Kinetics of Acyl Migration in Monoglycerides and Dependence on Acyl Chainlength

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ABSTRACT: The chemical rearrangement reaction of β- into α-monoglycerides is described. Four monoglycerides with different fatty acid chainlength have been investigated, and the equilibrium constant and the reaction velocity constants characterizing the reaction of the chemical rearrangement were estimated with a mathematical model. A 1:6.5 mixture of α/β-monoglycerides appeared to rearrange to a mixture of 1:1 α/β-monoglycerides within 24 h. Furthermore, the reaction velocity of the rearrangement reaction of β- to α-monoglycerides depended on the fatty acyl chainlength in the monoglyceride. *JAOCS 73,* 707–711 (1996).

KEY WORDS: Chemical rearrangement, equilibrium constant, monoglyceride, reaction velocity, reaction velocity constants.

Monoglycerides are widely used as emulsifiers in foods, pharmaceuticals, and cosmetics (1,2). At present, most monoglycerides are prepared by either a straightforward chemical esterification of glycerol and fatty acids or by glycerolysis of natural fats. During the past few decades, lipases also have been researched for their potential use in the production of monoglycerides. Various ways of lipase-catalyzed production of monoglycerides have been suggested, three of which are mentioned here. Monoglycerides can be formed by applying regioselective hydrolysis of triglycerides with a 1,3-specific lipase (3,4), resulting in a mixture of fatty acid mono- and diglycerides that must be purified. Alternatively, monoglycerides can be synthesized by interesterifying triglycerides with excess glycerol and a 1,3-specific lipase. The resulting mixture contains α- as well as β-monoglycerides (5). Finally, monoglycerides can be formed by esterifying free fatty acids with excess glycerol and a nonspecific lipase at certain conditions, yielding predominantly α-monoglycerides (6).

In theory, regioselective hydrolysis only yields β-monoglycerides. However, upon exploring this reaction, α-monoglycerides are produced as well. The occurrence of α-monoglycerides can be explained by assuming some degree of nonspecific activity of the lipase. However, the *Rhizopus javanicus* lipase used is reported to be virtually 100% selective (7). This suggests that the α-monoglycerides are formed *via* an intramolecular rearrangement. However, it is not known how the rate and equilibrium of this rearrangement varies with monoglyceride species. In this paper, the rearrangement kinetics of β- into α-monoglycerides with respect to acyl chainlength is reported.

MATERIALS AND METHODS

Experiments were done with several triglycerides of varying fatty acyl chainlengths [Tributyrin; Serva (Heidelberg, Germany), purity 99%; tricaprylin, Fluka (Buchs, Switzerland), purity 97%; trilaurin, Fluka, purity 97%; and tripalmitin, Sigma (St. Louis, MO), purity 90%]. The 1,3-specific lipase of the mould *R. javanicus* was purchased from Biocatalysts Ltd. (Pontypridd, United Kingdom), and hexane (p.a.) was purchased from Merck (Darmstadt, Germany).

Hexane was used to dissolve 0.01 mole of triglycerides in a 250-mL flask to make a 20% (w/w) solution. To this solution, 1.0 g of the 1,3-specific (7) *R. javanicus* lipase and 130 mL water was added, and the two-phase reaction mixture was stirred for 30 min at 30°C. After decanting, the hexane phase was poured into a well-stirred thermostated vessel at 30°C. By collecting the reaction products in the organic phase, only the chemical rearrangement occurred because any remaining lipase activity in the sample was absent, as was checked with the standard tributyrin test (8). After performing a lipase-catalyzed 1,3-specific hydrolysis of triglycerides, the chemical rearrangement of β-monoglycerides into α-monoglycerides was monitored in the following way. Aliquots (1 mL) were removed from the vessel and immediately silylated by adding 220 μL *N,O-bis*(trimethylsilyl)trifluoroacetamide (Pierce, Oud-Beijerland, The Netherlands). The silylation step prevented any further chemical rearrangement of the monoglycerides during the ensuing analytical procedures. The monoglycerides were then analyzed by gas chromatography (Carlo Erba, Milano, Italy). For monobutyrin, monocaprylin, and monolaurin, a CPSIL 5 CB column (Chrompack, Bergen op Zoom, The Netherlands) was used with split/splitless injection, and the detector temperature was kept at 275°C. After

