Effect of CLA on the Cellular Lipids of *Saccharomyces cerevisiae*

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ABSTRACT: The yeast *Saccharomyces cerevisiae* was cultivated in the presence of free CLA that was either a pure *trans*-10,*cis*-12 isomer, a pure *cis*-9,*trans*-11 isomer, or a 1:1 mixture of the two, and the influence of these supplementations on the content and FA composition of the lipids in the yeast was determined. Neither the pure isomers nor their 1:1 mixture influenced the growth of the yeast, but the *trans*-10,*cis*-12 isomer reduced the amount of cellular lipids by 40%. The reduction in total cellular lipids by the *trans*-10,*cis*-12 CLA was due to a reduction in TAG. Both of the isomers were incorporated into the yeast lipids, reaching a proportion of about 33% in TAG. With the incorporation of CLA, the yeast reduced the amount and desaturation of endogenously synthesized FA. These clear and pronounced isomer-specific effects of CLA on the yeast suggest that yeast might be a useful model to obtain a more comprehensive view of the mechanisms of the action of CLA on lipid metabolism.

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KEY WORDS: CLA, conjugated linoleic acid, desaturation, lipid content, *Saccharomyces cerevisiae*, triglycerides, yeast.

Conjugated isomers of linoleic acid (CLA) have been reported to reduce adiposity in animals and humans (reviewed in Ref. 1), and *trans*-10,*cis*-12 CLA has been shown to be responsible for this antilipogenic effect. In particular, the amount of TAG has decreased when a diet containing *trans*-10,*cis*-12 CLA has been fed to test animals or humans. On the basis of numerous *in vivo* and *in vitro* studies, several hypotheses for this mechanism have emerged. The suggestions with the most support are modifications of the activity of enzymes essential in FA uptake and metabolism, increased energy expenditure, and decreased preadipocyte differentiation and proliferation (reviewed in Refs. 1 and 2). However, the exact mechanism of the fat or TAG reduction remains to be elucidated. Considering the effects of CLA isomers on mammalian metabolism, the results of studies carried out by different laboratories are somewhat conflicting. The effects of CLA on animal metabolism seem to be more or less isomer, dose, time, and species specific.

The yeast *Saccharomyces cerevisiae* has been a useful model to understand the synthesis of eukaryotic TAG and its role in various cell processes (3). During growth, *S. cerevisiae* is able to incorporate free long-chain FA from the culture medium and accumulate them without modification in TAG and, as a result, the cellular lipid content is increased (4). Therefore, the FA composition of TAG resembles the FA composition of the growth environment of the yeast. TAG are used as an energy reserves and sources of FA for the synthesis of membrane phospholipids (5,6). All the acquired FA are not necessarily suitable for use in membrane synthesis. Thus, the third function of TAG is to serve as a storage place for such FA (7).

However, less is known about how the yeast lipids are affected when the culture medium is supplemented with CLA, which is known to inhibit the development of adipose tissue in mammals. Elucidation of the effects of CLA on yeast lipids could reveal the potential of yeast as a model organism in CLA studies. Therefore, a study was conducted in which the growth medium *S. cerevisiae* was supplemented with one of two pure, biologically active CLA isomers or with their mixture. After cultivation, the effects on yeast lipid content, lipid class distribution, and FA composition of different lipid classes were determined. The study showed that CLA has isomer-specific effects on the content and composition of yeast lipids, thereby opening up the possibility of elucidating the phenomena at the gene and enzyme level.

EXPERIMENTAL PROCEDURES

Lipid compounds. Linoleic acid (LA) and a CLA isomer mixture [*cis*-9,*trans*-11 and *trans*-10,*cis*-12 (about 40–45% each), minor amounts of other CLA isomers, and LA] and were purchased from Sigma (St. Louis, MO). *Cis*-9,*trans*-11 CLA (>98%) and *trans*-10,*cis*-12 CLA (>98%) isomers were purchased from Matreya Inc. (Pleasant Gap, PA). For determination of the FA concentrations in separated lipid classes, a standard mixture was prepared from heptadecanoic acid (Sigma), triheptadecanoin (Sigma), L-α-PC (dipentadecanyl) (Sigma), and 1,3-dipentadecanoin (Sigma).

