Triacylglycerol Assembly from Binary Mixtures of Fatty Acids by *Apiotrichum curvatum*

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To observe how the stereospecific distribution of acyl groups in triglycerides is affected by the composition of fatty acids available for esterification, the oleaginous yeast *Apiotrichum curvatum* was grown on various binary mixtures of palmitic, stearic, oleic and linoleic acids as carbon sources, and the yeast triglycerides were analyzed. When oleic acid–linoleic acid mixtures in various ratios were used as substrates, the yeast grew well, and the composition of the intracellular triglycerides reflected the substrate composition, but more linoleate than oleate was deposited in the triglycerides. Oleate was favored over linoleate at the *sn*-2 position of the glycerol. With substrates containing palmitic and stearic acids, the yeast accumulated less oil, and incorporation of stearic acid into the triglycerides also was very limited. When mixtures of palmitic acid–oleic acid and palmitic acid–linoleic acid were used as substrates, the yeast triglyceride composition did not reflect that of the substrate, and the accumulation in the yeast of the unsaturated acid in the substrate was favored. Possibly, the yeast had more limited access to solid than to liquid substrates. For oleic acid–linoleic acid substrates, when the percentages of oleate and linoleate at the three glycerol positions were plotted *vs.* the percentage of these acyl groups in the total triglyceride, apparent linear relations were observed for most of the range, and the sums of the intercepts and slopes of the three lines for each acyl group were 0 and 3, respectively. Two mathematical models of triglyceride assembly are proposed, both of which fit the experimental data. One model assumes that for a certain proportion of the glycerol molecules, the acyl composition of the three *sn* positions is rigidly controlled independently of the substrate concentration. The other assumes that the various acyl groups are distributed on the three *sn* positions of glycerol with different affinities.

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Triglyceride structure affects the consistency, stability and nutritional value of fats and oils (1–3), but because of the complexity of triglyceride mixtures, a complete analysis of triglycerides is seldom achieved. Stereospecific analysis of the acyl composition on the three positions of glycerol has revealed that the distribution of acyl groups is not random (3,4). It has been suggested that the restraints manifest in the stereospecific distribution account for the triglyceride structure, but analyses of olive oil suggest that other restrictions on the distribution also occur (5). Various proposals have been made to account for the distribution of acyl groups on the positions of glycerol, especially in oilseed, where the triglyceride supposedly is simply deposited during seed development. The distribution should reflect the biosynthetic pathways resulting in triglycerides and the specificity of the enzymes involved in the various steps, but the process is complicated by changes with time in the proportions of the acyl groups being deposited and by exchange reactions among the various lipid classes involved in the biosynthesis (6). Studies with labeled precursors and intermediates have led to helpful insights into triglyceride synthesis, but such studies often are limited by the insolubility of lipid substrates in water and the nonphysiological conditions sometimes required for the experiments.

A number of generalizations can be made about the observed distribution of acyl groups on glycerol, and these have been reviewed by Litchfield (3). One of these generalizations notes that, for a group of individual plants or animals with different acyl group compositions, there are linear relations between the percentages of a particular acyl group on the three glycerol positions and the percentage of that acyl group in the whole triglyceride mixture. Such linear relations have been reported in corn (7), soybean (4,8), oat (8), cruciferae (9) and peanut (10,11) oils.

The oleaginous yeast *Apiotrichum curvatum* ATCC 20509 (formerly known as *Candida curvata D*) will accumulate oil when grown on sugar or fat substrates if nitrogen for growth is limiting (12). It also will grow on fatty acids with 14–20 carbon chain lengths and accumulate triglycerides, but it grows best on palmitic, oleic or linoleic acid (13). It tends to deposit the same acyl groups that are in its substrate with minor modifications. In the yeast triglycerides, oleate tends to be favored on the *sn*-2 position of the glycerol, and saturated fatty acids are strongly favored on the *sn*-1 and *sn*-3 positions.

The characteristics of this yeast make it possible to study the effect of a wide range of oleate/linoleate ratios on the stereospecific distribution in the triglycerides, and the results of such a study are reported in this paper. From a few simple assumptions about triglyceride assembly, two models were derived that account for many of the relations observed in yeast and oilseeds.

MATERIALS AND METHODS

Apiotrichum curvatum was maintained as refrigerated slant cultures on yeast extract/dextrose/peptone/agar (1:2:2:1.5, by wt) and transferred monthly. The constituents of the basal medium were (g/L): KH_2PO_4, 2.5; $MgSO_4 \cdot 7H_2O$, 1.0; asparagine, 0.8; $CaCl_2 \cdot 2H_2O$, 0.2; NaCl, 0.06; $FeCl_3 \cdot 6H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.002; $ZnSO_4 \cdot 7H_2O$, 0.001; thiamine-HCl, 0.001; and $CuSO_4 \cdot 5H_2O$, 0.0001 (12). The basal medium was adjusted to pH 5.5 and supplemented with 18 g/L of fatty acids purchased from Sigma Chemical Co. (St. Louis, MO). When linoleic acid was used as a carbon source, it was supplemented with 1,000 ppm of butylated hydroxyanisole to prevent oxidation during incubation.

