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The Effect of Temperature on the Enantioselectivity of Lipase-Catalyzed Reactions; Case Study: Isopropylidene Glycerol Reaction

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Abstract: Commercial lipase (triacylglycerol lipase (EC 3.1.1.3) of *Burkholderia cepacia* (40 U/mg) in its crude form has been used in the kinetic resolution of enzyme-catalyzed reaction of 1,2-*O*-isopropylidene-sn-glycerol and vinyl acetate as acyl donor in the organic solvent *n*-hexane. It was observed that the enantioselectivity is in the range of 2.295 to 2.235 while $\Delta\Delta G_{D,L}$ -73.408 to -75.682 kJ/mol at 35 °C and 55 °C, respectively . This shows that any increase in the reaction temperature led to an increased final conversion, but it has no effect on the enantioselectivity of the reaction. Also, the thermodynamic effect of temperature on the Gibbs free energy in the lipase-catalyzed kinetic resolution of the reaction between racemic isopropylidene glycerol and vinyl acetate remains in the small range. By using this type of analysis, the researchers may predict if they should increase or decrease the temperature to enhance the selectivity of enzyme in catalyzing a reaction.

Keywords: Enantioselectivity, lipase, temperature, isopropylidenglycerin , kinetic resolution, *Burkholderia cepacia.*

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INTRODUCTION

One of the elusive hallmarks in formation of life within the prebiotic era on the Earth is how Nature chose a specific chirality (or handedness) or called biological homochirality. Thereof the homochirality of amino acids (L-enantiomers), sugars (D-enantiomers), proteins, and DNA became one of the biochemical characteristic properties in the life on Earth (1,2). Although Nature prefers almost exclusively stereochemical imperative chiral molecules in living organisms as single enantiomers, yet the left- and right-handed molecules of a compound will deterministically form in equimolar composition (a racemic mixture) when they are synthesized in the laboratory in the absence of some type of directing template (3,4).

However, about a century later it is drastically determined that the phenomenon of chirality implements a key role in pharmaceutical, agricultural, food, and other chemical industries as well as in the life of plants and animals (5–7). Since it is evident that the chirality is a fundamental characteristic of life processes, the individual enantiomers of chiral chemicals in a racemic mixture may divulge very different bioactivities and/or biotoxicities (8). It means that one enantiomer may be active (eutomer) while the other one (distomer) might be inactive, useless, harmful, or toxic (poisonous), sometimes in certain cases producing undesired side effects (9–11).

Over the last two decades, stereochemistry has been gaining prime importance in chemical technologies associated with the synthesis, separation, identification, and analysis of target eutomers from undesired distomers in a racemic compound, (12), particularly in the fields of contemporary pharmaceutical, agrochemical, food, smell, material sciences, and many other rapidly expanding areas of research (13–15).

Accordingly, it became necessary to search an appropriate process to separate racemic compounds into their enantiomers to produce optically active compounds. Therefore, the different methods to differentiate between various enantiomers can be used like crystallization (14,16,17), separation with membranes (18–21), liquid-liquid extraction (22), capillary electrophoresis (23,24), chromatography (25), and kinetic resolution (KR)(26–33). Among these methods, the resolutions based on kinetic effects in chemical reactions can be one of several major types but are typically divided between enzyme and inorganic catalyzed systems. The enzyme-catalyzed transformations to produce enantiomerically pure compounds have been progressively considered in the

manufacture of a wide range of single enantiomers in the industry. Kinetic resolution is defined as a process where the two enantiomers of a racemate are transferred to the product much faster than the other (34). Due to the structural diversity of chiral compounds, in the frame of substrate specificity, a huge amount of enzymes were recently used for enantioseparation to determine their activity and selectivity in the kinetic (35).

Among these numerous amounts of enzymes, the kinetic resolution using lipases provide high enantiomeric excess (ee) and can be cost effective compared to other techniques. However, there are some factors that affect activity and selectivity of lipase-catalyzed reactions, including the nature of the acylating agent, temperature, pH and solvent selection (26). This paper scrutinizes if there is any temperature effect on the kinetic resolution of lipases in the transesterification of isopropylideneglycerin with vinyl acetate as acyl donor.

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are a versatile group of biocatalysts, which are ubiquitous enzymes catalyzing the hydrolysis of fats and oils (36). The number of available lipases has increased considerably since the 1980s. Their natural physiologic function is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol during digestion (37,38). Lipases are frequently used in lipid modification and in organic synthesis. Enzymes in this class have been shown to be 1,3- regioselective for triglycerides, selective for fatty acid chain length and degree of fatty acid saturation (36,39).

In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification, inter-esterification, and transesterification reactions in non-aqueous media. The broad substrate specificity makes lipases usable in a wide range of applications, and thus their market is still growing (40). This versatility makes lipases the enzymes of choice for potential applications in the dairy and food industries, in the production flavor and aroma components, in oleo-chemical industry, in medical applications (37,41–43), in the detergent, leather, textile, cosmetic, and paper industries (38,44), and in the production of optically active compounds for the agrochemical and pharmaceutical industries (38,45,46). Beyond all these applications, they are widely used in the synthesis of organic compounds (47) to produce homochiral compounds from racemates via enantiomeric discrimination or from prochiral or meso compounds via enantiotropic differentiation. The resolution of racemic compounds *via* hydrolysis in aqueous media or trans/esterification in organic media cannot always be achieved in a highly enantioselective manner (48,49). Enantioselectivity can be improved by several methods, *e.g.*, the screening of enzymes (50,51), the modification of substrates (17,18), or the modulation of reaction conditions.

Temperature, which is an easily controllable parameter in the experimental conditions, is a potential factor that may affect the enantioselectivity of the enzymatic reactions (49,52). However, its effect on the stereoselectivity of enzymatic transformations has not been investigated sufficiently (48,53–55). There have been remarkably few systematic studies on the effects of temperature variation on the stereochemistry of enzymatic reactions (56). Some examples of an improvement of enantioselectivity by temperature-dependent reversal of stereochemistry were observed (57–59).

Eyring's transition state theory (60) defines the relation of temperature with the reaction rate constant as:

$$k = \kappa \frac{k_B T}{h} K \qquad \text{(Eq. 1)}$$

where k= reaction rate constant, κ= transmission coefficient, k_b= Boltzmann constant, T= temperature, K= equilibrium constant.

