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New trends in biocatalysis in the presence of cyclodextrins

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Abstract - For the first time, biocatalytic regio- and asymmetric cycloadditions have been attained. It is found that the regio- and enantioselectivity of these cycloadditions can be improved in the presence of cyclodextrins. An interpretation of these results and the role played by the cyclodextrins are described. Attempts have also been made to extend this modelling of biocatalytic reactions involving cyclodextrins to other asymmetric addition reactions in organic synthesis.

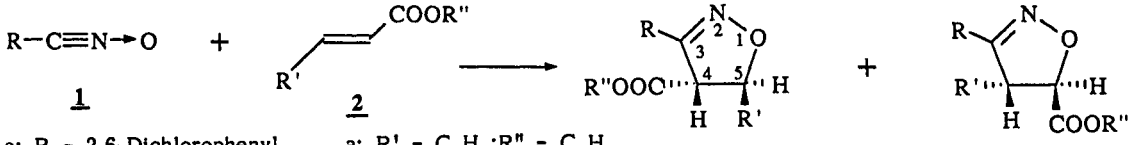
INTRODUCTION

Of recent, the catalytic activity of enzymes and microorganisms has been the focus of interest in organic synthesis due to their specificity, mild conditions and excellent stereoselectivities. Though a number of biocatalysts are known till now to catalyse an array of chemical reactions, their utility has not been explored for studying various selectivities of the versatile dipolar cycloaddition reactions and asymmetric additions to C=C bonds for generating useful chiral synthons. Nonetheless, the existing nonenzymatic methodologies are still not ideal to steer the selectivity of cycloadditions in the desired direction. Hence, the development of biocatalysts for highly regio- and enantioselective cycloadditions will be one of the important goals of organic synthesis in future. These biocatalytic cycloadditions apart from yielding higher selectivities can be performed under mild conditions without any chiral auxiliary either in dipole or in dipolarophile. In addition, since these cycloadditions involve two adends ie. dipole and dipolarophile, the scope of modelling these biocatalytic cycloadditions will also be rather good by using artificial enzymes such as cyclodextrins as additional binding cavities to rigidly fix the geometries of dipole and dipolarophile separately to further enhance the selectivities. In this lecture, I will be presenting some of our results obtained by modelling of biocatalytic reactions.

I. BIOCATALYTIC CYCLOADDITION REACTIONS

I.1 Biocatalytic regioselective 1,3-dipolar cycloaddition of nitrileoxides to α,β-unsaturated esters: reversal of regioselectivity in the presence of cyclodextrin

During our studies on biocatalytic reactions, we have found for the first time that Baker's yeast (Saccharomyces cerevisiae) catalyses the regioselective 1,3-dipolar cycloaddition reaction such as nitrileoxides 1 to cinnamic esters 2. Higher regioselectivities can be achieved by varying the substitution in the dipolarophile.



a: R = 2,6-Dichlorophenyl a: R' = C_6H_5;R" = C_2H_5
b: R = 2,4,6-Trimethylphenyl b: R' = 4-$H_3CC_6H_4$;R" = C_2H_5
c: R = 2,4,6-Trimethoxyphenyl c: R' = C_6H_5; R" = $C(CH_3)_3$

The nitrileoxide 1 and cinnamic ester 2 are taken in equimolar ratio in 30% ethanol and Baker's yeast in pH 7.2 phosphate buffer is added and incubated at 37°C for 30h. The mixture is then extracted with chloroform and purified by flash chromatography. The cycloaddition of nitrileoxides 1 a-c with cinnamic ester 2 c (where R = t-butyl) proceed to give exclusively the regio isomer 3, whereas cinnamic esters 2 a,b (where R=ethyl) give rise to a mixture of isomers 3 and 4 with preponderance of the former.

However, when the nitrileoxide contained electron releasing groups as in 1 a,c only one regioisomer 3 was formed. The isomer 4 could not be obtained exclusively in any case. At this stage a novel approach has been adopted to improve the regioselectivity of the biocatalytic cycloaddition of ethyl cinnamates 2 a,b with nitrileoxide 1 b by forming Host-Guest complex of ethyl cinnamates with cyclodextrin. This has led to total reversal of regioselectivity.