A seed culture was prepared by inoculating about 5×10^5 cells from a slant culture into 100 mL of heat-

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sterilized basal medium with the fatty acids isolated from saponified corn oil as a carbon source. The culture was grown in 250-mL flasks in a Labline orbital shaker (Melrose Park, IL) at 32°C and 180 rpm. The seed culture was in late logarithmic growth after about 2 d, at which time its optical density at 440 nm was normally 9 to 10. One milliliter of 48-h seed culture was inoculated into 100 mL of medium containing the substrate lipid to be tested. The test cultures were grown for 7 d under the same conditions used for the seed culture.

Residual oleic or linoleic acid was separated from the culture, and the yeast oil was extracted according to Hammond *et al.* (12) by sequential extraction with ethanol, hexane and benzene. Fatty acid mixtures having palmitic and stearic acid were emulsified with 5 g/L gum acacia by blending after sterilization, and agitation was decreased to 140 rpm. When emulsified substrates were used, the yeast cell mass could not be separated completely; so after removal of as much of the cell mass as possible by centrifugation, the supernatant was evaporated in a rotary evaporator, and the residue was pooled with the cell mass recovered by centrifugation. Extraction of lipid in the pooled cel mass was accomplished as before.

The amount of triglyceride in yeast oil was determined by thin-layer chromatography (13). Aliquots of the ethanol extract and pooled hexane and benzene extracts were applied to layers 1.0 mm thick. The plates were developed in hexane/diethyl ether/acetic acid (50:50:1, by vol), and bands were visualized by spraying with 0.2% dichlorofluorescein in ethanol and viewed under ultraviolet light. The triglyceride bands were scraped from the plates and eluted with diethyl ether, and the residue was weighed after evaporation of the ether under nitrogen.

Stereospecific analysis was done according to Christie and Moore (14). For fatty acid analysis, glycerides were transesterified by the method of Frey and Hammond (15), and the methyl esters were analyzed on a Varian Model 3700 Gas Chromatograph (Sugarland, TX) equipped with a 1.8 m × 3.3 mm column of 10% SP-2330 on Chromosorb WAW (Supelco, Bellefonte, PA) and a flame-ionization detector.

RESULTS AND DISCUSSION

Structure of triglycerides from A. curvatum *grown on oleic acid–linoleic acid mixtures.* Table 1 shows the fatty acid composition and stereospecific distribution of the acyl groups in triglycerides from *A. curvatum* grown on pure oleic acid or linoleic acid and on mixtures of oleic and linoleic acids as carbon sources. Saturated acyl groups in the yeast triglycerides amounted to <6% in all instances. Figure 1 shows the percentages of oleate and linoleate in the yeast triglycerides plotted *vs.* the percentages of these fatty acids in the substrate. The intercept of the oleate line is very near zero, but that of the linoleate line is about +8%. Seemingly, linoleate is accumulated in slightly greater amounts than oleate from binary mixtures of the acids, and when one of the pure acids was the substrate, there was greater conversion of oleate to linoleate than of linoleate to oleate by the yeast. The plots had almost the same slopes and correlation coefficients (0.892 and 0.9988 for oleate and 0.900 and 0.9984 for linoleate, respectively).

TABLE 1

Stereospecific Analysis of Triglycerides from *Apiotrichum curvatum* Grown on Oleic Acid and Linoleic Acid at Various Ratios

18:1/18:2 ratio		Acyl composition			
		16:0	18:0	18:1	18:2
100:0	TG[a]	0.8	0.7	92.2	6.3
	sn-1	2.4	0.6	91.1	5.9
	sn-2	—[b]	—	93.5	6.0
	sn-3	—	1.5	91.6	7.0
94:6	TG	3.4	2.6	83.5	10.5
	sn-1	N.A.[c]			
	sn-2	—	—	92.9	7.1
	sn-3	N.A.			
85:15	TG	1.3	1.0	76.9	20.8
	sn-1	3.4	1.4	66.0	29.3
	sn-2	0.5	0.2	86.6	12.6
	sn-3	—	1.4	78.1	20.5
76:24	TG	1.4	1.1	65.7	31.9
	sn-1	2.9	1.4	56.9	38.9
	sn-2	—	—	80.8	19.2
	sn-3	1.3	1.9	59.4	37.6
67:33	TG	1.1	1.3	57.1	40.5
	sn-1	2.0	1.2	50.3	46.6
	sn-2	—	—	73.0	27.0
	sn-3	1.3	2.7	48.0	47.9
45:55	TG	0.8	0.8	39.6	58.8
	sn-1	1.5	0.9	30.9	66.7
	sn-2	0.2	0.1	56.1	43.5
	sn-3	0.7	1.4	31.8	66.2
33:67	TG	0.8	1.0	29.0	69.2
	sn-1	1.6	0.9	22.8	74.8
	sn-2	—	—	43.8	56.2
	sn-3	0.8	2.1	20.4	76.6
15:85	TG	1.3	1.3	13.8	83.6
	sn-1	3.6	2.1	8.7	85.7
	sn-2	0.5	0.3	27.6	71.6
	sn-3	−0.2	1.5	5.1	93.5
10:90	TG	1.2	1.6	9.0	88.2
	sn-1	3.6	2.4	7.5	86.5
	sn-2	—	—	14.7	85.3
	sn-3	—	2.4	4.8	92.8
0:100	TG	0.9	1.5	1.3	96.3
	sn-1	2.3	1.5	0.7	95.5
	sn-2	—	—	2.2	97.8
	sn-3	0.4	3.0	1.0	95.6