The equilibrium constant is related with Gibbs free energy through Van't Hoff equation.

$$\Delta G = -RTlnK \qquad \text{(Eq. 2)}$$

Enzymatic enantioselectivity E is defined as the ratio of specificity constants of the two competing enantiomers (61). Aydemir modified this concept showing that the enantioselectivity is especially the ratio of kinetic constants of reactions for the competing racemates at the activated enzyme site (62). The specificity constant of an enzyme for its substrates is defined as the ratio k_{cat}/K for the D and L racemates (59,63).

$$E = \frac{D}{L} = \left(k_{cat}/K\right)_D / \left(k_{cat}/K\right)_L \qquad \text{(Eq. 3)}$$

The kinetic constant, k_{cat}/K, is related to the thermodynamic term ΔG, as shown in following equation from transition-state theory (64).

$$\Delta\Delta G = -RTlnE \qquad \text{(Eq. 4)}$$

where $\Delta\Delta G^*$ is the difference in free energy of activation between the D and L racemates (59).

The temperature dependence of the activation free energy is given by Gibbs-Helmholtz equation:

$$\Delta\Delta G^* = \Delta\Delta H^* - T\Delta\Delta S^* \qquad \text{(Eq. 5)}$$

Substituting Eq. (4) into Eq. (5), the relationship between enantioselectivity, enthalpy, and entropy is derived (56):

$$lnE = \left(\frac{\Delta\Delta S^*}{R}\right) - \left(\frac{\Delta\Delta H^*}{RT}\right) \qquad \text{(Eq. 6)}$$

if no enantiomeric discrimination of the enzyme between the D and L isomers occurs, then E = 1 and $\Delta\Delta G^* = 0$. In this case, the enthalpy and entropy contributions are equal to

$$T_r = \Delta\Delta H^*/\Delta S^* \qquad \text{(Eq. 7)}$$

The temperature is thus the "racemic temperature" (56,65). This analysis predicts that temperature dependent inversion of stereochemical configuration occurs. At temperatures below T_r., the $\Delta\Delta G^*$ is dominated by $\Delta\Delta H^*$, and the E value

of product will decrease as T increases, until it reaches unity at T_r. In contrast, at temperatures above T_r. the $\Delta\Delta G^*$ is dominated by $T\Delta\Delta S^*$, and the E value increases as T increases. Therefore, the optimization of enantiomeric enzyme catalyzed reactions may require either the raising or lowering the reaction temperature (56,66).

The influence of the reaction temperature on the enantioselectivity appears to depend on the nature of the reaction involved (67). Increasing the temperature normally leads to an increase of the enzymatic reaction rate, and obviously resulted in a higher reaction rate and a higher final conversion (57). At the same time, the enantioselectivity often decreases and a loss of enzyme stability can be observed (68).

Identification of $\Delta\Delta G_{D,L}$ as the free energy difference that determines the enantiomeric ratio opens the possibility to predict E (69). Studies on the temperature dependence of E allow for a thermodynamic analysis for the enantioselectivity of enzymes, which is caused by enthalpic and entropic activation energy differences of the enantiomers. These studies have also revealed the entropic contributions to be nearly as big as the enthalpic contribution, whereas the entropic activation energy depends on the interactions with solvent molecules and enantiomers in transition state at the active site (63,70). Although this is a dichotomy between enthalpy and entropy which results in the observed temperature dependence (65), the enthalpic and the entropic components of the differential activation free energy, $\Delta\Delta G_{D,L}$ were both important to the overall success of the kinetic resolution of the enantiomers (70).

Although increasing the temperature usually decreased the enantioselectivity, high enantioselectivity can be expected even at high temperatures if the structure of the substrate is ideal from the mechanistic point of view (71). The acyl donor may greatly influence the enantioselectivity and reaction rate of acylation (72). A slight elongation of the alkyl chain of the vinyl esters caused dramatic changes in the enantioselectivity (73). It was the highest when vinyl acetate was used as acyl donor and became lower with the chain length of the fatty acid moiety (52). The position of the double bond has also affected the reaction rate and enantioselectivity (42). The bulky aromatic group allowed only one enantiomer to fit in the active site, whereas for aliphatic compounds the enzyme could not distinguish well between both forms (43). An addition of a suitable amount of water can alter dramatically the behavior of their enantioselectivities as a function of the temperature (55).

As well as the effect of the structure of substrate by the medium engineering point of view, the temperature effect on the enantioselectivity discriminates itself quite differently depending on the type of reaction. As given on the (Table 2), it is reported that E value may increase or decrease or is unaffected with lowering or increasing the reaction temperature.

The non-covalent interactions of the substrate with the residues at the active site determine the thermodynamic and kinetic properties of the complex (74). Above-mentioned Equation 4 gives the relationship between enantioselectivity and temperature via the free activation energy $\Delta\Delta G$. The equations 5 and 6 relate further the free energy to enthalpic and entropy contributions. Ottosson (70) has studied that the enthalpic and the entropic components of the differential activation free energy, $\Delta\Delta G_{D,L}$ are both important to the overall success of the kinetic resolution of the enantiomers. The knowledge of how this enzyme distinguishes between enantiomers and the roles of enthalpy and entropy on a molecular level (75).

Table 1: some research for the change of E with variation in temperature.

Temperature	Enantioselectivity	Ref.
high	high	(76,77)
high	low	(58,71,78–81)
low	High	(82,83)
low	low	(84)
high or low	no change	(67,85,86)

If the $T\Delta\Delta S$ and $\Delta\Delta H$ terms for a desired reaction forming enantiomeric products are closely balanced, then the reaction will be subject to stereochemical modulation by changes in temperature. If the $\Delta\Delta G$ is dominated by the $T\Delta\Delta S$ term, then reactions will show the maximal stereochemical discrimination at the highest temperature compatible with the stability of the enzyme – cofactor – substrate system. If the substituent has polar groups that interact with the enzyme by ionic attraction or by hydrogen bonding, the $\Delta\Delta H$ term will be quite large and will dominate the free energy of activation, resulting in little or no effect of temperature. If the major contributor to $\Delta\Delta G$ is $\Delta\Delta H$, then the stereochemical purity of the reaction product will be maximal at the lowest temperature at which the enzyme exhibits useful reactivity (56).