Yeast strain and culture conditions. The wild-type baker's yeast, *S. cerevisiae* strain B-72021, was kindly provided by the Technical Research Centre of Finland (Espoo, Finland). The yeast was cultivated in Worth broth (malt extract 15 mg/mL, maltose 12.75 mg/mL, dextrin 2.75 mg/mL, peptone 0.75 mg/mL, ammonium chloride 1.0 mg/mL, and glycerol 3.1 mg/mL, pH 5.0; Merck, Darmstadt, Germany) for 17 h at 30°C by shaking at 270 rpm. For CLA and LA incorporation studies, 1 mL of the yeast culture was added to 50 mL of Worth broth

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supplemented with 0.05–0.4 mg/mL CLA isomer mixture (dissolved in ethanol prior to the supplementation), pure *cis*-9,*trans*-11 CLA, pure *trans*-10,*cis*-12 CLA, or LA and cultivated for 24 h. The concentration of 0.05 g FA per liter of growth medium represented 2% of the dry cell mass at the end of the cultivation. The control cells were cultivated in Worth broth without FA supplementation. The cultivations were performed in duplicate.

Extraction of yeast lipids. The cells obtained from the 50-mL culture by centrifugation were washed twice with tap water and freeze-dried. Total lipids were extracted by direct saponification and methylated as described in the following section. For the separation of lipid classes, the lipids were extracted from 50 mg of the dry cell mass with 5.7 mL of dichloromethane/methanol (2:1) for 18 h at ambient temperature by shaking at 240 rpm. The supernatant, containing at least 80% of total lipids, was evaporated under a nitrogen atmosphere.

Separation of lipid classes. TLC was used to separate the lipid classes. The lipid sample extracted from the 50-mg portion of the dry cell mass was dissolved into 200 μL of dichloromethane/methanol (100:1). The sample was supplemented with the standard mixture just mentioned and applied to a silica gel plate (Kieselgel 60; Merck). The plate was left to develop with petroleum ether/diethylether/acetic acid (80:30:1), and the spots containing TAG, FFA, and polar lipids (PL) were visualized after spraying with 0.01% rhodamine 6G under UV light, scraped off, and used for the FA analysis.

Determination of FA composition. To determine the concentrations of individual FA in different lipid classes, the FA were converted into methyl esters and analyzed by GC as described by Suutari *et al*. (8). In this procedure, the FA were saponified with 3.7 M NaOH in 49% MeOH at 100°C for 30 min and then methylated with 3.3 M HCl in 48% MeOH at 80°C for 10 min. The methyl esters were extracted in a hexane/methyl-*tert*-butyl ether solution (1:1), and the extract was washed with aqueous alkali. Analysis of the FAME was performed by a Hewlett-Packard model 6890 gas chromatograph equipped with an HP-FFAP column (25 m, 0.2 mm i.d., 0.33 mm film thickness; Agilent Technologies, Palo Alto, CA) and an FID. The temperature in the column was raised from 70 to 200°C at a rate of 25°C/min.

RESULTS AND DISCUSSION

Effect of cis-9,trans-11 and trans-10,cis-12 CLA on the growth of the yeast. Saccharomyces cerevisiae was cultivated for 24 h in the presence of *cis*-9,*trans*-11 CLA or *trans*-10,*cis*-12 CLA, or a CLA isomer mixture containing the same isomers at a 1:1 ratio. At the end of the cultivation, the dry cell masses were 2.9 mg/mL in both the nonsupplemented culture and the cultures initially containing 0.2 mg/mL of either the CLA isomer mixture or *cis*-9,*trans*-11 CLA. The corresponding value with the *trans*-10,*cis*-12 isomer was 2.5 mg/mL. These data suggest that the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 CLA isomers had hardly any effect on yeast growth.