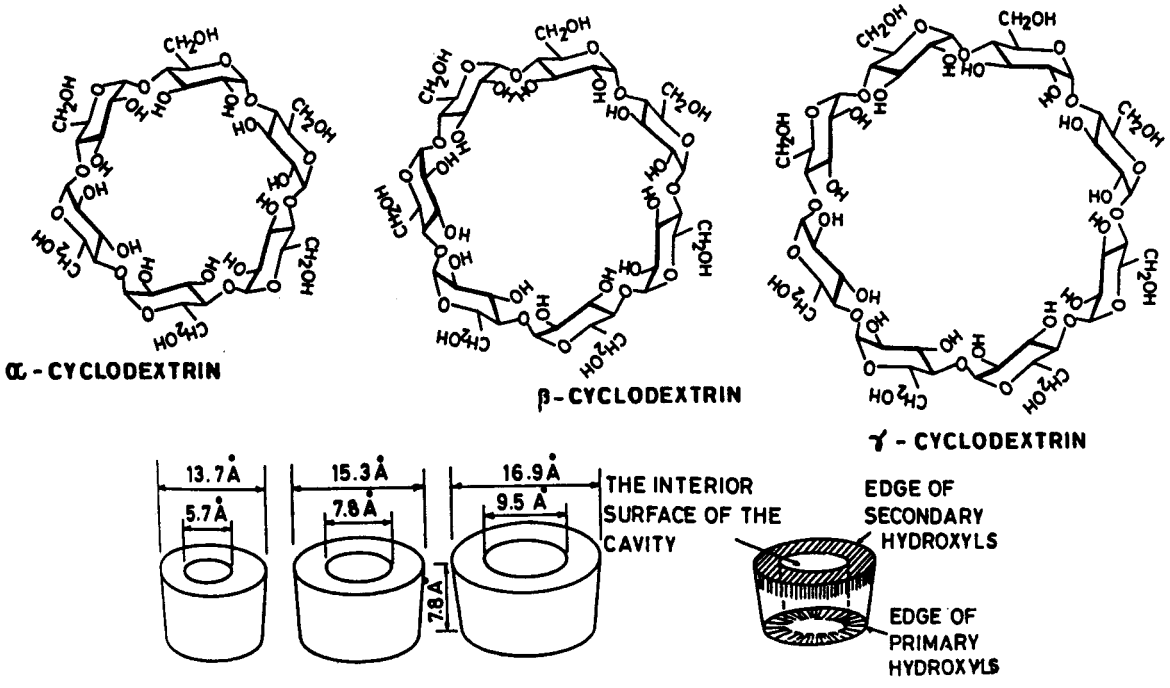


Fig. 1. Cyclodextrins as enzymes models

Cyclodextrins which have excited much interest in various biomimetic syntheses due to their ability to bind substrates selectively and catalyse chemical reactions (ref. 1-3) have been used as artificial enzymes for the dipolarophile. Cyclodextrins (Fig. 1) are cyclic oligosaccharides and the most commonly encountered are i) α-Cyclodextrin (six glucose units) ii)β-Cyclodextrin (seven glucose units) and iii)γ-Cyclodextrin (eight glucose units) . The molecular geometry and electronic structure of cyclodextrins is such that the interior surface of the cavity is hydrophobic (apolar) and the external surface is hydrophilic (polar) . In aqueous solution the cavity contains water molecules in an energetically unfavourable polar - apolar association and the driving force for the complex formation is displacement of water molecules by the more hydrophobic guest molecule to attain "apolar - apolar" association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state. The important criterion for complex formation is the "dimensional fit" between host cavity and guest molecule. The inclusion complexes can be formed by i) saturation in aqueous solution and ii) slurry mixing method. These complexes can be characterised by X-ray, NMR and other methods.

In the present case, a 1:1 complex of the dipolarophile with β-cyclodextrin is made in aqueous medium and is added to the buffer solution of nitrileoxide and Baker's yeast, incubated at 37°C for 30h and workedout. This has yielded surprisingly the regioisomer 4, so far not possible to generate exclusively by the cycloaddition of 1b to 2a,b. Thus it is shown for the first time that the regioselectivity in cycloadditions can be totally changed in the reverse direction without altering the substituents by fixing the dipolarophile in cyclodextrin which acts as an artificial enzyme and dipole in the binding cavity of the biocatalyst (ref. 4). These cycloadditions do not proceed in the presence of cyclodextrin alone.

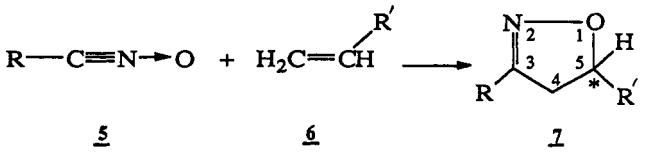
These results have been interpreted in terms of FMO theory. It is seen from the results of the cycloaddition of nitrileoxides 1a-c and tert-butylcinnamate 2c that the regioselective effect is by $LUMO_{(dipole)} - HOMO_{(dipolarophile)}$ to form exclusively the regioisomer 3. It is also observed from the experimental results that the selectivity decreases in the cycloaddition of nitrileoxide 1b with ethyl substituted esters 2a,b.

