# TISSUE SPECIFIC DIFFERENCES IN RATE LIMITING FACTORS OF GLUTAMATE METABOLISM IN ENZYME SYSTEMS OF KIDNEY AND LIVER

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In most animal tissues, glutamate dehydrogenase and glutamate transaminases are considered to be the major enzymes acting on glutamate as a common substrate. Since properties of these enzymes are well known, it should be possible to predict the enzyme chemical mechanism of metabolic flux of glutamate in various in vitro enzyme systems. Deviation from a predictable pattern is an experimental measure of multienzymatic control mechanisms. The following predictions can be made: a) Glutamate oxidation initiated by glutamate dehydrogenase in a multienzyme system requires some "pulling mechanism" capable of efficient removal of $\alpha$-ketoglutarate, $NH_4^+$ and reduced pyridine nucleotide, since the apparent equilibrium of the dehydrogenase (app. $K_{eq}$ at pH 7.4 is in the order of $10^{-8}$) does not favor continued metabolic flux, which, however, is readily observed experimentally. Although the ubiquitous " $\alpha$-ketoglutarate oxidase" system could be expected to provide this pulling mechanism, the oxidation of glutamate to $CO_2$, $H_2O$ and $NH_4^+$ under quasi-physiological conditions cannot be demonstrated experimentally, and, as first described by Krebs and Krebs and Bellamy (1), in most in vitro enzyme systems the following stoichiometry is approximated:

$$\text{glutamate} + 1.5\ O_2 = CO_2 + \text{aspartate}$$

b) If in a multienzyme system glutamate is to be metabolized exclusively by initial transamination, the second substrate, oxaloacetate (or pyruvate), needs to be provided by some other enzyme system.

Since the Krebs-Bellamy equation implies the operation of complex restrictive mechanisms not predictable from results obtained with isolated enzymes, we have employed this "system" as a detector of metabolic regulatory mechanisms in various animal tissues. It was assumed that a study of this ubiquitous system in different genetically differentiated tissues might yield information which defines in biochemical terms metabolic specificity.

The following results were obtained. 1) Metabolic flux of glutamate in enzyme systems of rat kidney is completely blocked by selective inhibition of either glutamate dehydrogenase or glutamate-oxaloacetate transaminase alone (2). In other words, in kidney systems the

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two enzymes do not act independently on the common substrate, but behave as members of a sequentially coupled (linear) multienzyme system (cf. 2, p. 2902). 2) Oxaloacetate as a single added substrate is rapidly oxidized by isolated kidney mitochondria but not at all by liver mitochondria (see ref. 3).

From these experimental observations an enzyme chemical mechanism of glutamate utilization in both kidney and liver tissues, actually reflecting tissue specific differences, was formulated. In enzyme systems of kidney, glutamate enters into a metabolic path by initial oxidation by glutamate dehydrogenase. Oxaloacetate subsequently derived from glutamate then reacts with another molecule of glutamate by transamination (cf. 2). This mechanism not only eliminates the hindrance of flux imposed by the "unfavorable" equilibrium of glutamate dehydrogenase (see a), but also postulates that in kidney no other source of oxaloacetate is made available for transamination except that which originates from added glutamate (see b). Quite a different mechanism operates in liver systems, where under conditions when oxidative phosphorylation is intact, the dehydrogenase and transaminases are connected by a distributive type of kinetic system (cf. 4). According to this mechanism, glutamate in liver systems can react with either glutamate dehydrogenase or transaminase or with both simultaneously.

One of the reasons for this metabolic pattern in liver is connected to the inability of liver mitochondria to oxidize oxaloacetate, which if formed from sources other than glutamate is then available for transamination with glutamate (3). The causes of apparent "metabolic discrimination" between various sources of oxaloacetate in mitochondria prepared from different tissues is the subject of separate studies (ref. 4). The present communication deals with only one aspect of metabolic regulation of glutamate metabolism in kidney and liver systems, i.e., the relevance of enzyme content to metabolic flux in both types of tissue.

EXPERIMENTAL PROCEDURE. Preparation of tissue homogenates of rat kidney cortex and liver and isolation of mitochondria and cytoplasmic fraction have been described earlier (2). Metabolic flux was determined under previously defined pseudo-steady state conditions (2) by determining $O_2$ uptake manometrically and glutamate utilization by direct chemical analysis (cf. 2) in Warburg type respirometers during 30 minutes of incubation at $30^{\circ}$. When "initial rates" of oxidations catalyzed by mitochondria or homogenates were measured, $O_2$ consumption was determined polarographically as described earlier (2) and also in a subsequent paper (3). Composition of reaction systems is summarized in the legend of the Table. Analyses for glutamate aspartate transaminase were carried out according to Velick and Vavra (5) in both cytoplasmic and mitochondrial extracts. The latter were prepared by sonic disintegration of isolated mitochondria, followed by sedimentation of insoluble material at 144,000 x g, as described in an earlier paper (6). Glutamate dehydrogenase of mitochondrial extracts was determined in presence of $10^{-4}$M ADP (also present during sonic extraction) by spectrophotometric kinetic analysis of NADH or NADPH formation in presence of $10^{-2}$M glutamate at pH 7.4 (cf. 7). Enzyme content

was calculated from experimentally determined apparent $V_{max}$ values and expressed as specific activity.

