Evidence for Orientation-Conserved Transfer in the TCA Cycle in *Saccharomyces cerevisiae*: ^{13}C NMR Studies†

B. Sumegi,‡,§ A. D. Sherry,‡,∥ C. R. Malloy,∥ and P. A. Srere*,⊥

Pre-Clinical Science Unit of the Department of Veterans Affairs Medical Center and Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, and Department of Chemistry, The University of Texas at Dallas, Dallas, Texas 75216, Department of Biochemistry, University Medical School, Pècs, Szigeti ut 12, H-7624 Pècs, Hungary, and Rogers Magnetic Resonance Center, 5801 Forest Park Road, Dallas, Texas 75235-9085

Received June 15, 1993; Revised Manuscript Received July 16, 1993®

ABSTRACT: When [4-^{13}C]glutamate is incubated with yeast cells, the [^{13}C]aspartate formed shows a [2-^{13}C]/[3-^{13}C] ratio that is greater than 1. If the conversion occurs through the Krebs TCA cycle with the symmetrical intermediates succinate and fumarate, the ratio is expected to be 1. The addition of fluoroacetate to the incubation increases the ratio further. When the mutants Acol$^-$ or CS1$^-$ are used, little or no aspartate is formed. When [2-^{13}C]acetate is incubated with yeast, the C2-/C3-aspartate ratio is 1.5 at 2 min and decreases to 1.0 after 10 min of incubation. These results indicate that orientation-conserved transfer occurs at the succinate thiokinase and succinate dehydrogenase steps of the Krebs TCA cycle.

There is mounting evidence that an organization of Krebs TCA[1] cycle and associated enzymes exists within the mitochondrial matrix (Srere, 1987, 1990; Sumegi & Porpaczy, 1990). These experiments include the following: (1) *in vitro* demonstration of specific interactions between sequential enzymes (Halper & Srere, 1976; Fahien *et al.*, 1989; Fahien & Smith, 1974; Beeckmans *et al.*, 1989; Beeckmans & Kanarek, 1981; Porpaczy *et al.*, 1983; Sumegi & Alkonyi, 1983; Sumegi *et al.*, 1980; Tyiska *et al.*, 1986; Hearl & Churchich, 1984); (2) binding of Krebs cycle enzymes to specific proteins on the inner surface of the inner mitochondrial membrane (Halper & Srere, 1976; Fahien *et al.*, 1989; Fahien & Smith, 1974; Beeckmans *et al.*, 1989; Beeckmans & Kanarek, 1981; Porpaczy *et al.*, 1983, 1987; Sumegi & Alkonyi, 1983; Sumegi *et al.*, 1980; Tyiska *et al.*, 1986; Hearl & Churchich, 1984; D'Souza & Srere, 1983b; Fukushima *et al.*, 1989; Srere & Sumegi, 1986; Kispal *et al.*, 1986; Sumegi & Srere, 1984a,b); (3) cross-linking studies of mitochondria showing the binding of Krebs TCA cycle enzymes to the inner membrane (D'Souza & Srere, 1983a); (4) kinetic enhancement of organized mitochondrial preparations compared to an equivalent soluble system (Srere, 1985; Porpaczy *et al.*, 1983; Sumegi *et al.*, 1980; Fukushima *et al.*, 1989; Tompa *et al.*, 1987; Cohen *et al.*, 1987); and (5) genetic manipulation of yeast cells which show the need for the TCA cycle enzyme proteins as well as the enzymatic activities for acetate growth (Kispal *et al.*, 1989; Srere *et al.*, 1987).

It was recently reported that the metabolism of [2-^{13}C]- or [3-^{13}C]propionate in yeast gave rise to asymmetrically labeled [2-^{13}C]- or [3-^{13}C]alanine (Sumegi *et al.*, 1990). This result was surprising since the accepted path of conversion of propionate to alanine includes the symmetrical intermediates succinate and fumarate, which one would expect to lead to equal labeling between carbons 2 and 3 of alanine whether one started with [2-^{13}C]- or [3-^{13}C]propionate. Numerous isotopic studies over the years had indicated that the isotope of asymmetrically labeled intermediates which entered the Krebs TCA cycle became randomized in the succinate and/or fumarate steps of that pathway (Ajl & Kamen, 1951; Ehrensward *et al.*, 1951; Lorber *et al.*, 1950; Bernhard & Tompa, 1990). If the Krebs TCA cycle enzymes are highly organized, then one could visualize an orientation conserved transfer of intermediates between succinate thiokinase, succinate dehydrogenase, and fumarase to explain this unexpected result. This would mean that the intermediates succinate and fumarate would be transferred from one active site to another without their release to a bulk phase where rotation of the molecules would occur.