In this work, the effect of temperature on the reaction conditions on the transesterification of isopropylideneglycerin (IPG), catalyzed by *Burkholderia cepacia* lipase (BCL), previously known as *Pseudomonas cepacia*, has been investigated. IPG, [+]-Solketal (1,2-O-isopropylidene-sn-glycerol (IPG); [+]-2,2-dimethyl-1,3-dioxolane-4-methanol) (Figure 1), is an important starting compound for the preparation of many C_3-synthons which are widely applied in organic synthesis (87), as an interesting chiral intermediate for pharmaceutical industries, since it is an important starting chiral synthon in the synthesis of diglycerides, glyceryl phosphates, tetraoxaspiroundecanes, and of many biologically active compounds, such as phospholipids, β-adrenoceptor antagonists propranolol, and platelet aggregating factors (88–91). The esterification of isopropylidene-glycerin (IPG)

with vinyl acetate as an acyl donor (92–94) in *n*-hexane (95–97) has been examined, and the effect of temperature on the enantioselectivity of *B.Cepacia* lipase for D, L-IPG was investigated.

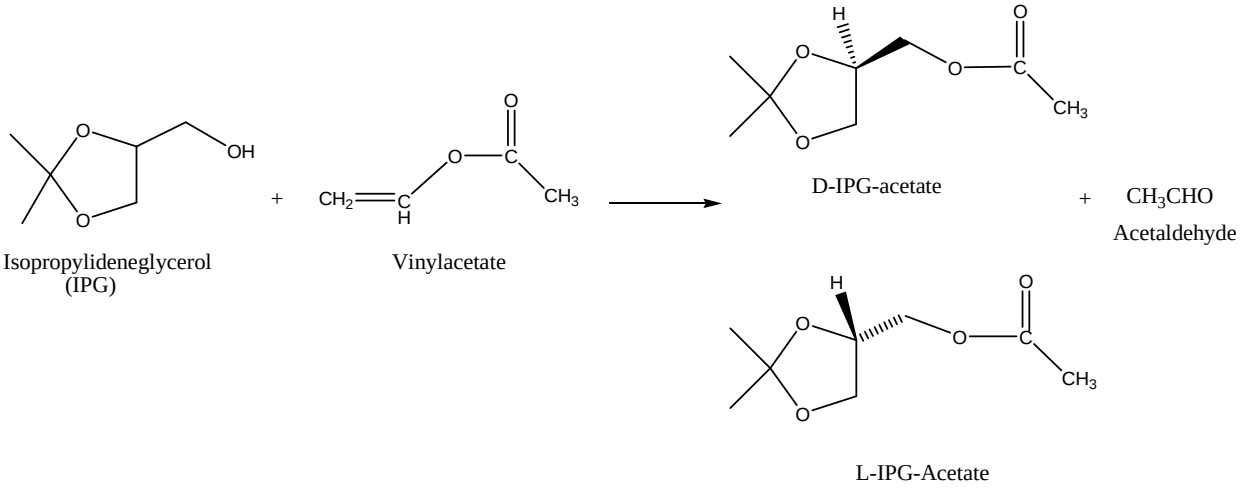

Figure 1: Reaction of Isopropylidenglycerin with vinyl acetate.

MATERIALS AND METHODS

Chemicals and Lipase

Lipase from *Burkholderia cepacia* (40 U/mg, Fluka) was used in its crude form. The organic solvent *n*-hexane (Fluka), 1,2-*O*-isopropylidene-sn-glycerol (Fluka), vinyl acetate as acyl donor (Merck) were used without any further purification.

The analysis has been performed by gas chromatography (CC-14A, Shimadzu) with a chiral column of FS-Hydrodex® ß-3P, (Heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-ß-cyclodextrin) with a length of 25 m and an inside diameter of 0.25 mm (Macherey-Nagel, Düren, Germany).

Reactions in organic solvents

Preliminary experiments of related reactions in organic solvent were carried out in a 20 mL volume of a glass vessel sealed with a rubber stopper. In the experiments, 10 mmol of racemate (IPG) and 30 mmol of excess component vinyl acetate as an acylating agent were mixed to complete the total reaction medium of 10 mL with *n*-hexane. By adding 50 mg of *Burkholderia cepacia* lipase, the reaction started. The reaction mixture is incubated in water bath and agitated with magnetic stirring. The magnetic stirrer speed was 600 rpm. Samples withdrawn during the reaction were centrifugated and diluted with acetone before gas chromatographic (GC) analysis.

Determination of enantiomeric excess and conversion

Samples from the reaction mixture were diluted with acetone. Enantiomeric purities were calculated from peak areas determined by gas chromatography using a chiral stationary phase (FS-Hydrodex® ß-3P, Macherey-Nagel, Germany). From the detected data, the conversion was calculated as described by Chen et al. (68).

RESULTS

Lipase-catalyzed trans-esterification between D, L-IPG and vinyl acetate was studied. The product IPG-acetate and acetaldehyde as a by-product were produced during this reaction. As shown in Figure 1, the overall reaction is irreversible and therefore shifts itself towards product formation (96,98). Transesterification in *n*-hexane was performed at different temperatures viz. 35 °C, 40 °C, 45 °C, 50 °C and 55 °C, respectively. The conversion and the enantioselectivity of BCL was calculated as described by Chen (99) and Aydemir's enantioselectivity definition (62).