Effect of the CLA isomer mixture on lipid accumulation. The presence of the CLA isomer mixture resulted in a 15% reduction in total cellular lipids in comparison with cells from the nonsupplemented control culture (Fig. 1). With a 0.05 mg/mL supplementation, the cellular lipid content was 97 mg/g (dry weight (d.w.)], whereas the lipid content in the cells from the nonsupplemented culture was 114 mg/g (d.w.). The lipid content did not decrease further even though the initial concentration of the CLA isomer mixture was raised to 0.4 mg/mL. The high supplemented doses in comparison with the total lipid content of the yeast suggest that this organism was either relatively insensitive to the isomer mixture or that the influence of one CLA isomer may, at least to some extent, have been counteracted by the other. To discriminate between these alternatives, the isomers present in the isomer mixture, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, were included separately as pure isomers in the following tests.

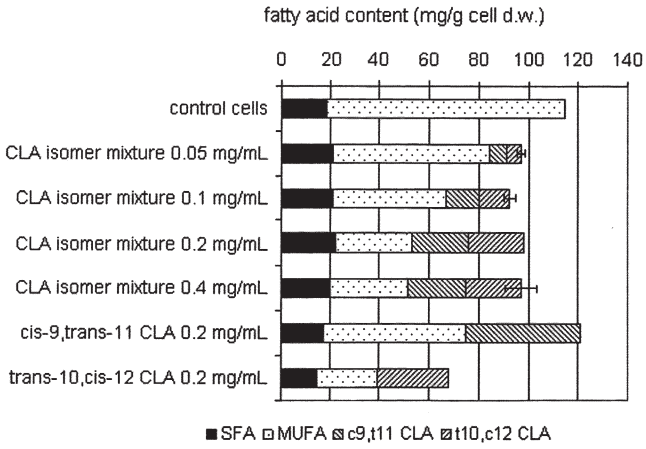

FIG. 1. Effect of the CLA isomer mixture and the pure CLA isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12) on the lipid content and FA composition of *Saccharomyces cerevisiae*. Data are averages of duplicate samples of duplicate cultivations. SD were calculated from the sum of the total FA. The lipid content in the control cells was significantly higher than in the cells grown in the presence of the CLA isomer mixture ($P < 0.05$) or *trans*-10,*cis*-12 CLA ($P < 0.0005$). SFA, saturated FA; MUFA, monounsaturated FA.

Effect of cis-9,trans-11 and trans-10,cis-12 CLA on lipid accumulation. In *S. cerevisiae*, the effects of the isomers were clearly different from each other. The yeast grown in the presence of 0.2 mg/mL *cis*-9,*trans*-11 CLA contained 6% more lipids (121 mg/g d.w.) than the same yeast grown in the nonsupplemented medium (Fig. 1). This difference was statistically insignificant. Instead, *trans*-10,*cis*-12 CLA at the same initial concentration caused a 40% reduction ($P < 0.0005$) in cellular lipid content (from 114 to 68 mg/g). Thus, the lipid-reducing effect was solely attributed to the *trans*-10,*cis*-12 isomer. In several studies in animals, humans, and cell cultures, the CLA used was an isomer mixture (1). The present results, although obtained with a single-cell organism, support the recent view that much of the lipid-reducing effect may be lost by using the CLA isomer mixture.