Another explanation for these data would be that a pathway for the direct conversion of propionate to pyruvate existed—a pathway that contained no symmetrical intermediates. Such metabolic pathways for propionate have been described in certain organisms (Halarnkar & Blomquist, 1989) but not in *Saccharomyces cerevisiae*. In order to differentiate between the two explanations, we have examined the hypothesis of orientation-conserved transfer in the TCA cycle by using other labeled compounds which could asymmetrically label succinyl-CoA and determine its metabolic fate in yeast. For this purpose, we have used [4-^{13}C]glutamate and [2-^{13}C]acetate and determined the distribution of ^{13}C in aspartate in wild-type yeast, both with and without fluoroacetate and also in several TCA cycle yeast mutant strains. The results reported in this article support the previous work with propionate, which

† This work was supported by grants from the Hungarian Science Foundation (OTKA T5057, TOO 60181, TOO 6360, and T2010) (B.S.), the Department of Veterans Affairs, NIDDK (DK11313), and the National Science Foundation (MCB-9117385) (P.A.S.), and the NIH (HL34557) (A.D.S.).

* Author to whom correspondence should be addressed at the Department of Veterans Affairs Medical Center, 4500 South Lancaster Rd., Dallas, TX 75216. Telephone: (214) 376-5451, x5605. Fax: (214) 372-9534.

‡ Department of Chemistry, The University of Texas at Dallas.

§ University Medical School, Pécs.

∥ Rogers Magnetic Resonance Center.

⊥ Department of Veterans Affairs Medical Center and Department of Biochemistry, The University of Texas at Dallas.

® Abstract published in *Advance ACS Abstracts,* November 1, 1993.

[1] Abbreviations: CS, citrate synthase; CS1$^-$, mutant lacking mitochondrial CS; Acon, aconitase; Acon1$^-$, yeast strain lacking mitochondrial Acon; TCA, tricarboxylic acid; *S. cerevisiae, Saccharomyces cerevisiae*; ICDH, isocitrate dehydrogenase; ICDH1$^-$, deletion mutant of mitochondrial ICDH; ICL$^-$, mutant lacking isocitrate lyase; (NAD)ICDH1$^-$, deletion mutation of mitochondrial (NAD)-isocitrate dehydrogenase; α-KG, α-ketoglutarate; α-KGDC, α-ketoglutarate dehydrogenase complex; MDH, malate dehydrogenase.

0006-2960/93/0432-12725$04.00/0

indicates that orientation-conserved transfer occurs in the Krebs TCA cycle metabolon.

MATERIALS AND METHODS

Materials. L-[4-^{13}C]glutamate was purchased from Texas Isotopes (Farmers Branch, TX), [2-^{13}C]acetate was from MSD Isotope (St. Louis, MO), and monofluoroacetate was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity commercially available.

Yeast Strains and Culture Conditions. Saccharomyces cerevisiae MMY011(*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*) and the mutants Aco1$^-$, ICDH1$^-$, and ICL$^-$ (same genotype except aco1-2, idh2-1, and icl, respectively) were a generous gift from Dr. Mark McCammon (Department of Biochemistry and Molecular Biology, University of Arkansas Medical Sciences). The CS1$^-$ cells were PSY142 (*Matα leu2-2,112 lys2-801 ura3-52 cs1*). The cells were grown in standard YP medium with 0.25% glucose and 2% galactose as carbon sources. Cells were harvested in the late-log phase by centrifugation at 4000 rpm for 10 min.

Glutamate and Acetate Oxidation. One gram of packed yeast cells, either wild type or mutants, was resuspended in 10 mL of pH-balanced (pH 6.2) minimal medium containing 3 or 5 mg of L-[4-^{13}C]glutamate or [2-^{13}C]acetate. The resuspended yeast cells were incubated at 30 °C for the times indicated in the figures and tables and shaken vigorously to supply oxygen. The reaction was stopped with 4% perchloric acid. After standard extraction procedures, the neutralized supernatant was lyophilized and redissolved in 0.6 mL of D_2O for NMR studies.