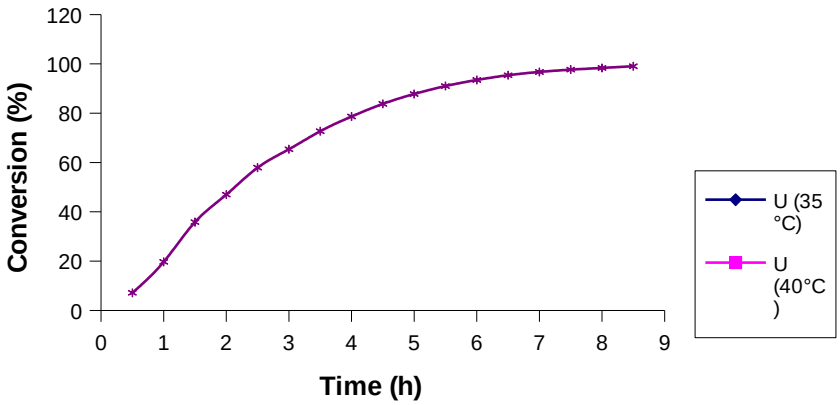

Figure 2. Conversion vs. time profile of IPG at different temperatures.
(10 mmol IPG, 30 mmol vinyl acetate, 50 mg Lipase BC. 10 mL solution)

The rate of a chemical reaction increases with rising temperature according to Van't Hoff equation. In this work, it is determined that the reaction rate and the conversion were risen at the same time with increasing temperature till 50 °C, then decreases above this temperature (Figure 2). The detected optimum temperature 50 °C is convenient with the lipase properties on the prospect of the enzyme supplying company (Fluka). Above this temperature, the activity of the lipase descends resulting in decrease of the conversion. That might possibly result in the fact that the enzyme structure starts to be destroyed along with the rising temperature above 50 °C.

Table 2: Temperature vs. E values.

Temp .(°C)	Max. conversion	E value	$\Delta\Delta G_{D,L}$ (kj)
35	61	2.295	-73.408
40	65	2.267	-73.497
45	69	2.254	-74.147
50	93	2.242	-74.818
55	79	2.235	-75.682

The enantiomeric ratio was determined according to Rakels *et.al.* with the following equation (100).

$$E = \frac{\ln\left[\frac{1-eeS}{1+\left(\frac{eeS}{eeP}\right)}\right]}{\ln\left[\frac{1+eeS}{1+\left(\frac{eeS}{eeP}\right)}\right]} \qquad \text{(Eq. 8)}$$

The results of the experiments to determine the temperature dependency of enantioselectivity in the esterification of IPG were given in Figure 3. The conversion reached from 61% at 35 °C to the maximum conversion of 93% at 50 °C, after 4 hours of reaction time. As a result, it became evident that the enantioselectivity (E) remained almost unchanged with the temperature in the mean value of 2.26, tending to convert more L-form than D- form of the IPG (Table 2).

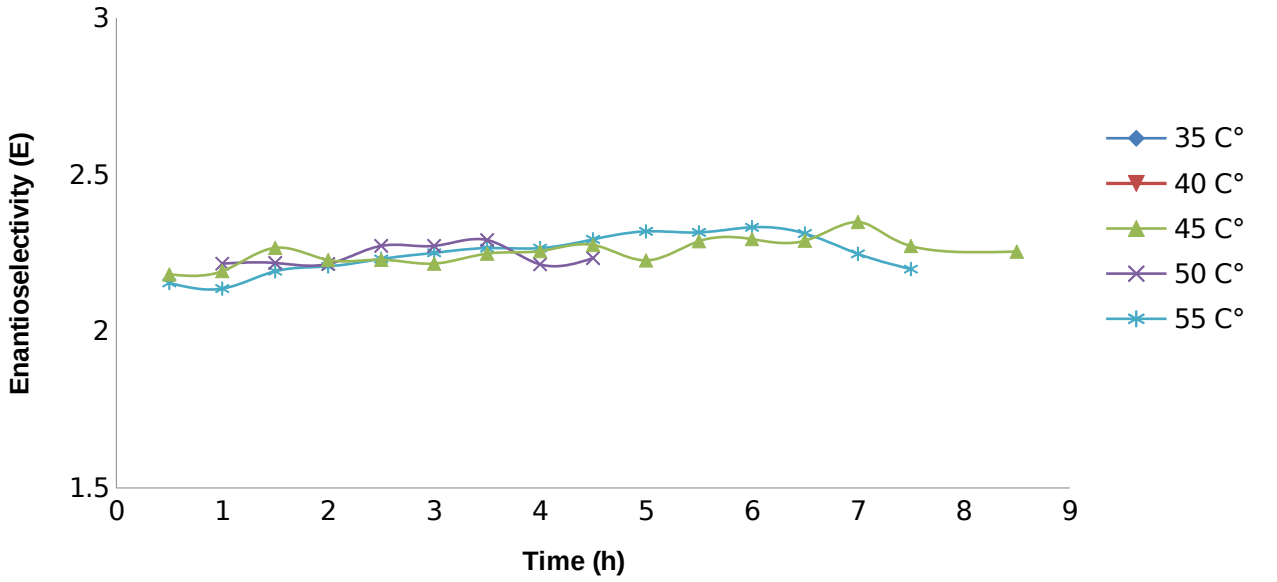

Figure 3: Enantioselectivity vs. time at different temperatures.

CONCLUSION

The enhancement of enantioselectivity to produce the desired racemic product is recently studied by many laboratories. In order to achieve the enhancement, the physical conditions of the reaction medium has been altered. The acyl donor, solvent type, the effect of water content on the enzyme flexibility, and the temperature are the commonly studied parameters. Among these parameters, it is examined that the enantioselectivity alters irregularly with temperature. Thus, it could be concluded that the molecular structure of the substrate indirectly determines the dependency of enantioselectivity on temperature, by defining the contribution of enthalpic or entropic effect of the activation energy. The enthalpic and entropic values are equal to each other at a certain temperature. Consequently, the enantiomeric ratio (E) value becomes 1. This temprature is called racemic tempreature, at which a racemate is formed. Above or below the racemic temperature, a decrease in temperature will cause either a decrease or increase in enantioselectivity. As a result it is thus suggested to consider the effects of temperature on the selectivity of enzymatic reactions (101) in the future works.