[a]Triglyceride. [b]Not detected. [c]Data not available.

In the triglycerides from the yeast grown on oleic acid–linoleic acid mixtures, oleate was favored at the *sn*-2 position compared with linoleate. The same trend was reported in oat oil (8), but in many seed oils, linoleate is favored in the *sn*-2 position (3). The percentages of oleate and linoleate in the *sn*-1, *sn*-2 and *sn*-3 positions were linearly related to the total percentages of these acyl groups in the triglyceride over a certain range. For oleate, the range was approximately 15–70%, and for linoleate it was approximately 25–80%. The slopes, intercepts and correlation coefficients for the linear ranges of oleate and linoleate are given in Table 2. The sign of the intercepts (positive or negative) in Table 2 indicates whether the placement of an acyl group in a certain position is favored or not. Slopes greater than 1 indicate that, as the amount of an acyl group in the whole oil increases, there is a tendency

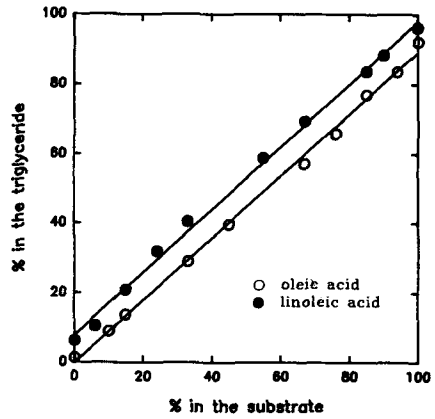

FIG. 1. The percentage of oleate and linoleate in the *Apiotrichum curvatum* triglycerides *vs.* their percentage in the substrates on which the yeast was grown. Standard linear regression lines are shown.

to place more of it in a particular glycerol position than is present in the whole oil. A slope less than 1 indicates the opposite. The slopes in Table 2 are close to 1, but slopes that deviate significantly from 1 are not unusual in vegetable oils (4,8).

Theoretical treatment of glyceride distribution. Fatemi and Hammond (4) observed that, if the slopes and intercepts of plots, such as those described in the preceding paragraph, were determined for soybean oil, the slopes for a particular acyl group for the three positions would total 3 and the intercepts would total 0. The use of plant varieties for such plots limits the range of the data to the range of fatty acid compositions that are available. Pan and Hammond (8) pointed out that, although these plots were linear over the range that could be observed, it was impossible for them to be linear over their entire range and that the lines must bend toward 0,0 and 1,1 at their extremes. The data obtained with *A. curvatum* have made it possible to examine a much longer range and to verify this prediction.

Two models are proposed to account for the data shown in Tables 1 and 2 and Figure 1. Model 1 can be used to explain the apparently linear relations, with slopes of the lines in Table 2 adding to 3.0 and intercepts adding to 0.0. The derivation is based on the simple hypothesis (Model 1) that, for some fraction of the glycerol molecules, there are enzymes that determine which acyl groups appear at the *sn*-1, *sn*-2 and *sn*-3 positions independently of the substrate concentration. This might be accomplished either by controlling which acyl group gets attached or by possible conversion of acyl groups from one form to another after attachment to the glycerol backbone. An alternate, simple model (Model 2) derived from basic chemical kinetics assumes that the glycerol positions are filled in proportion to substrate concentration but that the three *sn* positions of glycerol have different affinities for the various acyl groups. Model 2 leads to nonlinear relations, all of which pass through 0,0 and 1,1; however, Model 2 fits the seemingly linear data rather well. Both models assume that there are just two acyl groups, A and B, and that they are present in the substrate in the proportions α:β, respectively, with $\alpha + \beta = 1$. However, as a first approximation, the models can be used when more than two acyl groups are being studied, by letting A denote one specific acyl group and B denote all of the remaining acyl groups collectively. For the data in Table 1, A is identified with oleate and B with linoleate. B is favored at positions *sn*-1 and *sn*-3, and A is favored at position *sn*-2. The models can be easily adapted to other positional preferences.