Effect of cis-9,trans-11 and trans-10,cis-12 CLA on lipid class composition. The yeast cultivated without a FA supplementation had the following lipid class composition: TAG 57

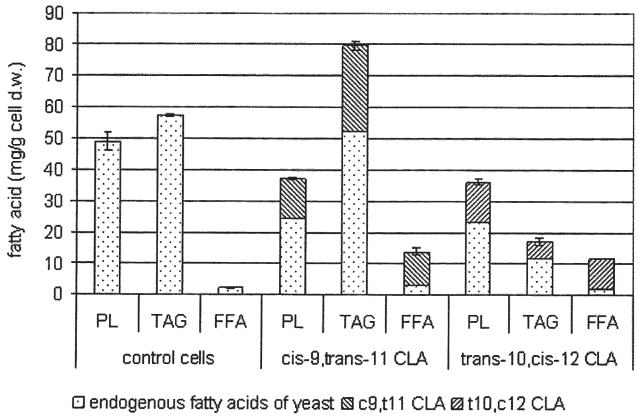

FIG. 2. Amounts of TAG, polar lipids (PL), and FFA in *S. cerevisiae* grown in the presence of the *cis*-9,*trans*-11 or the *trans*-10,*cis*-12 CLA isomer (0.2 mg/mL growth medium). The data are averages of triplicate samples of duplicate cultivations.

mg/g d.w. (50%), PL 49 mg/g (43%), and FFA 4 mg/g (3%) (Fig. 2). By considering the magnitude of the reduction in total cellular lipids by *trans*-10,*cis*-12 CLA (40%), we assumed that a significant reduction had occurred in the TAG class. Figure 2 shows that this reduction in the TAG fraction was 70% (from 57 to 17 mg/g). *Cis*-9,*trans*-11 CLA increased the TAG fraction by 40%, from 57 to 80 mg/g. Moreover, both isomers slightly reduced the amount of PL, from 49 to 37 mg/g, and increased the amount of FFA over fivefold (Fig. 2).

In *S. cerevisiae*, TAG and PL are formed from the common precursor PA (3). In the synthesis of TAG, PA is converted to TAG through a DAG intermediate by phosphatidate phosphatase and DAG acyltransferases. Because PL were not dramatically reduced and, moreover, because they also consisted of the *trans*-10,*cis*-12 isomer, it is possible that the lipid-reducing effect of the *trans*-10,*cis*-12 isomer might occur between the stages from PA to the finished TAG.

Effect of cis-9,trans-11 and trans-10,cis-12 CLA on FA composition. Both of the CLA isomers present in the CLA isomer mixture were incorporated into the acyl lipid pool during growth of the yeast. Figure 1 shows that when the initial concentration of the CLA isomer mixture was 0.2 mg/mL, the proportions of both isomers among the yeast total FA reached their maxima. Then 46% of all cellular FA, 45 mg/g (d.w.), consisted of the two CLA isomers (Table 1), and both isomers were found in nearly equal proportions. With the CLA isomer mixture (0.2 mg/mL), the proportions of the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers were 26 and 21% in the FA of TAG, 22 and 21% in the FA of PL, and 45 and 43% in FFA fraction, respectively.

When the cells were grown in the presence of a single CLA isomer (0.2 mg/mL) instead of the CLA isomer mixture, the proportion of the *cis*-9,*trans*-11 isomer was 38% of all FA and that of the *trans*-10,*cis*-12 isomer was 42% (Table 1). When present as a single isomer (0.2 mg/mL), both the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers were incorporated into TAG and PL and were also present as FFA. Notably, both of the isomers reached equal proportions among the FA of TAG, PL, and FFA, 34, 33, and 80% respectively (Fig. 2).

With the incorporation of the exogenous *cis*-9,*trans*-11 isomer, the amounts of yeast endogenous FA were decreased by 35%, and with the *trans*-10,*cis*-12 isomer the corresponding reduction was 66% (Fig. 1). Thus, both isomers had the capability to reduce the amount of yeast endogenous lipids. This reductive effect of the *cis*-9,*trans*-11 isomer did not appear as a reduced total lipid content since the isomer itself reserved a 38% proportion of total FA and largely compensated for the 35% reduction in endogenous FA. However, with the *trans*-10,*cis*-12 isomer, the reduction in endogenous FA was more pronounced (66%) and the incorporation of the isomer itself (42% of all FA) was not high enough to compensate for the reduction. Thus, the net result appeared as a reduction in the total cellular FA.

