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GLUTAMATE OXIDATION IN THE DIFFERENTIATING SLIME MOLD

I. STUDIES *IN VIVO*

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SUMMARY

Over the course of differentiation of *Dictyostelium discoideum*, (a) the conversion of glutamate to CO_2 *in vivo* increases 7-fold, based on protein, (b) the concentration of intracellular glutamate increases about 10-fold. Alterations in the S/K_m ratio of glutamic acid dehydrogenase during development are such as to account for the observed increase in glutamate oxidation *in vivo*.

INTRODUCTION

The endogenous metabolism of the slime mold during multicellular differentiation involves a re-orientation in catabolic pathways resulting in the conversion of endogenous protein to carbohydrate. One might predict that such an altered metabolism would necessitate an increase in the rate of such key reactions as that of glutamic acid dehydrogenase, an important bridge for the entry of amino acids into the citric acid cycle. Earlier studies on the concentration of this enzyme in extracts prepared at various developmental stages showed no significant differences[1]. However, since the concentration of an enzyme *in vitro* under optimal assay conditions does not necessarily reflect enzyme activity *in vivo*, a study was made of the rate of $^{14}CO_2$ evolution from [1-^{14}C]glutamate in the presence of intact cells. The concentration of intracellular glutamate during development was also determined, and the results suggest that *in vivo* glutamate availability limits enzyme activities in this pathway.

MATERIALS AND METHODS

DL-[1-^{14}C]Glutamate (3 mC/mmole) was obtained from Volk Radiochemical Company. *Dictyostelium discoideum* was grown on a rich medium in the presence of *Escherichia coli*, washed and replated on 2% agar[2].

$^{14}CO_2$ determination

A typical experiment to determine $^{14}CO_2$ evolution from [1-^{14}C]glutamate was carried out as follows: cells were removed from one 2% agar plate (5.5 inch diameter)

with 3.0 ml of BONNER's salt solution[3]. 2.5 ml of this suspension were diluted with 2.5 ml of 0.02 M sodium phosphate buffer (pH 6.0). 1 ml of this mixture was placed in the main vessel of 4 Warburg flasks (7 ml capacity), which contained 60 μl 14% NaOH in the center well and 200 μl of 5 N H_2SO_4 in the side arm. The reaction was started by the addition of 10 μl of a solution of labeled glutamate (about 10^6 counts per min/10 μl), followed by stoppering the vessel immediately and incubation at 18°. Acid was tipped into each flask at 15-min intervals, and 1.5 h after each acid addition 30 μl of NaOH were removed for counting. This sample was added to 0.17 ml H_2O in a scintillation vial, to which was then added 10 ml of a scintillation gel mixture[4,5]. ^{14}C activity was measured in the Packard Tri-Carb Liquid Scintillation Spectrometer, model 314-DC.

Specific radioactivity of internal glutamate pool

Cells from six 2% agar plates were removed by washing with a total of 3 ml of BONNER's salt solution. 2 ml of this suspension was mixed with 1.6 ml H_2O, 0.4 ml 0.1 M sodium phosphate, (pH 6) and 40 μl of a solution of labeled glutamate in a 125-ml Erlenmeyer flask. This mixture is comparable to the one in the Warburg flasks, and was incubated for 1 h at 18° while the time course of $^{14}CO_2$ evolution was being determined. The mixture was then diluted with 40 ml of chilled, nonradioactive 0.002 M DL-glutamate, centrifuged, and the cells washed twice with cold salt solution. Further washing did not significantly lower the radioactivity of the cells. The pellet was suspended in 2.5 ml H_2O and mixed with 0.37 ml of 30% perchloric acid. After centrifugation the supernatant solution was neutralized to pH 6.5 with 5 M KOH and the potassium perchlorate removed. The supernatant solution was put slowly through a column containing Dowex-1 formate (resin bed 2.5 cm high in a column 0.6 cm in diameter). The column was rinsed with 3.0 ml H_2O. The acidic amino acids were eluted with 2 ml of 0.1 M formic acid followed by 2.0 ml H_2O, which were collected in the same evaporating dish. This procedure gives a recovery of glutamate of at least 90%. 10% of the eluted material (glutamate + aspartate) was counted and the remainder, together with glutamate standards, chromatographed over night on Whatman No. 1 filter paper in a solvent mixture composed of methanol–water–pyridine (80:20:4). After chromatography the paper was dried, steamed 2 min, and dipped in freshly made 0.5% ninhydrin in acetone. After color development at 65° for 30 min, the glutamate spots were cut out and each one was eluted by shaking for 20 min in 10 ml of 75% ethanol containing 0.2 mg% $CuSO_4 \cdot 5H_2O$. Color intensity was read in the Klett–Summerson colorimeter with a No. 54 filter.

RESULTS

A typical experiment in which $^{14}CO_2$ evolution from [1-^{14}C]glutamate was determined *in vivo* is shown in Fig. 1. Over a 1-h period the rate is linear at each stage of development using comparable cell aliquots. Although it would appear that preculminating cells are the most active in converting glutamate to CO_2, this is not the case. After 1 h exposure to extracellular labeled glutamate, the isolated intracellular glutamate was higher in specific radioactivity at preculmination than at the other two stages of development (Table 1). Based on the specific radioactivity of the intracellular glutamate, there is an increase of about 5-fold per cell aliquot (or 7-fold/mg protein over

the course of differentiation, since the protein content per cell decreases during development[6].