NMR Spectroscopy. High-resolution ^{13}C NMR spectra were recorded on a GN-500 spectrometer at 11.75 T. Spectra were acquired using a 45° carbon pulse and a 4.5-s delay between pulses to insure nonsaturating conditions. ^{13}C spectra of natural abundance aspartate and glutamate samples at pH 7 collected with the same spectral parameters as those used for the yeast extracts gave equal areas (within 5%) for Asp C2 and C3 and for Glu C2 and C3. Therefore, it was not necessary to correct the resonance areas found in the extract spectra for small differences in nuclear Overhauser enhancements. All samples were maintained at 25 °C during data acquisition.

RESULTS

Figure 1 shows the accepted pathway for glutamate oxidation in yeast cells. As usually depicted, isotope scrambling of C1 and C4 and of C2 and C3 occurs at the symmetrical intermediates, succinate and fumarate. Both malate and oxalacetate would be expected to have a similar equilibration of isotope. If we assume that there is orientation-conserved transfer in the TCA cycle, and it occurs as suggested before on the basis of the [2-^{13}C]- and [3-^{13}C]propionate oxidation data (Sumegi *et al.*, 1990), then we would predict that [4-^{13}C]-glutamate is preferentially converted to [2-^{13}C]malate and [2-^{13}C]oxalacetate. However, if the oxalacetate formed is recycled and converted to α-KG and oxalacetate again, then the enrichment in the C2 and C3 of α-KG will be equal. That is, upon recycling, the glutamate oxidation data cannot differentiate between orientation-conserved transfer or scrambling in the TCA cycle. However, if we block the cycle before the formation of α-KG from isocitrate, the labeling pattern of glutamate oxidation could differentiate between orientation-conserved transfer and scrambling in the TCA cycle.

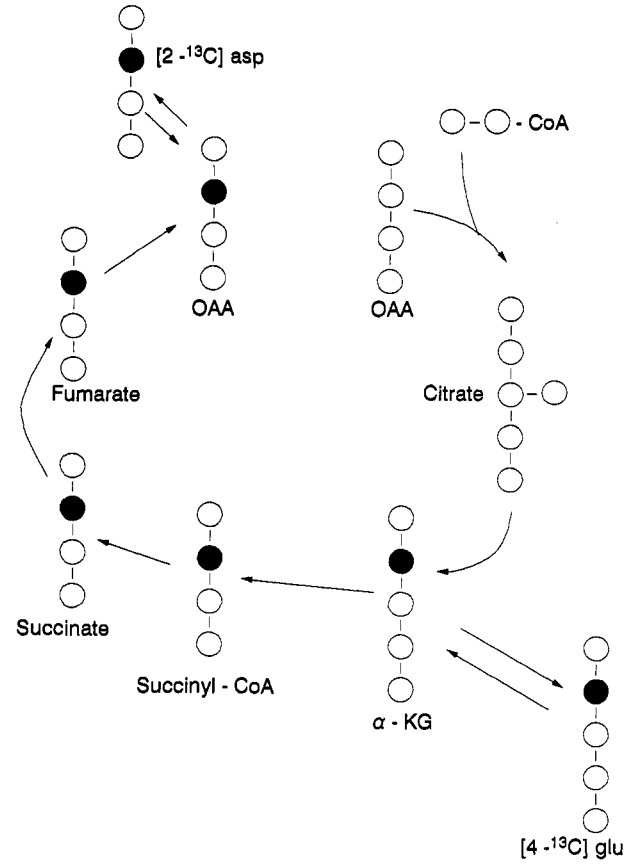

FIGURE 1: Glutamate conversion to aspartate through the Krebs TCA cycle. The C4 of glutamate, shown as ●, becomes the C2 of aspartate during the first turn.