The steering of regioselectivity of cycloaddition of nitrileoxide 1b and ethyl cinnamate 2a,b to give exclusively the regioisomer 4 by forming an inclusion complex of the dipolarophile with β-cyclodextrin may be explained by the dependence of the coefficients in frontier MOs on effective hydrogen bonding of β-cyclodextrin hydroxyls with the ester group. Thus, the ability to control the regioselectivity of biocatalytic cycloaddition reactions by using β-cyclodextrin as an artificial enzyme may find applications in various stereocontrolled syntheses.

I.2 Biocatalytic asymmetric cycloaddition of nitrileoxides to C=C bond: improved chiral recognition in the presence of cyclodextrin

The results on biocatalytic regioselective cycloadditions have prompted us to investigate the biocatalytic asymmetric cycloadditions which would proceed without the presence of any chiral auxiliary either in dipole or in dipolarophile. Attempts have been made for the first time to gain access into biocatalytic asymmetric cycloaddition reactions by utilizing Baker's yeast (Saccharomyces cerevisiae) which is earlier known to induce chirality in various transformations.

The asymmetric 1,3-dipolar cycloaddition of nitrileoxides 5 to the active C=C bond of 2- and 4-vinylpyridines 6 have been carried out in the presence of Baker's yeast, since this reaction gives heterocyclic substituted optically active 2-isoxazolines 7 which represent the masked form of an array of different functionalities.



5 6 7

R = 2,4,6-Trimethylphenyl,
2,4,6-Trimethoxyphenyl
& 2,6-Dichlorophenyl
R' = 2-Pyridyl & 4-Pyridyl

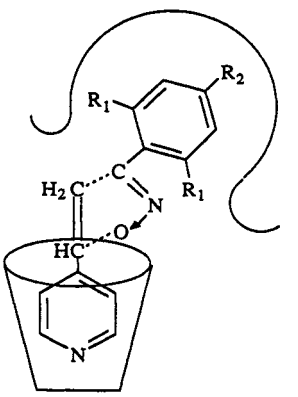


Fig. 2 Biocatalytic asymmetric cycloaddition using β-cyclodextrin

The regioselective formation of only 5-pyridyl substituted 2-isoxazolines 7 also fits the LUMO (dipole) - HOMO (dipolarophile) interaction with the preferred attachment of the dipolarophilic carbon of greater spatial requirement of vinylpyridine ie. α-carbon with the oxygen of the nitrileoxide leading to the sterically most stable transition state. The nitrileoxide 5 and vinylpyridine 6 are taken in equimolar ratio in 30% ethanol and incubated at 37°C with Baker's yeast in pH 7.2 phosphate buffer for 20h, extracted with chloroform and purified by flash chromatography. The optically active 2-isoxazolines 3 thus obtained are shown in Table-1. Amongst the compounds studied, only 2-isoxazoline formed from 2,6-dichlorophenyl nitrileoxide and 4-vinylpyridine has shown modest enantioselectivity with an ee upto 25%. This is due to poor chiral recognition during cycloaddition. Hence, a new concept has been envisaged in biocatalytic asymmetric cycloadditions for improving the stereoselectivity by adopting two host cavities so that the geometry of both dipole and dipolarophile are fixed during cycloaddition for attaining better enantioselectivity (Fig. 2) . This is further confirmed by the experimental results.

The inclusion complex of 4-vinylpyridine with β-cyclodextrin has been prepared and characterised. The aqueous solution containing the inclusion complex of 4-vinylpyridine with cyclodextrin is then added to the buffer solution containing nitrileoxide and Baker's yeast. It is incubated at 37°C for 20h and worked out. It is again 2-isoxazoline obtained from 2,6-dichlorophenyl nitrileoxide has shown higher enatioselectivity with an ee upto 64%.

Thus it has been demonstrated for the first time that Baker's yeast can be used as chiral catalyst in asymmetric cycloaddition reactions and that the chiral recognition during cycloaddition can be improved by using cyclodextrin as an additional binding cavity (ref. 5).