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injection, the oven temperature was kept at 160°C for 10 min, followed by an increase of the oven temperature to 250°C at a rate of 25°C/min and a constant temperature of 250°C for 15 min. Helium was used as carrier gas. An HT-SIMDIST column (Chrompack) was used for the analysis of monopalmitin, and the oven temperature was 210°C at the moment of injection. After 10 min, the temperature was increased by 25°C/min up to 360°C, and was maintained at 360°C for 4 min. The injector and detector temperature was set at 400°C, and hydrogen was chosen as carrier gas. For the α- and β-monoglyceride, concentrations were calculated by means of the internal standard method. The response factors for both monoglycerides were determined by using the respective pure α-monoglyceride, assuming that the response factors for the corresponding β-monoglycerides are equal.

RESULTS AND DISCUSSION

The time courses of β-monoglycerides' rearrangement to α-monoglycerides are depicted in Figure 1. Within 24 h of reaction, a mixture that was initially dominated by the β-monoglyceride was converted into a mixture of equal amounts of α- and β-monoglycerides. After prolonged reaction time, equilibrium was reached, and the ratio of α- to β-monoglycerides varied between 4.6 and 8.5 (Table 1).

For the rearrangement of monoglycerides, a first-order reaction rate model based on the reaction scheme shown in Scheme 1 was assumed. This reaction scheme yields the following reactions, where $[\alpha - M]$ = amount of α-monoglyceride and $[\beta - M]$ = amount of β-monoglyceride, all expressed in mmole:

$$\frac{d[\beta-M]}{dt}=-k_{+1}\bullet[\beta-M]+k_{-1}\bullet[\alpha-M] \qquad [1]$$

At equilibrium,

$$\frac{d[\beta-M]}{dt}=-k_{+1}\bullet[\beta-M]+k_{-1}\bullet[\alpha-M]=0 \qquad [2]$$

Therefore, the equilibrium constant K follows:

$$K=\frac{k_{+1}}{k_{-1}}=\frac{[\alpha-M]}{[\beta-M]} \qquad [3]$$

The equilibrium constant K can be calculated from the measured concentration, and is given in Table 1.

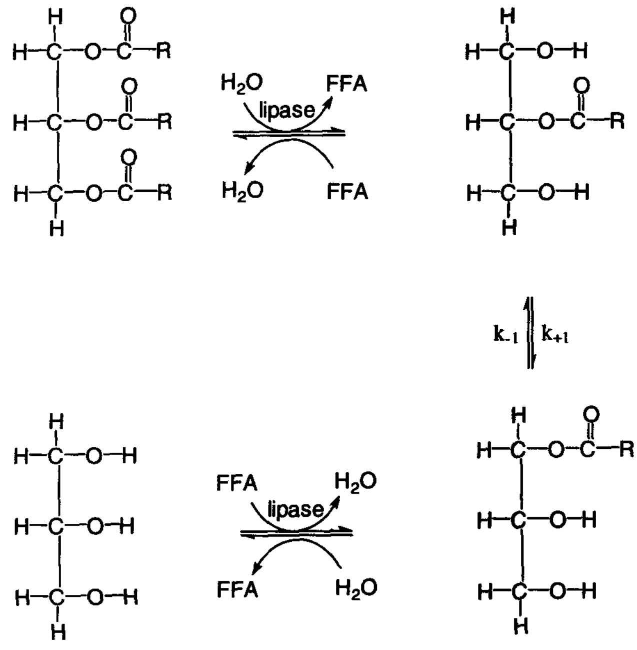
According to the extremely limited literature on rearrangement reactions of long-chain fatty acid monoglycerides, an equilibrium situation is reached at a composition of about 88% α-monoglyceride, and 12% β-monoglyceride for long-chain fatty acids (9). As can be concluded from Table 1, our results compare reasonably well with those data. Apparently, the equilibrium constants of the rearrangement reactions are relatively unaffected by fatty acid chainlength. Looking at the equilibrium constants of this reaction, we also observe that they do not vary much for the different investigated monoglycerides.