In the present work, the effect of temperature on the lipase catalyzed reaction between isopropylidene glycerol and vinyl acetate was analyzed thermodynamically, since the activation energy $\Delta G_{D,L}$ of each enantiomer is related to temperature and entropy ($T\Delta S$), the value of $\Delta G_{D,L}$ has been calculated to analyze how it changes with enantioselectivity at temperatures between 35 – 55 °C. It is observed that the enantiomeric excess value is 2.295 and $\Delta G_{D,L}$= -73.408 kJ/mol at 35 °C, while EE= 2.235 and $\Delta G_{D,L}$= -75.682 kJ/mol. It shows that the higher enantioselectivty can be obtained at low temperatures (35 °C) having low entropy value. Since there is no huge amount of difference in EE or ΔG values calculated in this work, it can be interpreted that the reaction between vinyl acetate and isopropylidene glycerol is not strongly dependent on the temperature, and increase in temperature causes decrease in ΔG, because $T\Delta S$ becomes greater than ΔH ($\Delta\Delta H^* < T\Delta\Delta S^*$). Finally, this work adds that low temperatures are suggested for the selectivity of one enatiomer to other in the reaction studied.

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REFERENCES

1. Yun Y, Gellman AJ. Enantioselective Separation on Naturally Chiral Metal Surfaces: D,L -Aspartic Acid on Cu(3,1,17) $^{R\ \&\ S}$ Surfaces. Angew Chem Int Ed. 2013 Mar 18;52(12):3394–7. <DOI>.

2. Fujii N, Saito T. Homochirality and life. Chem Rec. 2004;4(5):267–78. <DOI>.

3. Blackmond DG. The origin of biological homochirality. Philos Trans R Soc B Biol Sci. 2011 Oct 27;366(1580):2878–84. <DOI>.

4. Sallembien Q, Bouteiller L, Crassous J, Raynal M. Possible chemical and physical scenarios towards biological homochirality. Chem Soc Rev. 2022;51(9):3436–76. <DOI>.

5. Armstrong DW, Chang CD, Li WY. Relevance of enantiomeric separations in food and beverage analyses. J Agric Food Chem. 1990 Aug;38(8):1674–7. <DOI>.

6. Nguyen LA, He H, Pham-Huy C. Chiral drugs: an overview. Int J Biomed Sci IJBS. 2006 Jun;2(2):85–100. PMID: 23674971.

7. Bachmanov AA, Bosak NP, Glendinning JI, Inoue M, Li X, Manita S, et al. Genetics of Amino Acid Taste and Appetite. Adv Nutr Int Rev J. 2016 Jul;7(4):806S-822S. <DOI>.

8. Wu Q, Lv H, Zhao L. Applications of carbon nano materials in chiral separation. Trends Anal Chem. 2020;129:115941. <DOI>.

9. Mwamwitwa KW, Kaibere RM, Fimbo AM, Sabitii W, Ntinginya NE, Mmbaga BT, et al. A retrospective cross-sectional study to

determine chirality status of registered medicines in Tanzania. Sci Rep. 2020 Dec;10(1):17834. <DOI>.

10. Kumar R. Effects of Stereoisomers on Drug Activity. Am J Biomed Sci Res. 2021 Jun 21;13(3):220–2. <DOI>.

11. Hancu G, Modroiu A. Chiral Switch: Between Therapeutical Benefit and Marketing Strategy. Pharmaceuticals. 2022 Feb 17;15(2):240. <DOI>.

12. Peepliwal A, Bagade S, Bonde C. A review: stereochemical consideration and eudismic ratio in chiral drug development. J Biomed Sci Res. 2010;2(1):29–45.

13. Genva M, Kenne Kemene T, Deleu M, Lins L, Fauconnier ML. Is It Possible to Predict the Odor of a Molecule on the Basis of its Structure? Int J Mol Sci. 2019 Jun 20;20(12):3018. <DOI>.

14. Bodák B, Breveglieri F, Mazzotti M. On the model-based design and comparison of crystallization-based deracemization techniques. Chem Eng Sci. 2022 Jun;254:117595. <DOI>.

15. Gogoi A, Mazumder N, Konwer S, Ranawat H, Chen NT, Zhuo GY. Enantiomeric Recognition and Separation by Chiral Nanoparticles. Molecules. 2019 Mar 13;24(6):1007. <DOI>.

16. Anderson NG. Developing Processes for Crystallization-Induced Asymmetric Transformation. Org Process Res Dev. 2005 Nov 1;9(6):800–13. <DOI>.

17. Robl S, Gou L, Gere A, Sordo M, Lorenz H, Mayer A, et al. Chiral separation by combining pertraction and preferential crystallization. Chem Eng Process Process Intensif. 2013 May;67:80–8. <DOI>.

18. Xie R, Chu LY, Deng JG. Membranes and membrane processes for chiral resolution. Chem Soc Rev. 2008;37(6):1243. <DOI>.

19. Liu T, Li Z, Wang J, Chen J, Guan M, Qiu H. Solid membranes for chiral separation: A review. Chem Eng J. 2021 Apr;410:128247.

20. Han H, Liu W, Xiao Y, Ma X, Wang Y. Advances of enantioselective solid membranes. New J Chem. 2021;45(15):6586–99. <DOI>.

21. Ong CS, Oor JZ, Tan SJ, Chew JW. Enantiomeric Separation of Racemic Mixtures Using Chiral-Selective and Organic-Solvent-Resistant Thin-Film Composite Membranes. ACS Appl Mater Interfaces. 2022 Mar 2;14(8):10875–85. <DOI>.

22. Tong S. Liquid-liquid chromatography in enantioseparations. J Chromatogr A. 2020 Aug;1626:461345. <DOI>.

23. Fanali S, Chankvetadze B. Some thoughts about enantioseparations in capillary electrophoresis. Electrophoresis. 2019 May 21;elps.201900144. <DOI>.

24. Bernardo-Bermejo S, Sánchez-López E, Castro-Puyana M, Marina ML. Chiral capillary electrophoresis. TrAC Trends Anal Chem. 2020 Mar;124:115807. <DOI>.

25. Ward TJ, Ward KD. Chiral Separations: A Review of Current Topics and Trends. Anal Chem. 2012 Jan 17;84(2):626–35. <DOI>.

26. Ahmed M, Kelly T, Ghanem A. Applications of enzymatic and non-enzymatic methods to access enantiomerically pure compounds using kinetic resolution and racemisation. Tetrahedron. 2012;68(34):6781–802. <DOI>.

27. Qayed WS, Aboraia AS, Abdel-Rahman HM, Youssef AF. Lipases-catalyzed enantioselective kinetic resolution of alcohols. J Chem Pharm Res. 2015;7(5):311–22.