TABLE 2

Linear Regression of the Percentage of an Acyl Group at the *sn* Positions of Glycerol *vs.* the Percentage of the Acyl Group in the Total Triglyceride[a]

Fatty acid	Position	Intercept	Slope	r^b
Oleate	*sn*-1	−4.76	0.94	0.9986
	sn-2	14.05	1.03	0.9992
	sn-3	−9.30	1.03	0.9991
	Sum	−0.01	3.00	
Linoleate	*sn*-1	10.02	0.93	0.9966
	sn-2	−14.04	1.01	0.9984
	sn-3	4.02	1.06	0.9994
	Sum	0.00	3.00	

[a]Ranges used for this linear regression were 13.8–65.7% for oleate and 31.9–83.6% for linoleate in the total triglyceride.
[b]Correlation coefficient.

Model 1. Assume that there are enzymes, E_1, E_2 and E_3, that control the character of the acyl groups attached to, respectively, the *sn*-1, *sn*-2 and *sn*-3 positions on certain fractions of the glycerol molecules. Assume that E_1 affects a proportion, p_1, of the glycerol molecules and ensures that acyl group B is attached to position *sn*-1. The action could be accomplished by selecting B and attaching it to *sn*-1, by blocking the attachment of A to *sn*-1, or by converting to B all acyl groups A at *sn*-1 in the fraction affected by E_1. The fraction, $1 - p_1$, of the glycerol molecules that is not affected by E_1 has A and B attached to *sn*-1 in the substrate proportions α:β. Enzyme E_2 acts at position *sn*-2 to ensure acyl group A is attached to *sn*-2 on a fraction p_2 of the glycerol molecules, and enzyme E_3 ensures that B appears at position *sn*-3 on a fraction p_3 of the glycerol molecules. It is not necessary to assume that the enzymes act independently, so that, for example, the fraction of the glycerol acted on by both E_1 and E_3 may be different from the fraction $p_1 \cdot p_3$ that would be expected from independence.

Analysis of Model 1. At position *sn*-1, the fraction p_1 of the glyceride molecules has acyl group B, and $1 - p_1$ of the molecules has acyl groups A and B distributed according to the ratio α:β. Therefore, the fraction, A_1, of glyceride molecules with acyl group A at *sn*-1 is $(1 - p_1)\alpha$. At position *sn*-2, the fraction p_2 of the molecules has A, and $1 - p_2$ of the molecules has A and B in the proportions α:β, so that the fraction A_2 of glyceride molecules with acyl group A at *sn*-2 is $p_2 + (1 - p_2)\alpha$. Analysis of position *sn*-3 is similar to *sn*-1, and the fraction, A_3, of glyceride molecules with acyl group A at *sn*-3 is $(1 - p_3)\alpha$. The fraction, A_*, of the total positions on the glyceride molecules on which fatty acid A appears is $(A_1 + A_2 + A_3)/3$. This yields Equations 1 and 2.

$$A_1 = (1 - p_1)\,\alpha$$

$$A_2 = (1 - p_2)\,\alpha + p_2$$

$$A_3 = (1 - p_3)\,\alpha \qquad [1]$$

$$A_* = \left(1 - \frac{p_1 + p_2 + p_3}{3}\right)\alpha + \frac{p_2}{3} \quad [2]$$

Because $A_* + B_* = 1$, one obtains from Equation 2:

$$B_* = \left(1 - \frac{p_1 + p_2 + p_3}{3}\right)\beta + \frac{p_1}{3} + \frac{p_3}{3} \quad [3]$$

and in a similar way one can obtain equations for B_1, B_2 and B_3 from Equations 1. Equations 2 and 3 describe linear relations as illustrated in Figure 1, in which the fraction of an acyl group in the triglyceride appears to be linearly related to the fraction of the acyl group in the substrate. Note that the slopes are equal, consistent with the observed slopes shown in Figure 1 of 0.90 and 0.892. Both intercepts are positive in Equations 2 and 3 at zero substrate concentrations, due to the hypothesized enzymatic specificity for the fatty acids. Only one intercept is positive in Figure 1, but it is this positive intercept that motivates a model in which an acyl group of a certain type is placed on the glyceride molecule independently of the amount in the substrate. The linear Equations 1 can be fit by the method of least squares to oleate data in Table 1 to get estimates of p_1, p_2 and p_3 for use in Equation 2. In a similar way, linoleate data from Table 2 can be used to get estimates of p_1, p_2 and p_3 for use in Equation 3. With these values, the graphs of Equations 2 and 3 are almost indistinguishable from the regression lines shown in Figure 1. The two sets of estimates are similar, (0.1994,0.0972,0.1672) and (0.1490,0.1001,0.1350), with the two estimates for p_2 being very close, since nearly all the acyl groups at *sn*-2 are either oleate or linoleate.