Table 1 Asymmetric cycloaddition of nitrileoxides to vinylpyridines

No	R (Product 7)	R'	yield % BY*	yield % BY+ β CD**	$[\alpha]_D^{20}$ deg. BY	$[\alpha]_D^{20}$ deg. BY+ β CD	ee % BY	ee % BY+ β CD
a	2,4,6-Trimethylphenyl	4-Pyridyl	82	81	+5.1	+25.8	4.5	22.6
b	2,4,6-Trimethoxyphenyl	4-Pyridyl	88	89	-11.3	-32.5	9.2	28.4
c	2,6-Dichlorophenyl	4-Pyridyl	83	85	+66.7	+160.0	25.6	64.0
d	2,4,6-Trimethylphenyl	2-Pyridyl	85	-	+2.8	-	2.5	-
e	2,4,6-Trimethoxyphenyl	2-Pyridyl	79	-	+5.6	-	3.6	-
f	2,6-Dichlorophenyl	2-Pyridyl	88	-	+11.5	-	8.8	-

*BY: Baker's yeast; ** β-CD: β-cyclodextrin

II. BIOCATALYTIC ASYMMETRIC ADDITION OF AMINES TO C=C BOND

II.1 Biocatalytic asymmetric synthesis of β-aminoacid esters by the addition of amines to α,β-unsaturated esters: enhanced chiral recognition by using cyclodextrin

To further extend the scope of our observations on biocatalytic cycloadditions, efforts are initiated to generate useful chiral synthons by nucleophilic addition to C=C bonds. In this context, our attention has been drawn to the growing importance of various optically active β-aminoacid esters as components of peptide antibiotics and as precursors of β-lactams (ref. 6-8).

β-Aminoacid esters 10 of appreciable enantioselectivity are synthesised by the biocatalytic addition of various amines 9 to C=C bond of α,β-unsaturated esters 8 in the presence of Baker's yeast (Saccharomyces cerevisiae) . These reactions proceed without the presence of any chiral auxiliary.

8 R^1 = a: C_6H_5;b:4-$H_3CC_6H_4$;c:4-$H_3COC_6H_4$

9 HNR^2R^3 = a:$H_2NCH_2C_6H_5$;b:$H_2NCH_2CH_2C_6H_5$;c:HN⟨pyrrolidine⟩

The ester 8 and the amine 9 are taken in equimolar ratio and incubated at 37°C with Baker's yeast in pH 7.2 phosphate buffer for 48h, extracted with ethylacetate and purified by flash chromatography. It is seen from the results obtained that the aminoacid ester 10b formed from ethylcinnamate 8a and 2-phenylethylamine 9b has shown highest enantioselectivity with an ee upto 60% (Table-2).

Table 2 Biocatalytic asymmetric synthesis of β-aminoacid esters

No	R^1 (Product 10)	NR^2R^3	yield % BY*	yield % BY+ β -CD**	$[\alpha]_D^{20}$ deg. BY	$[\alpha]_D^{20}$ deg. BY+ β-CD	ee % BY	ee % BY+ β-CD
a	C_6H_5	$HNCH_2C_6H_5$	72	70	-5.7	-10.3	40.0	72.5
b	C_6H_5	$HN(CH_2)_2C_6H_5$	41	42	+4.0	+ 4.5	60.0	67.2
c	C_6H_5	N⟨pyrrolidine⟩	68	71	+6.7	+19.7	7.5	22.0
d	4-$H_3CC_6H_4$	$HNCH_2C_6H_5$	71	72	-4.8	- 9.4	24.1	47.2
e	4-$H_3CC_6H_4$	$HN(CH_2)_2C_6H_5$	42	39	+8.0	+11.5	20.0	28.5
f	4-$H_3COC_6H_4$	$HNCH_2C_6H_5$	72	71	-4.0	- 6.1	15.0	23.1
g	4-$H_3COC_6H_4$	$HN(CH_2)_2C_6H_5$	43	42	+7.5	+17.3	11.1	25.8
h	4-$H_3COC_6H_4$	N⟨pyrrolidine⟩	69	70	+15.1	+18.4	33.0	40.0

*BY: Baker's; ** β-CD: β-cyclodextrin

To improve the chiral recognition further, we envisioned at this stage a successful implementation of the strategy of the combination of natural and artificial enzymes by fixing the geometry of the ester 8 in β-cyclodextrin and the amine 9 with the biocatalyst. This should lead to preferential attack by the amine from only one of the enantiotopic faces of the prochiral ester rigidly fixed in the cyclodextrin cavity and thus result in higher enantioselectivity. This is indeed found to be the case. The aqueous solution containing the inclusion complex of the ester with the cyclodextrin is added to the buffer solution containing the amine and Baker's yeast, incubated at 37°C for 48h and worked out. The aminoester 10a obtained from ethylcinnamate 8a and benzylamine 9a has shown enhanced enantioselectivity with an ee upto 72%. This higher asymmetric bias observed for the benzylamine addition may be due to favourable control of geometry in the approach of the substrate to the active site (ref. 9).

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