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Temperature and solvent effects on enzyme stereoselectivity: inversion temperature in kinetic resolutions with lipases

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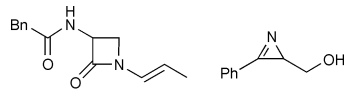
Eyring plots of ln *E vs.* 1/*T* show inversion temperature in kinetic resolution by lipases, demonstrating that clustering effects in the substrate solvation manage the enzymatic selectivity.

Over the past few years, the use of enzymes in organic synthesis has become increasingly important owing to the basic discovery that enzymatic reactions can occur in organic solvents as well as in water.[1] Indeed, enzymatic selectivity is remarkably dependent on the organic solvent[2] and many attempts have been made to explain this influence.[3] However, less attention has been paid to the effect of temperature on selectivity.[4]

We report here the results of our studies on the effect of temperature on the kinetic resolution of racemic β-lactam **1** by



(*R*,*S*)-**1** (*R*,*S*)-**2**

PGA (Penicillin G-Acylase). Furthermore, we also reanalyzed another enzymatic process that has been reported previously, involving the kinetic resolution of azirinol **2** by lipase PS.[5]

Over the past decade, the dependence of stereoselectivity on temperature has been evaluated in several non-enzymatic reactions; *e.g. cis*-dihydroxylation of olefins,[6] reduction of ketones[7] and nucleophilic addition to imines[8] and carbonyl compounds.[9] By plotting the logarithm of selectivity *vs.* the reciprocal of *T*, according to the modified Eyring equation [eqn. (1)], a peculiar behavior is sometimes observed: the existence of two linear regions intersecting at a point defining a temperature denoted the inversion temperature (T_{inv}), whose significance is still a matter of debate.[10]

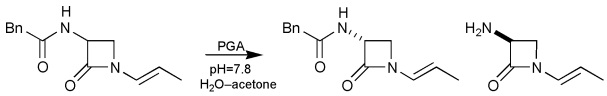
$$\ln S = -\Delta\Delta H^{\ddagger}/RT + \Delta\Delta S^{\ddagger}/R \qquad (1)$$

This break point leads to two sets of activation parameters: one for $T < T_{inv}$ and the other for $T > T_{inv}$. Our preceding studies demonstrated that the presence of an inversion temperature accounts for a particular effect of the reaction solvent on the stereoselectivity. We have proposed that T_{inv} constitutes a transition temperature between two different solute–solvent clusters, which act as two distinct supramolecules with different thermodynamic properties, reactivity and, therefore, different trends of stereoselectivity with temperature.[11]

We have recently shown[12] that in nucleophilic addition to aldehydes, T_{inv} depends mainly on the aldehyde–solvent couple and its value may be determined by ^{13}C NMR analysis using variable temperature (VT) experiments. NMR techniques provide a powerful method for investigating the local electronic environment of the molecular structure, and nuclear shielding is a sensitive probe of intermolecular interactions and solvent effects.[13] We found that the variation of the C=O chemical shift of the studied aldehydes with temperature presents two linear trends, whose intersection (referred to here as T_{NMR}) lies near the T_{inv} of the same substrate–solvent system, within the experimental error. Therefore, we considered that T_{NMR} and T_{inv} might have a common origin, and could in fact represent two independent experimental observations concerning the same solvation-clustering phenomenon.

We report here the first experimental example of inversion temperature in the field of stereoselective enzymatic reactions[14] and also confirm the presence of interconversion of solute–solvent supramolecules by means of VT ^{13}C NMR analysis.

As part of an ongoing project on the total synthesis of new β-lactam antibiotics,[15] we became interested in the kinetic resolution of racemic 1-*N*-(propen-1-yl)-3-*N*-(phenylacetoxy)-aminoazetidin-2-one **1** by PGA (supported on Eupergit) (Scheme 1). The experiments were carried out by incubating the substrate in phosphate buffer (pH = 7.8) and acetone (2:1 ratio) at different temperatures. Conversion of the reaction was monitored by titration of the phenylacetic acid formed with NaOH solution and the enantiomeric excesses of **3** and unreacted **1** were determined by HPLC analysis on a chiral column.[16] With an increase in temperature, we observed an initial linear lowering of the selectivity ratio *E*[17] until it reached a minimum value of 24 corresponding to $T = 301$ K (Fig. 1). Upon further warming, *E* increased, to reach a maximum value of 130 at $T = 328$ K. Least-squares analysis of these results enabled us to determine that $T_{inv} = 301$ K. Notably, the highest enantioselectivity is reached at the highest temperature.[18] Usually, $E = 100$ is considered the minimum satisfactory value for the industrial application of an enzymatic process. Thus, in our case, a slight variation in temperature made an inadequate enzymatic method very efficient.