TABLE 1
The Ratio α- to β-Monoglycerides[a]

Monoglyceride	α-Monoglyceride (wt%)	β-Monoglyceride (wt%)	K (–)	k_{+1} (h^{-1})
Monobutyrin	82	18	4.6	0.0236
Monocaprylin	89	11	8.5	0.0283
Monolaurin	87	13	6.8	0.0566
Monopalmitin	84	16	5.3	0.0436

[a] At equilibrium and the determined reaction and equilibrium constants for different chainlength of the fatty acid moiety in the monoglyceride.

The measured data of the α- and β-monoglyceride concentrations with time, as given in Figure 1, were fitted to the mathematical model presented in Equation 1 by using least square analysis, whereupon the rate constants were determined (10). The equilibrium constants were estimated by dividing the measured α- and β-monoglyceride concentrations after 168 h of reaction. The curves drawn in Figure 1 are the best fits according to this model. In Figure 2, the rate constants of the chemical rearrangement reaction of the four monoglycerides are shown as a function of the chainlength of the monoglyceride's fatty acyl moiety. Figure 2 suggests a dependence between the chainlength of the monoglyceride's fatty acyl moiety and the rate constant of the chemical rearrangement reaction. This relation can be illustrated by a straight line as shown in Figure 2. The slope of this line, which was calculated to be 0.002, gives the increase velocity for the chemical rearrangement reaction with increasing number of carbon atoms in the monoglyceride's fatty acyl moiety. This straight line only represents a trend for the products with 4 to 16 carbon atoms in the glyceride fatty acyl moiety. It may be expected that, with a further increase of the fatty acid