To get equations descriptive of the linear relations found in Table 2, one eliminates α between the expressions for A_i, i = 1, 2 and 3, in Equations 1 and A_* in Equation 2, and obtains Equations 4.

$$A_1 = \frac{3(1 - p_1)}{3 - p_1 - p_2 - p_3}A_* - \frac{(1 - p_1)p_2}{3 - p_1 - p_2 - p_3}$$

$$A_2 = \frac{3(1 - p_2)}{3 - p_1 - p_2 - p_3}A_* + \frac{(2 - p_1 - p_3)p_2}{3 - p_1 - p_2 - p_3}$$

$$A_3 = \frac{3(1 - p_3)}{3 - p_1 - p_2 - p_3}A_* - \frac{(1 - p_3)p_2}{3 - p_1 - p_2 - p_3} \quad [4]$$

Observe that the sum of the slopes is 3 and that the intercepts sum to 0, as is true of the data shown in Table 2. Equations 4 yield values of A_i between 0 and 1 when $p_2/3 < A_* < 1 - p_1/3 - p_3/3$. Figure 2 is a plot of the oleate on the three *sn* positions of glycerol *vs.* the oleate in the total triglyceride with the linear Equations 4 fit to the data. The lines correspond to, and are almost exactly the same as, those in Table 2. Because the equations are not linear in p_1, p_2 and p_3, nonlinear least squares fitting procedures are required.

Model 2. Assume that the affinities for A and B at the *sn*-1 position are, respectively, K(1,A) and K(1,B). The number of A acyl groups that attach to *sn*-1 will depend on the substrate fraction α and the ratio of K(1,A) to K(1,B). The fraction of A on *sn*-1 is assumed to be Equation 5.

$$\frac{K(1,A)\alpha}{K(1,A)\alpha + K(1,B)\beta} = \frac{K(1,A)\alpha}{K(1,A)\alpha + K(1,B)(1 - \alpha)} = \frac{r_1\alpha}{r_1\alpha + 1 - \alpha} \quad [5]$$

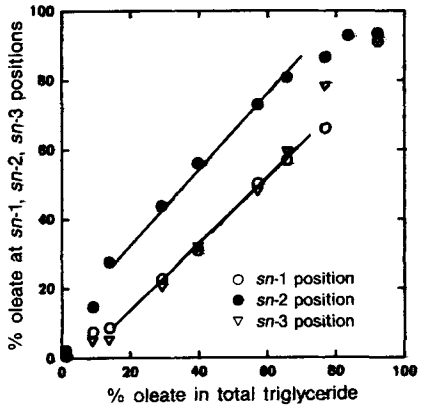

FIG. 2. The percentage of oleate in the *sn*-1, *sn*-2 and *sn*-3 positions of glycerol *vs.* the percentage of oleate in the total triglyceride fitted with Equations 4 with $p_1 = 0.2673$, $p_2 = 0.1657$, $p_3 = 0.2715$. The range of 13.8–65.7% oleate in the total triglyceride was used, as in Table 2.

where

$$r_1 = \frac{K(1,A)}{K(1,B)} \quad [6]$$

Assuming that B is preferred at *sn*-1 (meaning that the binding affinity for B at *sn*-1 is higher than for A), one would have $r_1 < 1.0$. Similar notation is assumed for *sn*-2 and *sn*-3, with $r_2 > 1.0$ and $r_3 < 1.0$.

Analysis of Model 2. From the definitions in Model 2 and of A_*, A_1, A_2 and A_3, one immediately obtains Equations 7–9.

$$A_1 = \frac{r_1\alpha}{r_1\alpha + 1 - \alpha}$$

$$A_2 = \frac{r_2\alpha}{r_2\alpha + 1 - \alpha}$$

$$A_3 = \frac{r_3\alpha}{r_3\alpha + 1 - \alpha} \quad [7]$$

$$A_* = \frac{1}{3}\left[\frac{r_1\alpha}{r_1\alpha + 1 - \alpha} + \frac{r_2\alpha}{r_2\alpha + 1 - \alpha} + \frac{r_3\alpha}{r_3\alpha + 1 - \alpha}\right] \quad [8]$$

As in Model 1, From $A_* + B_* = 1$, one obtains an expression:

$$B_* = \frac{1}{3}\left[\frac{\beta}{r_1(1 - \beta) + \beta} + \frac{\beta}{r_2(1 - \beta) + \beta} + \frac{\beta}{r_3(1 - \beta) + \beta}\right] \quad [9]$$

and in a similar way one can get equations for B_1, B_2 and B_3. In Equation 8, the relation between A_* and α is not linear, and the graph passes through 0,0 and 1,1. One can use a nonlinear minimization computer code to do a least squares fit of Equations 7 to the oleate data of Table 1 in order to get estimates for r_1, r_2 and r_3 for use in Equation 8, and a fit of a similar set of equations to the linoleate data and for use in Equation 9. Doing so, we obtain a figure analogous to Figure 1, shown in Figure 3. It is apparent from the graphs that the relations are not linear and that the intercepts are all at 0,0 and at 1,1.