28. Verho O, Bäckvall JE. Chemoenzymatic Dynamic Kinetic Resolution: A Powerful Tool for the Preparation of Enantiomerically Pure Alcohols and Amines. J Am Chem Soc. 2015 Apr 1;137(12):3996–4009. <DOI>.

29. Hall M. Enzymatic strategies for asymmetric synthesis. RSC Chem Biol. 2021;2(4):958–89. <DOI>.

30. Mu R, Wang Z, Wamsley MC, Duke CN, Lii PH, Epley SE, et al. Application of Enzymes in Regioselective and Stereoselective Organic Reactions. Catalysts. 2020 Jul 24;10(8):832. <DOI>.

31. Bering L, Thompson J, Micklefield J. New reaction pathways by integrating chemo- and biocatalysis. Trends Chem. 2022 May;4(5):392–408. <DOI>.

32. Burek BO, Dawood AW, Hollmann F, Liese A, Holtmann D. Process Intensification as Game Changer in Enzyme Catalysis. Front Catal. 2022;2:1–18. <DOI>.

33. Wang F, Liu Y, Du C, Gao R. Current Strategies for Real-Time Enzyme Activation. Biomolecules. 2022 Apr 19;12(5):599. <DOI>.

34. Sikora A, Siódmiak T, Marszałł MP. Kinetic Resolution of Profens by EnantioselectiveEsterification Catalyzed by *Candida antarctica* and *Candida rugosa* Lipases: Kinetic resolution of anti-inflammatory drugs. Chirality. 2014 Oct;26(10):663–9. <DOI>.

35. Kovács B, Forró E, Fülöp F. Candida antarctica lipase B catalysed kinetic resolution of 1,2,3,4-tetrahydro-ß-carbolines: Substrate specificity. Tetrahedron. 2018 Nov;74(48):6873–7. <DOI>.

36. Reetz MT. Lipases as practical biocatalysts. Curr Opin Chem Biol. 2002 Apr;6(2):145–50. <DOI>.

37. Bornscheuer UT, Bessler C, Srinivas R, Hari Krishna S. Optimizing lipases and related enzymes for efficient application. Trends Biotechnol. 2002 Oct;20(10):433–7. <DOI>.

38. Houde A, Kademi A, Leblanc D. Lipases and Their Industrial Applications: An Overview. Appl Biochem Biotechnol. 2004;118(1–3):155–70. <DOI>.

39. Bornscheuer UT, Ordoñez GR, Hidalgo A, Gollin A, Lyon J, Hitchman TS, et al. Selectivity of lipases and esterases towards phenol esters. J Mol Catal B Enzym. 2005 Nov;36(1–6):8–13. <DOI>.

40. Knežević Z, Šiler-Marinković S, Mojović L. Immobilized Lipases as Practical Catalysts. APTEFF. 2004;35:151–64. <DOI>.

41. Mojović L, Šiler-Marinković S, Kukić G, Vunjak-Novaković G. Rhizopus arrhizus lipase-catalyzed interesterification of the midfraction of palm oil to a cocoa butter equivalent fat. Enzyme Microb Technol. 1993 May;15(5):438–43. <DOI>.

42. Knezevic ZD, Siler-Marinkovic SS, Mojovic LV. Kinetics of lipase-catalyzed hydrolysis of palm oil in lecithin/izooctane reversed micelles. Appl Microbiol Biotechnol. 1998 Mar 27;49(3):267–71. <DOI>.

43. Lortie R. Enzyme catalyzed esterification. Biotechnol Adv. 1997 Jan;15(1):1–15. <DOI>.

44. Rathi P, Saxena RK, Gupta R. A novel alkaline lipase from Burkholderia cepacia for detergent formulation. Process Biochem. 2001 Oct;37(2):187–92. <DOI>.

45. Zaks A, Dodds DR. Application of biocatalysis and biotransformations to the synthesis of pharmaceuticals. Drug Discov Today. 1997 Dec;2(12):513–31. <DOI>.

46. Rasor JP, Voss E. Enzyme-catalyzed processes in pharmaceutical industry. Appl Catal Gen. 2001 Nov;221(1–2):145–58. <DOI>.

47. Zuegg J, Hönig H, Schrag JD, Cygler M. Selectivity of lipases: Conformational analysis of suggested intermediates in ester hydrolysis of chiral primary and secondary alcohols. J Mol Catal B Enzym. 1997 Jun;3(1–4):83–98. <DOI>.

48. Miyazawa T, Kurita S, Shimaoka M, Ueji S, Yamada T. Resolution of racemic carboxylic acids via the lipase-catalyzed irreversible transesterification of vinyl esters. Chirality. 1999;11(7):554–60. <DOI>.

49. Miyazawa T, Imagawa K, Yanagihara R, Yamada T. Marked dependence on temperature of enantioselectivity in the Aspergillus oryzaeprotease-catalyzed hydrolysis of amino acid esters. Biotechnol Tech. 1997;11(12):931–3. <DOI>.

50. Sandoval G, Marty A. Screening methods for synthetic activity of lipases. Enzyme Microb Technol. 2007 Feb;40(3):390–3. <DOI>.

51. Berglund P. Controlling lipase enantioselectivity for organic synthesis. Biomol Eng. 2001 Aug;18(1):13–22. <DOI>.

52. Miyazawa T, Yukawa T, Ueji S, Yanagihara R, Yamada T. Resolution of 2-phenoxy-1-propanols by Pseudomonas sp. lipase-catalyzed highly enantioselective transesterification: influence of reaction conditions on the enantioselectivity toward primary alcohols. Biotechnol Lett. 1998;20(3):235–8. <DOI>.

53. Andrade MAC, Andrade FAC, S.Phillips R. Temperature and DMSO increase the enantioselectivity of hydrolysis of methyl alkyl dimethylmalonates catalyzed by pig liver esterase. Bioorg Med Chem Lett. 1991 Jan;1(7):373–6. <DOI>.

54. Holmberg E, Hult K. Temperature as an enantioselective parameter in enzymatic resolutions of racemic mixtures. Biotechnol Lett. 1991 May;13(5):323–6. <DOI>.

