Possible Relations Between the Direct Oxidation System of Acetate (MAS) and the Tricarboxylic Acid Cycle (TAC) in Experiments with Living Yeast Cells

The existence of a direct way of acetate oxidation in yeast[1] and in *E. coli*[2] has been demonstrated according to the following scheme:

Acetate → acetyl → glycolate → glyoxylate → formaldehyde → formyl → $H_2O + CO_2$.

All intermediates have been isolated. In experiments with yeast and labeled acetate, the distribution of radioactivity in the intermediates was in accord with the reported scheme[3]. It seemed of interest to establish whether the two systems occur simultaneously in the yeast cells by determining in the medium the TAC intermediates which may be present besides those of the MAS.

In fact paper chromatographic analysis have shown the presence of succinic, fumaric, malic and citric acid together with the C_2 and C_1 intermediates of the MAS, usually present. This report deals with the identification of these acids and the possible relations between the TAC and the MAS are discussed.

Details of the procedure used in the experiments and for the isolation of the intermediates of the MAS were described in previous papers[4].

The acetyl and the formyl were calculated respectively from the acetophenone and the benzaldehyde formed[3]. In the experiments of acetate oxidation without phenylhydrazine (blank test), the pH was maintained around 7 by dropwise addition of 10% sulphuric acid solution for at least 2 h. The separation of the di- and tricarboxylic acids from the medium was performed according to the method outlined for the glycolic acid[5], the time of the ether extraction lasting for 50–60 h, instead of 18–20 h. The conventional descending technique of BRYANT and OVERELL[6] for the paper chromatographic identification of the acids was used. Citric acid was determined by the method of TÄUFEL *et al.*[7], glycolic acid by the method of DAGLEY *et al.*[8]. Succinic, fumaric and malic acids were detected by visual comparison of the intensity of colour and the size of the spots obtained with those of standard solutions.

The results are summarized in the Table. It is interesting that the acids of the TAC and the glycolic acid of the MAS are simultaneously present in the experiments of acetate oxidation without phenylhydrazine, i.e. under normal physiological conditions. Since the same result is almost obtainable in the presence of phenylhydrazine,

Intermediates found in the medium during acetate oxidation with and without phenylhydrazine. Average results of four experiments.

Intermediates	Incubation without phenylhydrazine	Incubation with phenylhydrazine
	mg/l	mg/l
Acetyl	0·0	45·0
Formaldehyde	0·0	2·0
Formyl	0·0	4·0
Glycolic acid	1·5	3·0
Succinic acid	15·0	11·0
Fumaric acid	0·0	2·0
Malic acid	4·5	2·0
Citric acid	2·5	1·0

it may be concluded that this product has no or weak toxic action on the enzymes of the TAC.

The presence in the medium of the intermediates of the MAS besides those of the TAC (or also of the DAC) suggests that two acetate oxidation systems occur simultaneously in yeast cells. This result is consistent with the view already expressed by KREBS *et al.*[9]: 'That two mechanisms may occur in the same species side by side, the relative preponderance varying according to environmental conditions'. Recent findings of WIAME and BOURGEOIS[10], Mc QUILLEN and ROBERTS[11] and KATZ and CHAIKOFF[12] in accord with KREBS *et al.*[9] ascribe to the TAC energetic and synthetic functions. It is possible that this double function may also be assigned to the MAS. Probably, within the microbial cells more than one oxidative system occurs, each system having the purpose to produce some (or specific) intermediates for microbial synthesis. Therefore we suppose that the TAC supplies C_4, C_5 and C_6 fragments and the MAS C_1 and C_2 fragments simultaneously with the energy necessary for their utilization.

Furthermore we suppose that the isocitritase of yeast[13], and the malate synthetase of WONG and AJL[14], or a similar enzyme, may be the enzymes able to establish relations between the TAC and the MAS. In fact, isocitritase by splitting isocitrate into succinate and glyoxylate permits the passage from an intermediate of the TAC to another of the MAS and *vice versa*. The same observation accounts for the malate synthetase which synthetizes malate from acetate and glyoxylate.

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Zusammenfassung

Es wird nachgewiesen, dass in der Hefe die Oxydation des Azetates sowohl nach dem Krebs-Zyklus als auch direkt über Glykolsäure, Glyoxylsäure und Formaldehyd ablaufen kann. Dabei kommen Isozitronensäure-Lyase und Apfelsäure-Synthetase als Brücke zwischen den beiden Atmungssystemen in Frage.

[1] V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, Arch. Biochem. Biophys. *69*, 372 (1957). – V. BOLCATO and M. E. SCEVOLA, Naturwissenschaften *44*, 539 (1957). – V. BOLCATO and G. LEGGIERO, Ann. chimica *48*, 177 (1958).

[2] V. BOLCATO, E. BOSCHETTI, and E. MONTOYA, Ant. v. Leeuwenhoek *22*, 131 (1956).

[3] V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, Arch. Biochem. Biophys. *69*, 372 (1957).

[4] V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, Arch. Biochem. Biophys. *69*, 372 (1957). – V. BOLCATO, M. FRASCHINI, and G. BONIPERTI, Ant. v. Leeuwenhoek *22*, 419 (1956).

[5] V. BOLCATO and M. E. SCEVOLA, Naturwissenschaften *44*, 539 (1957).

[6] F. BRYANT and B. T. OVERELL, Biochim. Biophys. Acta *10*, 471 (1953).

[7] K. TÄUFEL, R. POHLONDEK-FABINI, and U. BEHNKE, Z. anal. Chem. *146*, 244 (1955).

[8] S. DAGLEY and A. RODGERS, Biochim. biophys. Acta *12*, 591 (1953).

[9] H. A. KREBS, S. GURIN, and L. EGGLESTON, Biochem. J. *51*, 614 (1952).

[10] J. M. WIAME and S. BOURGEOIS, Nature *172*, 310 (1953). – J. M. WIAME, Adv. Enzymol. *18*, 241 (1957).

[11] K. McQUILLEN and R. B. ROBERTS, J. biol. Chem. *207*, 81 (1954).

[12] J. KATZ and I. L. CHAIKOFF, Biochim. biophys. Acta *18*, 87 (1955).

[13] J. A. OLSON, Nature *174*, 695 (1954).

[14] D. T. O. WONG and S. J. AJL, J. Amer. chem. Soc. *78*, 3230 (1956).