RESULTS which show the relationship of metabolic flux and specific enzymatic activity are summarized in the Table. Numerical values represent micromoles of glutamate consumed per 1 g protein per 30 minutes incubation at $30^{\circ}$. Metabolic flux, in terms of $-\Delta O_{2(glu)}$, expresses the extra $O_2$ consumed per g protein in 30 minutes due to addition of glutamate. The $O_2$/glu ratio in kidney homogenates is approximately 1, in mitochondria in presence of added ATP 1.3, and 1.6 when the hexokinase + glucose + ADP system is employed. The $O_2$/glu ratio in all liver systems is close to unity. Columns 3 and 4 contain specific activities of glutamate dehydrogenase of solubilized mitochondrial extracts determined with both NAD and NADP, expressed in the same units as metabolic flux (i.e., columns 1 and 2). It was determined in separate experiments that mitochondria in kidney homogenates contribute 20 to 25% of total cellular protein, while this value in liver homogenates is 12 to 14% (8). Since glutamate dehydrogenase is an exclusively mitochondrial enzyme, it is readily calculated that in kidney homogenate metabolic flux (in terms of glutamate utilization) is limited by the amount of glutamate dehydrogenase (column 3). Transaminase is present in large excess. This is in good agreement with the proposed mechanism of enzymatic disposition of glutamate in kidney where transaminase is to provide an efficient catalytic link to systems removing products of the glutamate dehydrogenase reaction (cf. 2). It cannot be decided with certainty whether or not glutamate dehydrogenase in kidney uses NAD or NADP as coenzyme, although the specific activity with NAD appears to be well within the range of experimentally determined flux values, and thus can account for glutamate utilization. The low levels of NADP in kidney seem to provide further support for this assumption (9). In sharp contrast to kidney, in liver tissue there is no relationship between metabolic flux and enzyme contents. This observation together with the distributive multienzymatic behavior of liver systems (cf. 4) clearly demonstrates that even though individual enzymes acting on glutamate occur in most tissues, control mechanisms cannot be predicted merely from this descriptive type of information. It is anticipated that other metabolic "pathways" (or cycles) hitherto considered to function in the same manner in all tissues may also exhibit "tissue specific" patterns. It is, however, necessary to devise unconventional experimental models to discover these differences.

Results summarized in the Table contain further less obvious but interesting information. It is readily calculated that respiratory activity of kidney mitochondria ($-\Delta O_{2(glu)}$) operating in homogenates is at its maximum and identical with that of isolated mitochondria in presence of an ADP generating system. By direct measurements of the acceptor control ratio (cf. 10) of mitochondria in isolated state and in homogenates, it was shown that mitochondria in homogenates are not uncoupled, but are functioning in "state 3" (cf. 11). Liver systems again behave in an unusual fashion, since the calculated $-\Delta O_{2(glu)}$ for mitochondria

TABLE

RELATIONSHIP BETWEEN METABOLIC FLUX AND ENZYME CONTENT

| MULTIENZYME SYSTEM | METABOLIC FLUX | | MITOCHONDRIAL GDH (S.A.) | | CALCULATED GDH S.A. OF MITOCHONDRIA PRESENT IN HOMOGENATES | | GOT S.A. | |
|---|---|---|---|---|---|---|---|---|
| | $-\Delta O_2$(glu) | $-\Delta$glu | NAD | NADP | NAD | NADP | Mitoch. | Cytopl. |
| Kidney homogenate (ADP) | 500 | 600 | -- | -- | 1,100 | 540 | 43,000 | 83,000 |
| Kidney mitochondria (ADP) | 1,200 | 920 | 4,400 | 2,200 | -- | -- | -- | -- |
| Kidney mitochondria (ADP + Hk + glucose) | 1,900 | 1,200 | -- | -- | | | | |
| Liver homogenate (ADP) | 193 | 160 | -- | -- | 2,800 | 1.900 | 31,000 | 90,000 |
| Liver mitochondria (ADP) | 400 | 440 | 23,000 | 16,000 | -- | -- | -- | -- |
| Liver mitochondria (ADP + Hk + glucose) | 600 | 500 | | | | | | |

Metabolic flux was determined under pseudo-steady state conditions (cf. 2) in Warburg type respirometer flasks, each containing 20-30 mg protein (homogenate or mitochondria), 0.15 M KCl, 0.001 M $MgCl_2$, 0.03 M phosphate (pH 7.4), 0.0023 M ADP and 0.01 M glutamate in a final volume of 3.0 ml. Gas phase = air; temperature, 30°; time of incubation, 30 minutes. $CO_2$ was absorbed by KOH; ADP + Hk + glucose = 0.03 M glucose + 0.0023 M ADP + crystalline yeast hexokinase (0.24 mg protein/flask). Results are calculated in terms of μmoles of glutamate (or $O_2$) consumed per 1 g protein per 30 minutes.(= S. A.). GDH = glutamate dehydrogenase. GOT = glutamate oxaloacetate transaminase.

functioning in homogenates is 1350 instead of the experimentally obtained maximal value of 600 (in presence of ADP generating system). It is clear that under "pseudo-steady state" conditions an extramitochondrial metabolic system connected to glutamate pathways "distorts" $-\Delta O_{2(glu)}$ values, probably by contributing oxidizable substrates which are triggered by components of the glutamate system (4). That this may be the reason for the observed discrepancy in liver systems is supported by direct measurements of $-\Delta O_{2(glu)}$ values of liver mitochondria in isolated state and in homogenates under "initial rate" conditions (cf. 2). Values of 530 and 520 were obtained in both systems. In both isolated mitochondria and dilute homogenates, under "initial rate" (cf. 2) conditions addition of glutamate alone had no effect on endogenous $O_2$ uptake, but required ADP (and orthophosphate) for maximal respiration.

SUMMARY. In kidney tissue, the quantity of glutamate dehydrogenase limits metabolic flux of glutamate in accordance with predicted linear kinetic coupling of the dehydrogenase with transaminases (cf. 2). In liver tissue, however, control mechanisms of the glutamate system are not related in an obvious manner to the quantity of enzymes for which glutamate is a common substrate.

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