SCHEME 1

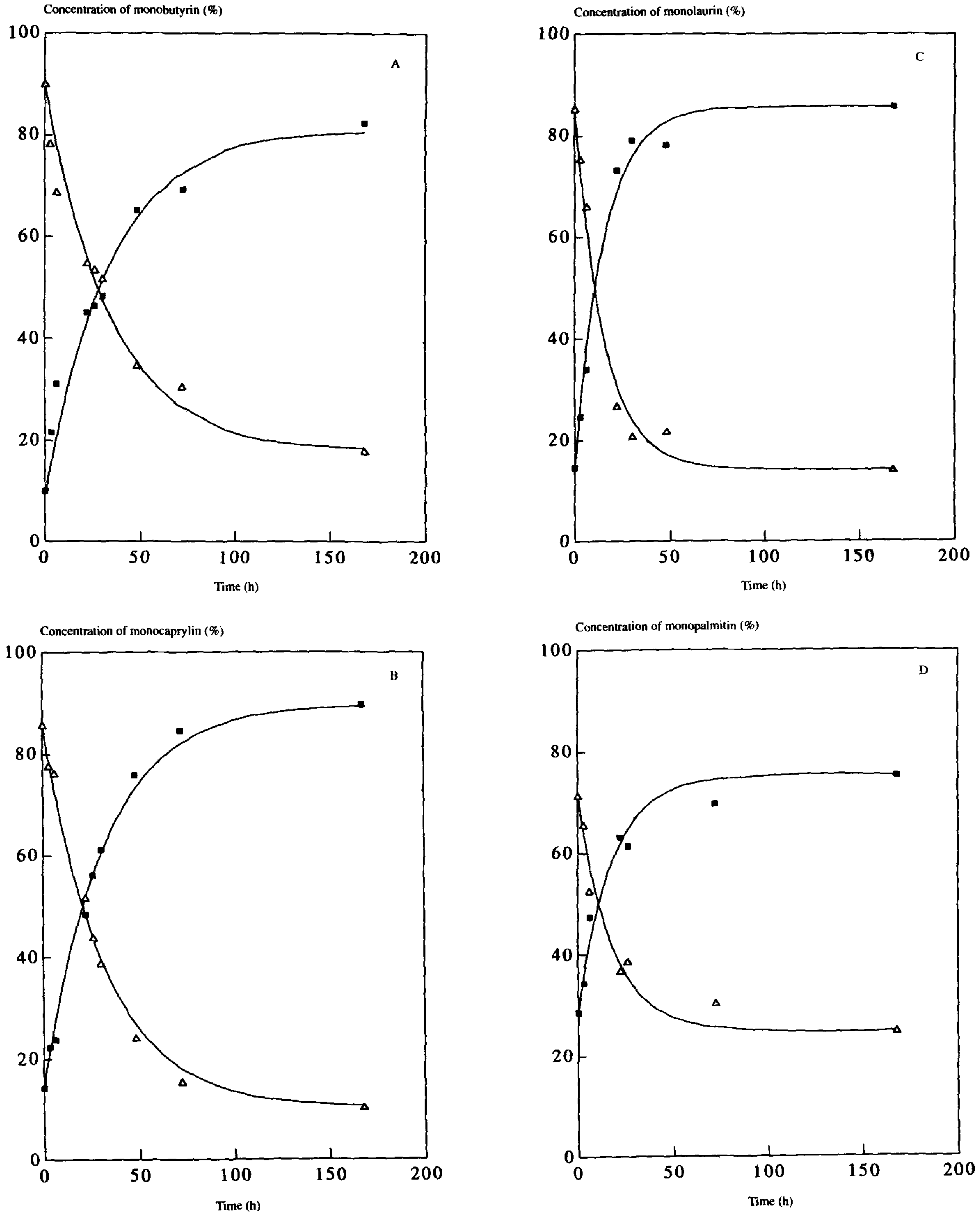


FIG. 1. Time course of the chemical rearrangement of monoglycerides. A, Monobutyrin; B, monocaprylin; C, monolaurin; and D, monopalmitin. α-Monoglycerides (■), β-monoglycerides (△), and the lines representing the values for α- and β-monoglycerides calculated by the model.

chainlength, the mobility becomes lower, hence the rearrangement rate would decrease.

Holmberg and Osterberg (3) investigated the 1,3-specific hydrolysis of palm oil by *Rhizopus delemar* at 35°C in a microemulsion system. They postulated that the rate of acyl group migration is comparable to the rates of di- to monoglyceride hydrolysis. Using the present work in combination with the rate constants of the specific hydrolysis of tricaprylin