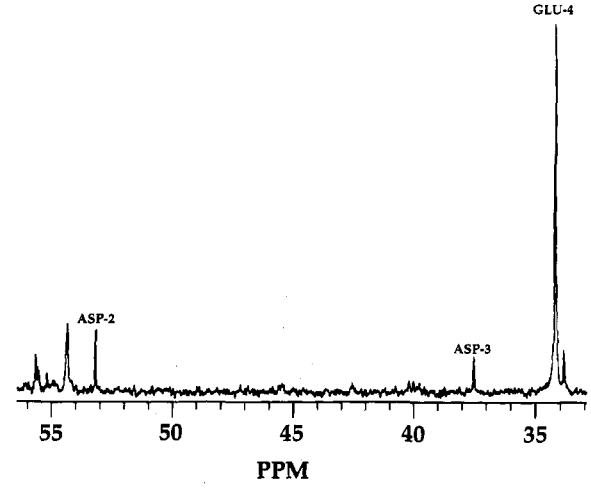

FIGURE 2: Oxidation of [4-^{13}C]glutamate in yeast cells with an inhibited TCA cycle. Yeast cell (W303a) pellets (2 g) were resuspended in 10 mL of yeast minimal medium containing 5 mg of [4-^{13}C]glutamate and 3 mg of fluoroacetate. After incubation for 25 min, the reaction was stopped with perchloric acid. The neutralized, freeze-dried perfusate was redissolved in D_2O and used for NMR spectroscopy as described in Materials and Methods. The aspartate C2 and C3 carbon resonances are labeled as ASP2 and ASP3, respectively. The largest resonance at 34 ppm is from [4-^{13}C]-glutamate, while the largest resonance near 54 ppm arises from natural abundance trimethylamino carbons.

To do this, oxidation of [4-^{13}C]glutamate was studied in wild-type yeast cells in the presence of 3 mM fluoroacetate (Figure 2). Fluoroacetate can be converted to fluoroacetyl-CoA, which is condensed with oxalacetate by CS to form fluorocitrate, a potent inhibitor of aconitase (Morrison &

Table I: Metabolism of [4-^{13}C]Glutamate in Wild-Type and Isocitrate Lyase Mutant Yeast Cells[a]

experiment	added fluoroacetate	aspartate C2	aspartate C3	C2/C3
		Wild-Type Cells		
1	–	142	100	1.42
2	–	112	100	1.12
3	+	152	100	1.52
4	+	178	100	1.78
		ICL1 Mutant Cells		
5	–	118	100	1.18

[a] Experimental conditions are the same as described in Figure 2.

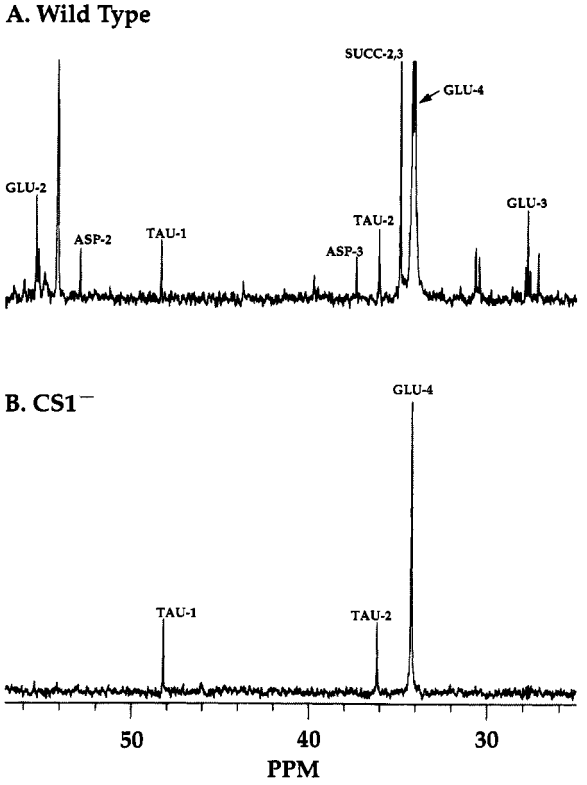

FIGURE 3: Oxidation of [4-^{13}C]glutamate in wild-type and CS1$^-$ yeast cells and determination of the products by ^{13}C NMR. Yeast cells were grown as described in Materials and Methods. In these experiments, yeast cells (1 g) were incubated in 10 mL of minimal medium for 10 min with good aeration in the presence of 3 mg of [4-^{13}C]glutamate. The C2,3 resonance of succinate is labeled SUCC-C2,3, and natural abundance taurine resonances (added to the extracts as an internal standard) are labeled TAU1 and TAU2.