55. Yasufuku Y, Ueji S ichi. Effect of temperature on lipase-catalyzed esterification in organic solvent. Biotechnol Lett [Internet]. 1995 Dec [cited 2022 Apr 2];17(12). <DOI>.

56. Phillips RS. Temperature modulation of the stereochemistry of enzymatic catalysis: Prospects for exploitation. Trends Biotechnol. 1996 Jan;14(1):13–6. <DOI>.

57. Bornscheuer U, Schapöhler S, Scheper T, Schügerl K. Influences of reaction conditions on the enantioselective transesterification using Pseudomonas cepacia lipase. Tetrahedron Asymmetry. 1991 Jan;2(10):1011–4. <DOI>.

58. Parmar VS, Prasad AK, Singh PK, Gupta S. Lipase-catalysed transesterifications using 2,2,2-trifluoroethyl butyrate: Effect of temperature on rate of reaction and enantioselectivity. Tetrahedron Asymmetry. 1992 Nov;3(11):1395–8. <DOI>.

59. Pham VT, Phillips RS. Effects of substrate structure and temperature on the stereospecificity of secondary alcohol dehydrogenase from Thermoanaerobacter ethanolicus. J Am Chem Soc. 1990 Apr;112(9):3629–32. <DOI>.

60. Eyring H. The Activated Complex in Chemical Reactions. J Chem Phys. 1935 Feb;3(2):107–15. <DOI>.

61. Straathof AJJ, Jongejan JA. The enantiomeric ratio: origin, determination and prediction. Enzyme Microb Technol. 1997 Dec;21(8):559–71. <DOI>.

62. Aydemir A. Modeling of Enzyme Catalyzed Racemic Reactions and Modifications of Enantioselectivity [Internet] [PhD Thesis]. [Hannover, Germany]: Gottfired Leibniz Universitat Hannover; 2010. Available from: <URL>.

63. Orrenius C, Hbffner F, Rotticci D, öhrner N, Norin T, Hult K. Chiral Recognition Of Alcohol Enantiomers In Acyl Transfer Reactions Catalysed By *Candida Antarctica* Lipase B. Biocatal Biotransformation. 1998 Jan;16(1):1–15. <DOI>.

64. Sih CJ, Chen CS. Microbial Asymmetric Catalysis? Enantioselective Reduction of Ketones [New Synthetic Methods (45)]. Angew Chem Int Ed Engl. 1984 Aug;23(8):570–8. <DOI>.

65. Pham VT, Phillips RS, Ljungdahl LG. Temperature-dependent enantiospecificity of secondary alcohol dehydrogenase from Thermoanaerobacter ethanolicus. J Am Chem Soc. 1989 Mar;111(5):1935–6. <DOI>.

66. Phillips RS. Temperature effects on stereochemistry of enzymatic reactions. Enzyme Microb Technol. 1992;14:417–9. <URL>.

67. Monterde MI, Brieva R, Sánchez VM, Bayod M, Gotor V. Enzymatic resolution of the chiral inductor 2-methoxy-2-phenylethanol. Tetrahedron Asymmetry. 2002 Jun;13(10):1091–6. <DOI>.

68. Lokotsch W, Fritsche K, Syldatk C. Resolution of d,l-menthol by interesterification with triacetin using the free and immobilized lipase of Candida cylindracea. Appl Microbiol Biotechnol. 1989 Oct;31–31(5–6):467–72. <DOI>.

69. Overbeeke PLA, Ottosson J, Hult K, Jongejan JA, Duine JA. The Temperature Dependence of Enzymatic Kinetic Resolutions Reveals the Relative Contribution of Enthalpy and Entropy to Enzymatic Enantioselectivity. Biocatal Biotransformation. 1998 Jan;17(1):61–79. <DOI>.

70. Ottosson J, Fransson L, Hult K. Substrate entropy in enzyme enantioselectivity: An experimental and molecular modeling study of a lipase. Protein Sci. 2002 Jun;11(6):1462–71. <DOI>.

71. Ema T. Rational strategies for highly enantioselective lipase-catalyzed kinetic resolutions of very bulky chiral compounds: substrate design and high-temperature biocatalysis. Tetrahedron Asymmetry. 2004 Sep;15(18):2765–70. <DOI>.

72. Miyazawa T, Kurita S, Ueji S, Yamada T, Kuwata S. Resolution of mandelic acids by lipase-catalysed transesterifications in organic media: inversion of enantioselectivity mediated by the acyl donor. J Chem Soc Perkin 1. 1992;(18):2253. <DOI>.

73. Ema T, Maeno S, Takaya Y, Sakai T, Utaka M. Significant effect of acyl groups on enantioselectivity in lipase-catalyzed transesterifications. Tetrahedron Asymmetry. 1996 Mar;7(3):625–8. <DOI>.

74. Lin C, Hiraga Y, Masaki K, Iefuji H, Ohkata K. Temperature-dependence of enantioselectivity and desymmetrization in the acetylation of 2-mono- and 2,2-di-substituted 1,3-propanediols by a novel lipase isolated from the yeast *Cryptococcus* spp. S-2. Biocatal Biotransformation. 2006 Jan;24(5):390–5. <DOI>.

75. Ottosson J. Enthalpy and entropy in enzyme catalysis, a study of lipase enantioselectivity [PhD Thesis]. [Department of Biotechnology, Stockholm, Sweden]: Royal Institute of Technology; 2001.

76. Yasufuku Y, Ueji S. Improvement (5-fold) of enantioselectivity for lipase-catalyzed esterification of a bulky substrate at 57 °C in organic solvent. Biotechnol Tech. 1996 Aug;10(8):625–8. <DOI>.

77. Yasufuku Y, Ueji S ichi. High Temperature-Induced High Enantioselectivity of Lipase for Esterifications of 2-Phenoxypropionic Acids in Organic Solvent. Bioorganic Chem. 1997 Apr;25(2):88–99. <DOI>.

78. Ema T, Yamaguchi K, Wakasa Y, Yabe A, Okada R, Fukumoto M, et al. Transition-state models are useful for versatile biocatalysts: kinetics and thermodynamics of enantioselective acylations of secondary alcohols catalyzed by lipase and subtilisin. J Mol Catal B Enzym. 2003 Jun;22(3–4):181–92. <DOI>.