In yeast, the repression of FA biosynthesis by the presence of exogenous FA is a well-known phenomenon (9,10). The present data on the CLA isomers also support this view and show that the efficiency of inhibition was largely isomer specific. On the other hand, the effect of the *trans*-10,*cis*-12 isomer appeared as a strong reduction in the TAG fraction, suggesting

TABLE 1
Proportions of FA (%) and Palmitic/Palmitoleic Acid and Stearic/Oleic Acid Ratios in *Saccharomyces cerevisiae* Grown in the Presence of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 Isomers of CLA or Linoleic Acid (LA) (0.2 mg/mL)[a]

	Control cells	*Cis*-9,*trans*-11 CLA	*Trans*-10,*cis*-12 CLA	CLA isomer mixture	LA
C14:0	Trace	Trace	Trace	Trace	Trace
C16:0	12.6 ± 0.3	11.5 ± 1.7	18.8 ± 0.4	18.5 ± 2.7	15.2 ± 1.3
C16:1	51.3 ± 0.5	29.3 ± 9.2	28.8 ± 1.4	21.0 ± 6.4	8.7 ± 4.4
C18:0	3.5 ± 0.2	3.0 ± 0.4	2.9 ± 0.1	4.0 ± 0.1	4.5 ± 0.2
C18:1	32.5 ± 0.6	18.2 ± 1.7	7.7 ± 0.1	10.5 ± 1.2	7.7 ± 0.9
LA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	63.3 ± 5.2
Cis-9,*trans*-11 CLA	0.0 ± 0.0	37.9 ± 5.9	0.0 ± 0.0	23.0 ± 5.9	0.0 ± 0.0
Trans-10,*cis*-12 CLA	0.0 ± 0.0	0.0 ± 0.0	41.9 ± 2.0	22.8 ± 5.9	0.0 ± 0.0
C16:0/C16:1	0.25 ± 0.00	0.39 ± 0.01	0.65 ± 0.02	0.88 ± 0.11	1.74 ± 0.18
C18:0/C18:1	0.11 ± 0.00	0.17 ± 0.00	0.38 ± 0.02	0.38 ± 0.06	0.58 ± 0.09

[a]The data are averages ± SD of duplicate samples of duplicate cultivations.

that the efficiency of acylation may also have been impaired, leading indirectly to an attenuation of FA synthesis.

Effect of cis-9,trans-11 and trans-10,cis-12 CLA on the ratio of saturated FA (SFA) to monounsaturated FA (MUFA). It has been noted in several *in vivo* and *in vitro* studies that CLA isomers, especially *trans*-10,*cis*-12 CLA, increase the ratio of SFA to MUFA in mammals, presumably owing to inhibition of Δ-9 desaturase activity or its transcription (11–13). Both of the CLA isomers were also found to increase the ratio of SFA to MUFA in *S. cerevisiae.* In the *trans*-10,*cis*-12 isomer, the increment was more pronounced than that caused by the *cis*-9,*trans*-11 isomer. However, one can question whether this phenomenon, which appears in both mammals and yeast, was specific to the CLA isomers used or was a common feature of PUFA. Therefore, the yeast was also cultivated in a medium containing 0.2 mg/mL LA. The ratio of SFA to MUFA in the endogenously synthesized lipids increased even more than in the presence of the CLA isomers (Table 1). In *S. cerevisiae*, the desaturation of FA has also been carried out by the enzyme Δ-9 desaturase encoded by the gene *OLE1* (14), and its activity was reported to be repressed by unsaturated FA (15). *Saccharomyces cerevisiae* is known to have the capability of maintaining constant FA unsaturation under varying growth conditions, probably to resist dramatic fluidity changes in its lipids (16). Under the pressure caused by the external supply of CLA, the increment in the ratio of SFA to MUFA may reflect the organism's response by the endogenous fatty acids to maintain lipid homeostasis. According to this view, owing to the conjugated double bond of the CLA isomers, we suggest that these isomers have no specific regulatory effect on FA desaturation, at least in yeast.

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