Peters, 1954). This amount of added fluoroacetate likely will not completely inhibit aconitase, but should decrease recycling of oxalacetate enough to test our hypothesis. As illustrated in Figure 2 and summarized in Table I, ^{13}C enrichment of aspartate C2 and C3 is clearly unequal in the presence of fluoroacetate, with aspartate C2 containing, on average, 1.65 times more ^{13}C than the aspartate C3. In the absence of fluoroacetate, this ratio also appeared to be different from 1 (the average in this case was 1.27, see Table I), but was significantly less than that observed in the presence of fluoroacetate.

Oxidation of [4-^{13}C]glutamate was also studied in three TCA cycle mutants, (NAD)ICDH1$^-$, Aco1$^-$, and CS1$^-$. In the wild-type strain, [2-^{13}C]- and [3-^{13}C]glutamate, [2,3-$^{13}C_2$]succinate, and [2-^{13}C]- and [3-^{13}C]aspartate (Figure 3) were detected in significant quantities. However, in the spectra of mutant cells, no [^{13}C]succinate was detected, and ^{13}C enrichment observed in the glutamate C2 and C3 and aspartate C2 and C3 resonances was significantly less (Table II and Figure 3).

Table II: Oxidation of [4-^{13}C]Glutamate in Yeast Mutants

yeast	total ^{13}C resonance areas[a] succinate	glutamate	aspartate
wild type	315	328	129
ICDH1$^-$	<10	160	31
Aco$^-$	<10	49	<10
CS1$^-$	<10	<10	<10

[a] Experimental conditions are the same as described in Figure 3. The numbers represent the total combined areas of the C2 and C3 resonances for each metabolite relative to a fixed amount of taurine added to each sample as an internal reference. The error in the determination of peak areas was less than 5% in these experiments.

In order to test further for orientation-conserved transfer in yeast cells, we also followed the change in the ratio [2-^{13}C]/[3-^{13}C]aspartate using [2-^{13}C]acetate as a precursor. As illustrated in Figure 4, one would predict that even if orientation-conserved transfer of symmetrical intermediates occurs on each turn of the cycle with high efficiency, any asymmetry detected in the enrichment of C2 versus C3 aspartate during early turnover should disappear after the cycle pools have turned over about 3 times. The ^{13}C NMR data presented in Table III show that this ratio decreased in a time-dependent manner.

DISCUSSION

Early experiments using stable and radiolabeled intermediates can be interpreted to indicate that randomization of the label occurred at the steps of the TCA cycle which generated the symmetrical intermediates, succinate and fumarate (Ajl & Kamen, 1951; Ehrensward *et al.*, 1951; Lorber *et al.*, 1950). Measures were not taken to avoid back-reactions, and experimental precision was considerably worse than present-day conditions allow. More recently, there was an attempt to detect "channeling" in the TCA cycle by studying the oxidation of [5-^{13}C]glutamate in intact liver mitochondria (Bernhard & Tompa, 1990). However, the experimental conditions used, high mitochondrial density, possible release and uptake of intermediates, and the use of H_2O_2 which could cause significant changes in the mitochondria structure, probably were not optimal for the detection of orientation-conserved transfer.

A large and undisputed literature exists that shows that sequential Krebs TCA cycle enzymes interact and that they interact with protein components of the inner membrane. Further experiments with disrupted animal mitochondria and with TCA cycle mutant yeast cells have shown that disruption of this organization slows the reaction rate of coupled systems for the first case and changes the phenotype in the second. These data do not support the notion of an unorganized solution of soluble Krebs TCA cycle enzymes. All of the data can be explained most easily by postulating the existence of an organized complex of these metabolic enzymes—a Krebs TCA cycle metabolon.