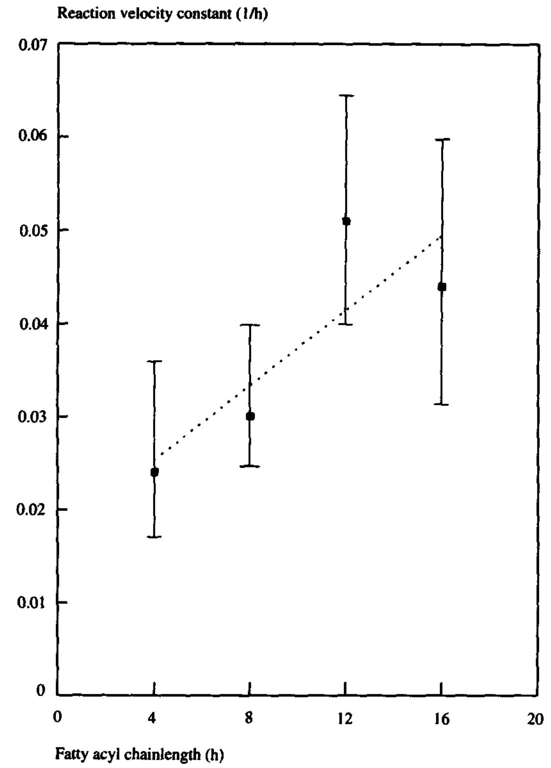


FIG. 2. The reaction velocity constants of the chemical rearrangement within monoglycerides with increasing fatty acyl chainlength (including the 95% confidence interval).

calculated in prior research (11), this hypothesis can easily be checked for tricaprylin. In Table 2, the rate constants for the three different hydrolysis steps are summarized.

When comparing the calculated rate constants of the rearrangement reaction with the rate constants of the specific hydrolysis of tricaprylin, the rate constants for the specific hydrolysis k_+^{ref} should be changed to (pseudo) first-order reaction constants k_+. For the reaction steps where water is crucial, k_+ can be estimated by assuming that the water concentration remains constant at 55.6 mole/L; thus, the first-order reaction rate k_+ is calculated as $k_+^{ref} \cdot [H_2O]$. The calculated first-order reaction rate constants are also shown in Table 2.

When the first-order reaction rate constant (k_+) of the di- to monoglyceride hydrolysis step is compared with the rate constant of the rearrangement of monocaprylin (Table 1), it can be concluded that the rate constant for the hydrolysis step is one order of magnitude higher than the rate constant for the chemical rearrangement reaction. However, the rate constant of the hydrolysis step of monoglyceride to glycerol and that of the chemical rearrangement are in the same order of magnitude. These results strongly suggest that the rearrangement reaction of β- to α-monoglyceride is the rate-limiting step in the further hydrolysis of monoglycerides to glycerol by 1,3-specific lipases, and does not involve the enzyme-catalyzed step.

TABLE 2
Rate Constants and (pseudo) First-Order Reaction Constants[a]

Step	T → D	D → M	M → G
$10^3\ k_+^{ref}$ (L • mole^{-1} • h^{-1})	5.6	4.5	0.7
k_+ (h^{-1}) = $[H_2O] \cdot k_+^{ref}$ (see text)	0.31	0.25	0.039

[a]For the specific hydrolysis of tricaprylin (Ref. 11). T, D, M: tri-, di-, and monoglyceride, respectively.

This paper illustrates that the preparation of 100% pure β-monoglycerides with a 1,3-specific lipase is difficult. As described, a high yield of β-monoglycerides can only be realized by using an oil/water mixture with an excess of 1,3-specific lipase and a short reaction time. The produced β-monoglycerides will spontaneously rearrange to α-monoglycerides. McNiell *et al.* (5) showed that the reaction temperature has a substantial effect on the production of monoglycerides *via* glycerolysis at 5°C. However, preliminary experiments in our laboratory on 1,3-specific hydrolysis at 5°C showed no significant increase of β- or α-monoglyceride concentration. The difference could be due to the presence of water, which gives rise to the production of free fatty acids.

The overall monoglyceride yield can be improved by extracting the α- and β-monoglycerides from the reaction mixture. Because the lipase is not present in the extraction phase, the hydrolysis of the α-monoglycerides by the 1,3-specific enzyme does not occur. Although, in this case, an increase in the overall monoglyceride yield can be observed, the relative amount of β-monoglyceride does not increase because a rearrangement of the monoglycerides also occurs in the extraction phase. This rearrangement can be influenced in a system with controlled thermodynamic activity (12). The yield of β-monoglyceride may increase substantially by addition of a specific solvent, as has been shown by Janssen *et al.* (13) and Millqvist *et al.* (12). In principal engineering tools, like distillation, water absorption by zeolites or membrane technology can be used for tailoring of the thermodynamic environment.

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