One can eliminate α between Equations 7 and Equation 8 and obtain the following equations analogous to

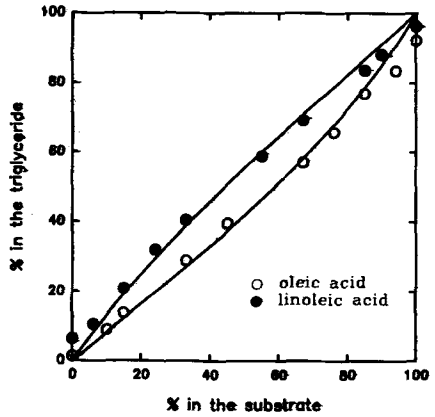

FIG. 3. The percentage of oleate and linoleate in yeast triglycerides *vs.* their percentage in the substrate with the graphs of Equations 8 and 9. The values of $r_{1,A} = 0.4651$, $r_{2,A} = 1.5460$, $r_{3,A} = 0.5041$ were obtained from fitting Equations 7 to oleate data in Table 1 and used in Equation 8. A similar procedure was used to obtain $r_{r,B} = 0.6468$, $r_{2,B} = 1.5689$, $r_{3,B} = 0.5768$ for use in Equation 9.

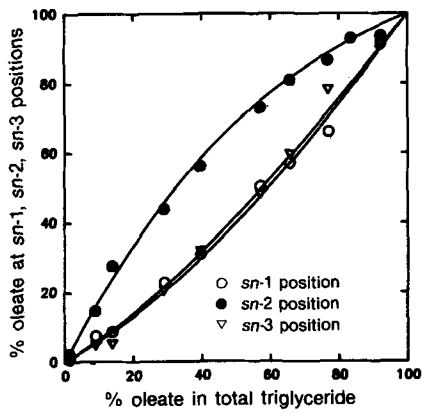

FIG. 4. The percentage of oleate in the *sn*-1, *sn*-2 and *sn*-3 positions of glycerol *vs.* the percentage of oleate in the total triglyceride fitted with Equations 10; $r_1 = 0.3146$, $r_2 = 1.0$ and $r_3 = 0.3380$.

Equations 4 and descriptive of the relations shown in Figure 2 (see Equations 10).

$$3A_* = A_1 + \frac{A_1 r_2}{(r_2 - r_1)A_1 + r_1} + \frac{A_1 r_3}{(r_3 - r_1)A_1 + r_1}$$

$$3A_* = \frac{A_2 r_1}{(r_1 - r_2)A_2 + r_2} + A_2 + \frac{A_2 r_3}{(r_3 - r_2)A_2 + r_2}$$

$$3A_* = \frac{A_3 r_1}{(r_1 - r_3)A_3 + r_3} + \frac{A_3 r_2}{(r_2 - r_3)A_3 + r_3} + A_3 \qquad [10]$$

To write A_i in terms of A_* requires computing the root of a cubic, and it is better to use a root-solving routine to compute the value of A_i, given A_*, as needed. These equations are homogeneous in the values r_1, r_2 and r_3; the relations defined for one set of values r_1, r_2 and r_3 will be exactly the same for another set of values Cr_1, Cr_2 and Cr_3 for any nonzero number C. Therefore, in fitting these equations to data, such as that represented in Figure 2, only the relative values of r_1, r_2 and r_3 will be obtained. We normalized the three equations by dividing by r_2, (equivalent to restricting r_2 to be 1.0), fit the equations to the data in the oleate column of Table 1 and obtained $r_1 = 0.3146$ and $r_3 = 0.3308$. These compare with the values in Figure 3, $r_{1,A}/r_{2,A} = 0.3008$ and $r_{3,A}/r_{2,A} = 0.3261$. The resulting curves are shown in Figure 4, where it can be seen that these curved lines fit the data very well.