If one could demonstrate orientation-conserved transfer of symmetrical intermediates in the operation of the postulated metabolon, then such evidence would help establish the existence of a TCA cycle metabolon and, further, provide a rationale for its metabolic importance. However, the absence of orientation-conserved transfer does not negate the evidence that has been accumulated for the existence of an organized complex of TCA cycle enzymes. One can think of many

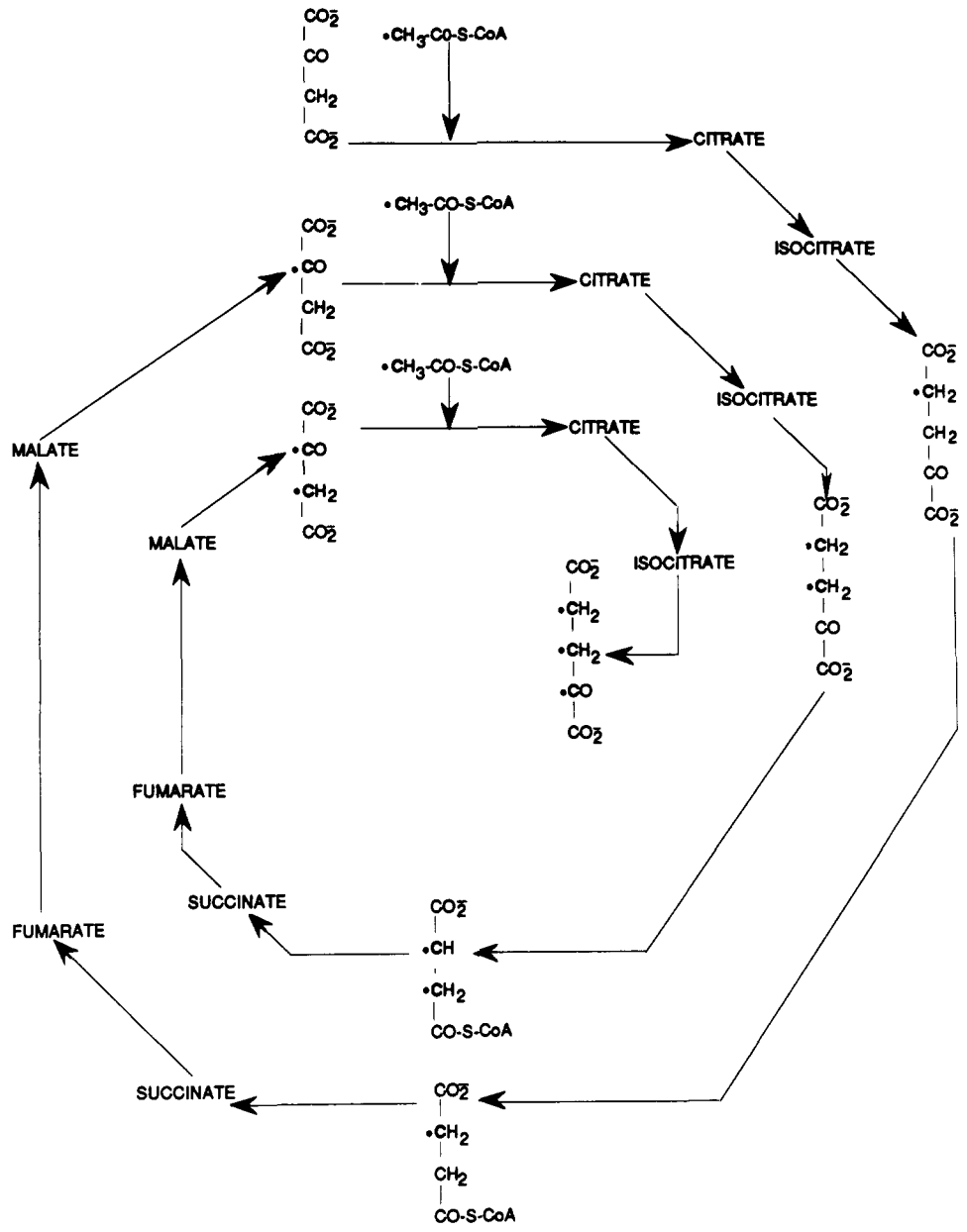

FIGURE 4: Oxidation of methyl-labeled acetyl-CoA through 2 $^1/_2$ turns of the Krebs TCA cycle *with* orientation-conserved transfer, showing that both methylenes of succinate become labeled.

Table III: Time Course of the Incorporation of [2-^{13}C]Acetate into Aspartate in *S. cerevisiae*[a]

time (min)	incorporation into aspartate (in arbitrary units)		aspartate C2/C3
	C2	C3	
2	16.3	10.8	1.51
4	34	24	1.41
6	65	61	1.06
10	100	100	1.00

[a] Experimental conditions are as described in Materials and Methods. Errors in the determination of peak area are less than 5% in these experiments.

experiments to test further for the *in vivo* existence of a complex of TCA cycle enzymes, and we are pursuing such a line of research.