(*R*,*S*)-**1** (*R*)-**1** (*S*)-**3**

Scheme 1

Next, we performed a series of VT ^{13}C NMR analyses of **1** in deuterated water and d_6-acetone (2:1 ratio). The δ value of the phenylacetoxy C=O chemical shift decreases with an increase in temperature between 278 and 323 K, and two linear regions may be clearly recognized by least-squares analysis (Fig. 2). T_{NMR} occurs at $T = 306$ K and corresponds to T_{inv}, within the experimental error. We looked for other studies on temperature dependent selectivity in enzymatic processes in the literature. In 1997 Sakai *et al.*[5] described the kinetic resolution of racemic phenyl-2*H*-azirine-2-methanol **2** in diethyl ether. The selective

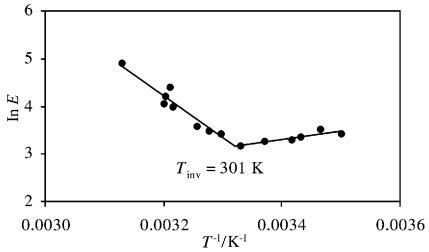


Fig. 1 Eyring plot for the selectivity ratio *E* in the kinetic resolution of **1** by PGA at different temperatures in H_2O–acetone (2:1).

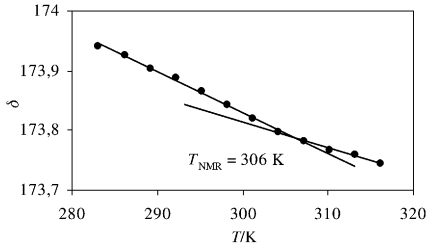


Fig. 2 Plot of the ^{13}C chemical shift of the phenylacetoxy C=O *vs. T* for **1** in D_2O–d_6-acetone (2:1).

acetylation reaction promoted by lipase Amano PS in the presence of vinyl acetate as an acylating agent, proceeded smoothly until 233 K, with progressively higher selectivity (Fig. 3). At T = 233 K, the enantioselection reached a maximum (E = 99) and then decreased. A least-squares analysis of the tabulated data allowed us to determine a T_{inv} at T = 229 K. To test for the presence of a T_{NMR}, we prepared **2** by the reported method[19] and studied the evolution of δ *vs.* T in d_{10}-diethyl ether. The results for the quaternary aromatic carbon[20] are reported in Fig. 4. Again, two linear regions exist and T_{NMR} occurs at T = 222 K, quite near to the corresponding T_{inv}. The different rates of variation of the chemical shift δ *vs.* T indicate an abrupt change in solute–solvent interactions, which reflects a change in substrate solvation.[21] The same solvation change causes the presence of the T_{inv} in an Eyring plot which regards the same substrate in the same solvent of the NMR analysis.

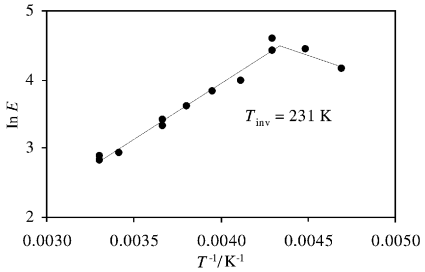


Fig. 3 Eyring plot for the selectivity ratio E in the kinetic resolution of **2** by lipase Amano PS at different temperatures in diethyl ether.

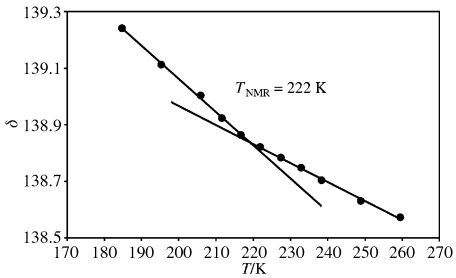


Fig. 4 Plot of the ^{13}C chemical shift of the quaternary aromatic carbon atom *vs. T* for **2** in d_{10}-diethyl ether.

Thus, once again the T_{inv} and the T_{NMR} appear as two independent experimental results due to the same phenomenon. Actually, we think that a reorganization between two differently ordered solvation clusters generates both the T_{inv} and the T_{NMR}. The T_{NMR} reveals the presence of dynamic phenomena on the ground state of solute–solvent clusters which thus appear to be much more structured than generally believed. The T_{inv} reveals the same dynamic phenomena, which now act on diastereomeric transition states leading to a different stereoselectivity below and above the T_{inv}.[22]

From the experimental data reported here, it results that even enzymatic reactions show temperature dependent phenomena of substrate solvation because of the presence of a T_{inv} and a corresponding T_{NMR}, so that even enzymatic reactions experience reorganization of different solute–solvent clusters depending on temperature. Because of the difference in selectivity above and below the T_{inv}, it follows that those solute–solvent clusters contribute to differentiate the Gibbs free energies of the two diastereomeric substrate–enzyme complex transition states. Thus our data provide evidence that solvent molecules are still present in substrate–enzyme transition states.

Our experimental data clearly suggest that enzymatic reactions are only a particular case of a more general phenomenon. Solvation always plays a fundamental role in stereoselectivity and the substrate cannot be considered a mere isolated species in solution, but rather a part of a more complex and well defined solute–solvent cluster.

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Notes and references

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