79. Sakai T. Enhancement of the enantioselectivity in lipase-catalyzed kinetic resolutions of 3-phenyl-2H-azirine-2-methanol by lowering the temperature to -40℃. J Org Chem. 1997;62:4906–7. <URL>.

80. Sakai T, Kishimoto T, Tanaka Y, Ema T, Utaka M. Low-temperature method for enhancement of enantioselectivity in the lipase-catalyzed kinetic resolutions of solketal and some chiral alcohols. Tetrahedron Lett. 1998 Oct;39(43):7881–4. <DOI>.

81. Yang H, Jönsson Å, Wehtje E, Adlercreutz P, Mattiasson B. The enantiomeric purity of alcohols formed by enzymatic reduction of ketones can be improved by optimisation of the temperature and by using a high co-substrate concentration. Biochim Biophys Acta BBA - Gen Subj. 1997 Jul;1336(1):51–8. <DOI>.

82. Sakai T. 'Low-temperature method' for a dramatic improvement in enantioselectivity in lipase-catalyzed reactions. Tetrahedron Asymmetry. 2004 Sep;15(18):2749–56. <DOI>.

83. Majumder A, Shah S, Gupta M. Enantioselective transacetylation of (R, S)-beta-citronellol by propanol rinsed immobilized Rhizomucor miehei lipase. Chem Cent J. 2007;1:10.

84. Boutelje J, Hjalmarsson M, Hult K, Lindbäck M, Norin T. Control of the stereoselectivity of pig liver esterase by different reaction conditions in the hydrolysis of cis-N-benzyl-2,5-bismethoxycarbonylpyrrolidine and structurally related diesters. Bioorganic Chem. 1988 Dec;16(4):364–75. <DOI>.

85. Barton MJ, Hamman JP, Fichter KC, Calton GJ. Enzymatic resolution of (R,S)-2-(4-hydroxyphenoxy) propionic acid. Enzyme Microb Technol. 1990 Aug;12(8):577–83. <DOI>.

86. Cipiciani A, Bellezza F, Fringuelli F, Silvestrini MG. Influence of pH and temperature on the enantioselectivity of propan-2-ol-treated Candida rugosa lipase in the kinetic resolution of (±)-4-acetoxy-[2,2]-paracyclophane. Tetrahedron Asymmetry. 2001 Sep;12(16):2277–81. <DOI>.

87. Jurczak J, Pikul S, Bauer T. Tetrahedron report number 195 (R)- and (S)-2,3-0-isopropylideneglyceraldehyde in stereoselective organic synthesis. Tetrahedron. 1986 Jan;42(2):447–88. <DOI>.

88. Schwarz KH, Kleiner K, Ludwig R, Schrötter E, Schick H. Synthesis of methyl (±)-2,3-O-isopropylideneglycerate by electrochemical oxidation of (±)-1,2-O-isopropylideneglycerol. Liebigs Ann Chem. 1991 May 16;1991(5):503–4. <DOI>.

89. Lemaire M, Jeminet G, Gourcy JG, Dauphin G. 2- and 8-functionalized 1,4,7,10-tetraoxaspiro[5.5]undecanes. Tetrahedron Asymmetry. 1993 Jan;4(9):2101–8. <DOI>.

90. García M. Synthesis of new ether glycerophospholipids structurally related to modulator. Tetrahedron. 1991;47(48):10023–34. <DOI>.

91. Dröge MJ, Bos R, Woerdenbag HJ, Quax WJ. Chiral gas chromatography for the determination of 1,2-O-isopropylidene-sn-glycerol stereoisomers. J Sep Sci. 2003 Jul 1;26(9–10):771–6. <DOI>.

92. Lundh M, Nordin O, Hedenström E, Högberg HE. Enzyme catalysed irreversible transesterifications with vinyl acetate. Are they really irreversible? Tetrahedron Asymmetry. 1995 Sep;6(9):2237–44. <DOI>.

93. Secundo F, Ottolina G, Riva S, Carrea G. The enantioselectivity of lipase PS in chlorinated solvents increases as a function of substrate conversion. Tetrahedron Asymmetry. 1997 Jul;8(13):2167–73. <DOI>.

94. Zanoni G, Agnelli F, Meriggi A, Vidari G. Enantioselective syntheses of isoprostane and iridoid lactones intermediates by enzymatic transesterification. Tetrahedron Asymmetry. 2001 Jul;12(12):1779–84. <DOI>.

95. Bornscheuer U, Stamatis H, Xenakis A, Yamane T, Kolisis FN. A comparison of different strategies for lipase-catalyzed synthesis of partial glycerides. Biotechnol Lett. 1994 Jul;16(7):697–702. <DOI>.

96. Tservistas M. Untersuchungen zum Einsatz von überkritischem Kohlendioxid als Medium für biokatalysierte Reaktionen [Dissertation]. [Hannover, Germany]: Leibniz Universität Hannover; 1997.

97. Capewell A, Wendel V, Bornscheuer U, Meyer HH, Scheper T. Lipase-catalyzed kinetic resolution of 3-hydroxy esters in organic solvents and supercritical carbon dioxide. Enzyme Microb Technol. 1996 Aug;19(3):181–6. <DOI>.

98. Yildirim A. Lipase Catalysed Transesterification of Isopropyledene Glycerol [Master of Science Thesis]. [Hannover, Germany]: Gottfired Leibniz Universitat Hannover; 2005.

99. Chen CS, Sih CJ. General Aspects and Optimization of Enantioselective Biocatalysis in Organic Solvents: The Use of Lipases[New Synthetic Methods(76)]. Angew Chem Int Ed Engl. 1989 Jun;28(6):695–707. <DOI>.

100. Rakels JLL, Straathof AJJ, Heijnen JJ. A simple method to determine the enantiomeric ratio in enantioselective biocatalysis. Enzyme Microb Technol. 1993 Dec;15(12):1051–6. <DOI>.

101. Persson M, Costes D, Wehtje E, Adlercreutz P. Effects of solvent, water activity and temperature on lipase and hydroxynitrile lyase enantioselectivity. Enzyme Microb Technol. 2002 Jun;30(7):916–23. <DOI>.