In this work, we chose to follow the metabolism of [4-^{13}C]-glutamate and [2-^{13}C]acetate in yeast by ^{13}C NMR. Even though glutamate is metabolized to many products in cells, no direct conversion of glutamate to aspartate (other than through the TCA cycle) has been reported. We found that, under the conditions we chose, aspartate was present at sufficient levels to allow detection and quantitation of its ^{13}C resonance areas by NMR. If the path were identical from glutamate-generated succinyl-CoA to oxalacetate, as was seen during propionate metabolism (Sumegi *et al.*, 1990), we would expect [4-^{13}C]glutamate to give rise to [2-^{13}C]-aspartate. Our data show that ^{13}C from [4-^{13}C]glutamate is preferentially found in aspartate C2, especially when the TCA cycle is blocked at aconitase by fluoroacetate (Figure 2). In the absence of fluoroacetate, the asymmetry found in aspartate C2 versus C3 was reduced, a result that is anticipated whenever the oxalacetate continues to equilibrate with TCA cycle pools. The asymmetry we observe here in aspartate suggests that orientation-conserved transfer of succinate and fumarate occurs in the TCA cycle with the same spatial orientation that was suggested by the data in our earlier propionate study (Sumegi *et al.*, 1990). More specifically, all of the data support the hypothesis that [3-^{13}C]succinyl-CoA (formed either from [4-^{13}C]glutamate or from [2-^{13}C]propionate) is preferentially

converted to [2-^{13}C]oxalacetate and, hence, to [2-^{13}C]-aspartate.

It is important to note here that, even if one has 100% conservation of orientation in the transfer of the symmetrical intermediates, several cycles of the pathway would lead to a labeling pattern that is indistinguishable from that expected when complete rotational randomization of the symmetrical intermediates occurs (Figure 4). Thus, one test for the conservation of orientation hypothesis would be a time-dependent change in the ratio of [2-^{13}C]aspartate/[3-^{13}C]-aspartate from a value significantly greater than one to a ratio that approaches unity with time. Indeed, we have demonstrated that here using [2-^{13}C]acetate (Table III).

The data on mutant cells reported here (Table II) are in accord with the data reported by Sumegi *et al.* (1990, 1992), using [^{13}C]propionate and [^{13}C]pyruvate as the enriched precursors. These data indicated that mutation in TCA cycle enzymes resulted in a drastic decrease in the production of metabolites associated with the Krebs TCA cycle, even though in some cases one would have expected significant formation of selected intermediates. Our interpretation was that deletion of an enzyme resulted in disruption of the metabolon, thereby resulting in a much greater effect than one would have expected from a system that was not organized. Although the NMR data obtained from mutant cells provide no new direct support for or against the orientation-conserved transfer of symmetrical intermediates in the cycle, the data do support the existence of an organized TCA cycle metabolon, which is a prerequisite for the type of highly organized transfer of TCA cycle intermediates we have detected in these experiments.

ACKNOWLEDGMENT

The authors thank Mrs. Penny Kerby for secretarial assistance.