In plots analogous to Figure 2, the slopes add to 3, and the intercepts add to 0. The data for Figure 2 are based on N measurements $\{A_{i,j}\}_{j=1,N}$ of oleate percentages at each position, *sn*-i, i = 1, 2 and 3, and computation of $A_{*,j} = (A_{1,j} + A_{2,j} + A_{3,j})/3$, j = 1, N. Similar plots are shown in (4,8,10,11) in which straight lines $A_i = m_i A_* + b_i$ are fit by the method of least squares to the data $\{A_{i,j}, A_{*,j}\}_{j=1,N}$ for i = 1, 2 and 3. We will show that for all such regressions, there is an algebraic identity that $m_1 + m_2 + m_3 = 3$ and $b_1 + b_2 + b_3 = 0$. From the method of least squares, the "normal equations" (16) used to obtain m_i and b_i (for i = 1, 2 or 3) are represented by the matrix equation, Equation 11.

$$\begin{bmatrix} \sum_{j=1}^{N} A_{*,j}^2 & \sum_{j=1}^{N} A_{*,j} \\ \sum_{j=1}^{N} A_{*,j} & \sum_{j=1}^{N} 1 \end{bmatrix} \begin{bmatrix} m_i \\ b_i \end{bmatrix} = \begin{bmatrix} \sum_{j=1}^{N} A_{*,j} A_{i,j} \\ \sum_{j=1}^{N} A_{i,j} \end{bmatrix} \qquad [11]$$

The matrix on the left is indepdent of i and is invertible. Adding the three equations for i = 1, 2 and 3, one obtains Equation 12.

$$\begin{bmatrix} \sum_{j=1}^{N} A_{*,j}^2 & \sum_{j=1}^{N} A_{*,j} \\ \sum_{j=1}^{N} A_{*,j} & \sum_{j=1}^{N} 1 \end{bmatrix} \begin{bmatrix} m_1 + m_2 + m_3 \\ b_1 + b_2 + b_3 \end{bmatrix} = \begin{bmatrix} \sum_{j=1}^{N} A_{*,j}(A_{1,j} + A_{2,j} + A_{3,j}) \\ \sum_{j=1}^{N} (A_{1,j} + A_{2,j} + A_{3,j}) \end{bmatrix} \qquad [12]$$

Using the definition of $A_{*,j} = (A_{1,j} + A_{2,j} + A_{3,j})/3$, one obtains Equation 13.

$$\begin{bmatrix} \sum_{j=1}^{N} A_{*,j}^2 & \sum_{j=1}^{N} A_{*,j} \\ \sum_{j=1}^{N} A_{*,j} & \sum_{j=1}^{N} 1 \end{bmatrix} \begin{bmatrix} m_1 + m_2 + m_3 \\ b_1 + b_2 + b_3 \end{bmatrix} = \begin{bmatrix} 3\sum_{j=1}^{N} A_{*,j}^2 \\ 3\sum_{j=1}^{N} A_{*,j} \end{bmatrix} \qquad [13]$$

This last matrix equation has the solution $m_1 + m_2 + m_3 = 3$ and $b_1 + b_2 + b_3 = 0$, which is unique because the matrix on the left is invertible.

Biological significance of the models. The biased distribution of acyl groups at the *sn* positions of glycerol has mostly been attributed to biases in the specificity of the enzymes involved (6). Model 2 shows that if these biases are the only factors operating, plots such as those in Figures 2 and 4 cannot be linear; however, the curvature

of the plot may be slight enough to appear linear over a short range of variation. The yeast data reported here are the only data available with enough range to demonstrate such curvature, and their fit, illustrated in Figure 4, support this model. Model 2 is also supported by reports that the amounts of individual triglycerides in seed fats agree fairly well with the amounts predicted by stereospecific analysis and 1-random-2-random-3-random distribution Equations 4.

For a truly linear outcome of plots, such as those in Figures 2 and 4, Model 1 teaches that a fixed fraction of the triglycerides must have a particular acyl group distribution that is uninfluenced by the amounts of fatty acids available for esterification. Such a distribution might occur, for example, if a fraction of the triglycerides is always formed from a certain pool of phosphatides with rigid acyl group compositions. This sort of assumption is supported by plots (*e.g.*, Fig. 1) that show a fixed bias in the incorporation of linoleate regardless of the amount available in the medium. This assumption also is supported by the seeming linearity of plots (*e.g.*, Fig. 2) for many seed oils. This model also might account for nonrandom biases in acyl group distribution that cannot be attributed to stereospecific distributions, such as those reported for olive oil (5).

Effect of substrate mixtures containing saturated fatty acid on the composition of yeast triglycerides. The other binary mixtures of fatty acids could not be studied over the wide range used for oleic acid–linoleic acid mixtures because the acyl group composition of *A. curvatum* triglycerides varied over a relatively narrow range, regardless of the ratios of fatty acids in the substrate. Also the emulsions of solid, saturated, high-melting acids used as substrates made it difficult to isolate the yeast triglyceride for stereospecific analysis. Table 3 shows the fatty acid composition of triglycerides isolated from *A. curvatum* grown on various binary combinations that included saturated fatty acids as carbon sources. When large proportions of stearic acid were present in the medium, the amount of accumulated yeast oil decreased (data not shown) in agreement with the previous observations by Lee *et al.* (13). Less than 10% stearic acid was observed in the yeast triglycerides even from a substrate with 75% stearic acid. Seemingly, the yeast reluctantly used and incorporated stearic acid into its triglycerides. In a study using cell-free extracts and spheroplasts, Holdsworth and Ratledge (17) reported that the activity of fatty acyl coenzyme A (CoA) synthetase in *A. curvatum* was some 6- to 8-fold lower with stearic acid than with palmitic, oleic and linoleic acids. The apparently poor substrate activity of this enzyme with stearic acid might account for the very limited utilization of stearic acid and its low incorporation into the triglyceride. In the stearic acid–oleic acid and palmitic acid–oleic acid mixtures, the presence of saturated acids seemed to decrease by 2–4% the linoleate found in the yeast triglyceride compared with that found for pure oleic acid as a substrate.