These data indicating increased activity in glutamate oxidation were in apparent conflict with our *in vitro* results (DPNH formation from glutamate oxidation) which showed a slight decrease in enzyme activity at the later stages of development. Since the K_m of glutamic acid dehydrogenase from the slime mold is very high ($2.5 \cdot 10^{-3}$),

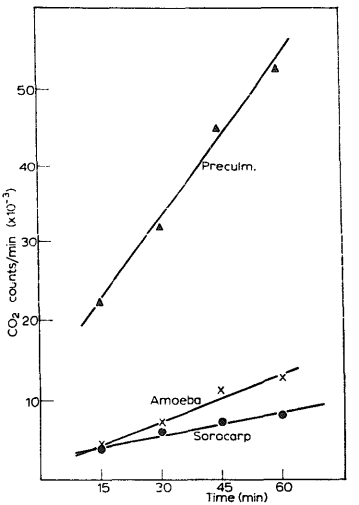

Fig. 1. The rate of $^{14}CO_2$ evolution as a function of time at 3 stages of differentiation (see METHODS).

TABLE I

CO_2 EVOLUTION FROM GLUTAMATE *in vivo*

Comparable cell aliquots were used at each stage of development. After exposure of the cells to exogenous labeled glutamate for 1 h, the intracellular glutamate was isolated in order to determine its specific radioactivity

Stage	*Cellular glutamate (counts/min/μmole ($\times 10^{-3}$))*	*CO_2 (counts/min/h ($\times 10^{-3}$))*	*μmole CO_2/h*
Amoeba	38.0	24.0	0.64
Preculm.	100.0	80.0	0.80
Sorocarp	4.1	12.0	2.92

(see ref. 7), and since preliminary observations suggested that intracellular glutamate accumulates in starving slime mold cells during differentiation, it appeared possible that the activity of this enzyme *in vivo* could be influenced by substrate concentration.

Quantitative determinations were made of the intracellular glutamate concentration present in differentiating cells at 3 stages of development. The method employed for the quantitative isolation of glutamate is described in METHODS. In order to express the results on a molar basis, the packed cell volume of each suspension was

TABLE II

INTRACELLULAR GLUTAMATE CONCENTRATION

See text and METHODS for experimental details.

Stage	*μmole/cell aliquot*	*Molarity* ($\times 10^4$)	$(S)/K_m$
Amoeba	0.11	0.7	0.028
Preculm.	0.16	1.6	0.064
Sorocarp	0.48	8.0	0.320

determined by centrifugation for 10 min at 1000 $\times$ *g*. (The volume of the cell decreases about 40% during differentiation.) Table II indicates the intracellular glutamate concentration over the course of differentiation and also presents the substrate to K_m ratio at each stage. Since the $(S)/K_m$ ratio is below 1 at all stages, it is clear that glutamic acid dehydrogenase activity is substrate-limited throughout development. Fig. 2 depicts both the $(S)/K_m$ ratio and the rate of CO_2 evolution as a function of the time of differentiation. In both cases, the major rise occurs between the preculmination and sorocarp stages.

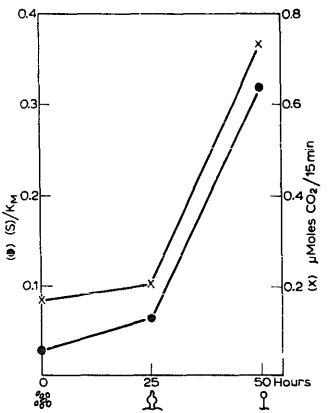

Fig. 2. The rate of CO_2 formation and the ratio $(S)/K_m$ (concentration intracellular glutamate/K_m of glutamic acid dehydrogenase) plotted as a function of time (or stage) of differentiation.

DISCUSSION

Although the over-all increase in μmoles CO_2/h from the amoeba to the sorocarp stage of development was similar in two other experiments such as the one described in Table I, the intracellular specific radioactivity of glutamate was quite variable. The cause(s) of alterations, as a function of differentiation, in the permeability to glutamate (or exchangeability of exogenous with endogenous glutamate[8]) is unknown. Clearly, one source of variation is due to the difficulty in choosing similar developmental stages from one experiment to another.

The data *in vivo* presented demonstrate a 7-fold increase in the conversion of glutamate to CO_2 throughout differentiation. Glutamic acid dehydrogenase, as assayed by DPNH formation, does not increase in concentration during development. However, alterations in the concentration of intracellular glutamate have been observed which could account for the enhanced enzyme activity *in vivo*. In fact, during differentiation the substrate to K_m ratio changes from 0.03 to 0.3, which theoretically can account for the observed 7-fold increase in the rate of a reaction. Furthermore, if the S/K_m ratio and the rate of CO_2 evolution are plotted as a function of the time of differentiation, the major rise in both curves occurs just after preculmination, suggesting a causal relationship. All the data are compatible with the conclusion that an increase in intracellular glutamate concentration during development is responsible for the observed increase in glutamate oxidation *in vivo*. However, this conclusion is based upon a comparison of our data on CO_2 evolution *in vivo* with our data on the activity and K_m of glutamic acid dehydrogenase *in vitro* as assayed by DPNH formation. It will be shown in the following paper that these two assay systems represent the same reaction.

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