REFERENCES

Ajl, S. J., & Kamen, M. D. (1951) *J. Biol. Chem. 189*, 845–857.
Beeckmans, S., & Kanarek, L. (1981) *Eur. J. Biochem. 117*, 427–435.
Beeckmans, S., VanDriessche, E., & Kanarek, L. (1989) *Eur. J. Biochem. 183*, 449–454.
Bernhard, S. A., & Tompa, P. (1990) *Arch. Biochem. Biophys. 276*, 191–198.
Cohen, N. S., Cheung, C.-W., & Raijman, L. (1987) *J. Biol. Chem. 262*, 203–208.
D'Souza, S. F., & Srere, P. A. (1983a) *Biochim. Biophys. Acta 724*, 40–51.
D'Souza, S. F., & Srere, P. A. (1983b) *J. Biol. Chem. 258*, 4706–4709.
Ehrensward, G., Reio, L., Saluste, E., & Stjernholm, R. (1951) *J. Biol. Chem. 189*, 93–108.
Fahien, L. A., & Smith, S. E. (1974) *J. Biol. Chem. 249*, 2696–2703.
Fahien, L. A., MacDonald, M. J., Teller, J. K., Fibich, B., & Fahien, C. M. (1989) *J. Biol. Chem. 264*, 12303–12312.
Fukushima, T., Decker, R. V., Anderson, W. M., & Spivey, H. O. (1989) *J. Biol. Chem. 264*, 16483–16488.
Halarnkar, P. P., & Blomquist, G. J. (1989) *Comput. Biochem. Physiol. 92B*, 227–231.
Halper, L. A., & Srere, P. A. (1976) *Arch. Biochem. Biophys. 184*, 529–534.
Hearl, W. G., & Churchich, J. E. (1984) *J. Biol. Chem. 260*, 11459–11463.
Jackson, S. A., Thomson, M. J., & Clegg, J. S. (1990) *FEBS Lett. 262*, 212–214.
Keys, D. A., & McAlister-Henn, L. (1988) *J. Bacteriol. 172*, 4280–4287.
Kispal, G., Sumegi, B., & Alkonyi, I. (1986) *J. Biol. Chem. 261*, 14209–14213.
Kispal, G., Evans, C. T., Malloy, C. R., & Srere, P. A. (1989) *J. Biol. Chem. 264*, 11204–11210.
Lorber, V., Lifson, V., Sakami, W., & Wood, H. G. (1950) *J. Biol. Chem. 183*, 531–538.
Morrison, J. F., & Peters, R. A. (1954) *Biochem. J. 58*, 473.
Porpaczy, Z., Sumegi, B., & Alkonyi, I. (1983) *Biochim. Biophys. Acta 749*, 172–179.
Porpaczy, Z., Sumegi, B., & Alkonyi, I. (1987) *J. Biol. Chem. 262*, 9509–9514.
Robinson, J. B., Jr., Inman, L., Sumegi, B., & Srere, P. A. (1987) *J. Biol. Chem. 262*, 1786–1790.
Srere, P. A. (1985) in *Organized Multienzyme Systems* (Welch, G. R., Ed.) pp 1–61, Academic Press, Inc., New York.
Srere, P. A. (1987) *Annu. Rev. Biochem. 56*, 89–124.
Srere, P. A., & Sumegi, B. (1986) in *Myocardial And Skeletal Muscle Bioenergetics* (Brautbar, N., Ed.) pp 13–25, Plenum Press, New York.
Srere, P. A., Sumegi, B., & Sherry, A. D. (1987) *Biochem. Soc. Trans. 54*, 173–182.
Sumegi, B., & Alkonyi, I. (1983) *Biochim. Biophys. Acta 749*, 163–171.
Sumegi, B., & Srere, P. A. (1984a) *J. Biol. Chem. 259*, 15040–15045.
Sumegi, B., & Srere, P. A. (1984b) *J. Biol. Chem. 259*, 8748–8752.
Sumegi, B., & Porpaczy, Z. (1990) in *Structural And Organizational Aspects Of Metabolic Regulation* (Srere, P. A., Jones, M. E., & Matthews, C., Eds.) Vol. 133, pp 229–244, Alan R. Liss, Inc., New York.
Sumegi, B., Gyocsi, L., & Alkonyi, I. (1980) *Biochim. Biophys. Acta 616*, 158-168.
Sumegi, B., Sherry, A. D., & Malloy, C. R. (1990) *Biochemistry 29*, 9106–9110.
Sumegi, B., Porpaczy, Z., & Alkonyi, I. (1991) *Biochim. Biophys. Acta 1081*, 121–128.
Sumegi, B., McCammon, M. T., Sherry, A. D., Keys, D. A., McAlister-Henn, L., & Srere, P. A. (1992) *Biochemistry 31*, 8720–8725.
Tompa, P., Batke, J., Welch, G. R., & Srere, P. A. (1987) *J. Biol. Chem. 262*, 6089–6092.
Tyiska, R. L., Williams, J. S., Brent, L. G., Hudson, A. P., Clark, B. J., Robinson, J. B., Jr., & Srere, P. A. (1986) in *The Organization Of Cell Metabolism* (Welch, G.R., & Clegg, J. S., Eds.) NATO Series A: Life Sciences, Vol. 127, pp 177–189, Plenum Press, New York.