Although Holdsworth and Ratledge (17) reported that the activities of fatty acyl CoA synthetase for palmitic and linoleic acids were similar, our microscopic observation of the yeast showed that they accumulated less lipid as the proportion of palmitic acid increased in the substrate. In palmitic acid–stearic acid mixtures, the yeast seemed to prefer palmitic over stearic acid because the yeast triglyceride contained much more palmitate than stearate. With palmitic acid–oleic acid or palmitic acid–linoleic acid substrates, the yeast contained only 15–18% palmitate when the proportion of palmitic acid was 25–50%. It is suggestive that the 15–18% palmitate found in these triglycerides corresponded to the solubility of palmitic acid in oleic acid at 33°C, namely, 15.7% (18). It appears likely that the access of the yeast to solid lipid substrate is less than for liquid fatty acids, and this influences the proportions of the acyl groups in the yeast triglyceride. The percentage of palmitate in the triglyceride increased to 25–40% when the proportion of palmitic acid in the substrate was 75%. Much more palmitate was found in combination with oleate than with linoleate.

Table 4 shows the stereospecific analyses of three triglycerides that we were able to isolate from yeast grown on mixtures containing palmitic acid and an unsaturated acid. The palmitate accumulated in the *sn*-1 and *sn*-3 positions, and the amounts of unsaturated acyl groups in these positions decreased relative to the amounts found for substrates of pure oleate or linoleate.

TABLE 3

Fatty Acid Composition of Triglyceride from *Apiotrichum curvatum* Grown on Various Combinations of Saturated and Unsaturated Fatty Acids as Carbon Sources[a]

Carbon source	Acyl composition				
	16:0	16:1	18:0	18:1	18:2
16:0/18:0 = 75:25	60.7	11.6	1.3	23.9	2.4
50:50	56.5	8.7	2.7	28.3	3.8
25:75	50.8	4.5	6.5	33.6	4.5
16:0/18:1 = 75:25	39.9	3.1	1.6	50.7	4.8
50:50	17.7	—	1.5	78.3	2.5
25:75	16.9	—	1.6	78.8	2.7
16:0/18:2 = 75:25	25.3	1.7	1.9	6.3	64.8
50:50	15.0	—	1.7	2.3	81.1
25:75	14.9	—	2.0	4.0	79.1
18:0/18:1 = 75:25	0.9	—	9.7	86.4	3.1
50:50	0.5	—	2.5	95.1	1.2
25:75	0.5	—	3.1	94.7	1.7
18:0/18:2 = 75:25	—	—	6.0	2.1	91.9
50:50	0.5	—	5.7	1.5	92.3
25:75	0.5	—	3.7	1.3	94.5
16:0 = 100	61.3	13.9	0.8	21.5	2.6
18:0 = 100	0.9	—	48.0	46.1	5.1
18:1 = 100	0.8	—	0.7	92.2	6.3
18:2 = 100	0.9	—	1.5	1.3	96.3

[a]The compositions observed for substrates of single fatty acids used in the mixtures are included for comparison.

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TABLE 4

Stereospecific Analysis of Triglycerides from *Apiotrichum curvatum* Grown on Mixtures of Palmitic Acid and Oleic Acid or Linoleic Acid

Carbon sources		Acyl composition				
		16:0	16:1	18:0	18:1	18:2
16:0/18:1 = 75:25	TG[a]	42.0	2.5	1.4	51.4	2.7
	sn-1	62.4	3.2	1.8	30.5	1.9
	sn-2	2.4	2.7	—[b]	88.7	5.4
	sn-3	61.0	1.6	2.4	35.0	0.8
16:0/18:2 = 75:25	TG	25.2	1.2	2.0	6.3	65.4
	sn-1	37.3	1.4	3.2	3.3	56.2
	sn-2	1.6	1.2	—	11.6	84.6
	sn-3	36.7	1.0	2.8	4.0	55.4
16:0/18:2 = 25:75	TG	15.1	—	2.2	4.1	78.6
	sn-1	26.6	—	2.6	2.1	68.8
	sn-2	0.8	—	—	6.0	93.4
	sn-3	17.9	—	4.0	4.2	73.6

[a]Triglyceride. [b]Not detected.

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