) Biochem. Z. 346, 41–49.
[8] Schuberth, J., Sollenberg, J., Sundwall, A. and Sörbo, B. (1965) J. Neurochem. 12, 451–454.
[9] Lipmann, F. and Duttle, I. C. (1945) J. Biol. Chem. 159, 21–28.
[10] Block, R. J., Durrum, E. L. and Zweig, G. (1955) A Manual of Paper Chromatography and Paper Electrophoresis, pp. 161–166.
[11] Moore, S. and Stein, W. H. (1951) J. Biol. Chem. 192, 663–681.
[12] Schweiger, H. G. and Rapoport, S. (1958) Hoppe-Seyler's Z. Physiol. Chem. 313, 97–108.
[13] Garfinkel, D. (1970) J. Theor. Biol. 29, 113–130.