Inclusion effects of cyclodextrins on the catalytic activity of 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*endo*-amine for the decarboxylation of oxalacetate

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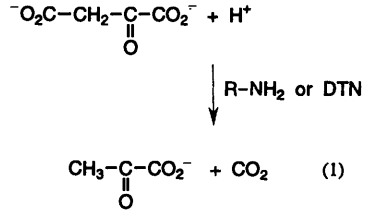
The inclusion effects of cyclodextrins (CD) on the catalytic activity of 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*endo*-amine (DTN) for the decarboxylation of oxalacetate have been studied. It was found that β-CD, consisting of seven glucose units, showed a marked enhancement of the catalytic activity of DTN by the inclusion, whereas α-CD showed neither inclusion nor enhancement, and γ-CD showed the inclusion phenomenon, but only with small enhancement. These observations are discussed in terms of the regulation of the DTN conformation by the inclusion.

Introduction

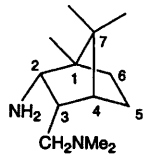
Amine-catalysed decarboxylation of oxalacetate (OA) has been the subject of continued investigation [eqn. (1)].[1-5] In the



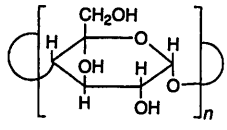
(1)

decarboxylation of OA, it is known that primary amines are the most effective catalysts, secondary amines are much less so, and tertiary amines are ineffective.[5-7] The reason is understood as being due to the formation of imine as an obligatory intermediate in the catalysis. The catalysis of amines having more than one primary amino group has also been investigated. The catalysis of ethylenediamine was studied in detail by Leussing and Raghavan.[2] For polyamines, Spetnagel and Klotz studied the catalysis of poly(ethylene imines).[1] More recently, we reported high catalytic activities of peraminocyclodextrins.[3] Very recently, Benner *et al.* reported the catalysis of a rationally designed polypeptide having five lysine residues, and they pointed out the importance of a neighbouring relationship between amino groups for efficient catalysis.[4]

The above literature examples suggest the importance of neighbouring *cis*-diamine or polyamine structures for the catalysts to be effective in the decarboxylation of β-ketoacids. As supporting evidence, we reported in the previous paper that 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*endo*-amine (DTN), a *cis*-diamine, is remarkably active as compared to the related 1,3-diamines including the *trans*-counterpart, and rationalized its high activity as being due to a cooperative action of two amino groups.[8] However, although the C^2–N and the C^3–C bonds of DTN are firmly fixed in an *endo–endo* configuration by bicyclic rings, the dimethylamino group can rotate around the C^3–C bond so that the conformations of two amino groups are not so firmly fixed. Therefore, we were interested in the possible control of the conformations of DTN by inclusion into CD cavities. The regulation of the reactivities by the conformational controls of guest molecules by CDs was already discussed by Bender and Komiyama for several cases.[9-11] We report here that the catalytic activity of DTN for the decarboxylation of OA [eqn. (1)] is markedly enhanced when DTN is included in the cavity of β-CD.



DTN



$n = 6$: α-CD
$n = 7$: β-CD
$n = 8$: γ-CD

Experimental

Materials and methods

Water for the kinetics studies was purified by deionization followed by distillation. Buffers for the kinetics experiments were prepared by using commercially available extra pure reagents: buffer(pH); acetic acid–sodium acetate (4:5). Oxalacetic acid (OA) obtained from Kishida Chem. (Japan) was found to be >98% pure by titration with standard NaOH, was used without further purification, and was stored under refrigeration. Cyclodextrins (CDs) used were: α-CD from Tokyo Kasei (Japan), 99% pure; β-CD from Tokyo Kasei, 99% pure; γ-CD from Kishida Chem. (Japan) 97% pure. The microwater contents in the CD samples were determined by Karl Fischer's method by using a Kyoto Densi MKA-3 apparatus. DTN was prepared according to the previous methods.[8] ^{1}H NMR spectra were recorded with a JEOL 400 MHz spectrophotometer.

Kinetics

The rates of the decarboxylation of OA were measured spectrophotometrically by monitoring the decrease of the absorbance of the enol form of OA at 260 nm on a Shimadzu UV-160A recording spectrophotometer according to the previous method.[1,3,8] All kinetic data were obtained in fully aqueous solutions. Acetate buffers (0.2 mol dm^{-3}) were used to maintain the desired pH. Each solution contained sufficient KCl to give a final ionic strength of 0.2 mol dm^{-3}. The reaction solutions containing DTN and CD were placed in a 1 cm cuvette. The cuvette was placed in a thermally regulated cell holder, and an aliquot from a freshly prepared stock solution of OA was added to the cuvette solution to initiate the decarboxylation reaction. The pseudo-first-order rate constants (k_{obs}) were calculated by using: $k_{obs} = 1/t \ln[(A_\infty - A_0)/(A_\infty - A_t)]$.

Results and discussion

Effect of varying CD concentrations on the rate constants

In the presence of a fixed concentration of the catalyst DTN, the concentrations of three CDs were varied as shown in Fig. 1. It is seen that α-CD showed essentially no effect on the rate and a slight rate increase with increasing the concentration of γ-CD, whereas a large rate increase occurs with increasing concentration of β-CD, giving a saturation curve. The rate increase by β-CD was also observed for other DTN concentrations giving similar saturation curves, as shown in Fig. 2. These saturation curves were found to be analysed by assuming a reaction Scheme 1 involving the complexation of DTN with β-CD, as represented by eqns. (2–5), where k_0 and k_c are the rate constants in the absence of β-CD and in the presence of the complex, respectively, and K is the association constant of complex. In Scheme 1, the known equilibria for the imine formation and the related steps are not shown for simplicity. The complexation of OA with β-CD in the absence of DTN is not considered because of a lack of spectroscopic evidence and it is unlikely for such a small and polar guest like OA. Actually the enol form of OA was measured at 260 nm in the presence or in the absence of CDs. The Boneni–Hildebrand plots of the curves of Fig. 2 based on eqn. (5) gave the corresponding values of $k_0 = 0.28$ mol^{-1} dm^3 s^{-1}, $k_c = 5.5$ mol^{-1} dm^3 s^{-1}, and $K = 80$ mol^{-1} dm^3. Thus, the activity of DTN is enhanced about 20-fold at pH 4.65, 25 °C when complexed with β-CD.

$$\text{DTN} + \beta\text{-CD} \underset{}{\overset{K}{\rightleftharpoons}} \text{complex} \quad (2)$$

$$\text{OA} + \text{complex} \xrightarrow{k_c} \text{products} \quad (3)$$

$$\text{OA} + \text{DTN} \xrightarrow{k_o} \text{products} \quad (4)$$

$$k_{obs} = k_o + \frac{kcK[\text{DTN}][\beta\text{-CD}]}{1 + K([\text{DTN}] + [\beta\text{-CD}])} \quad (5)$$

Scheme 1 The reaction scheme (2–4) and the rate eqn. (5)

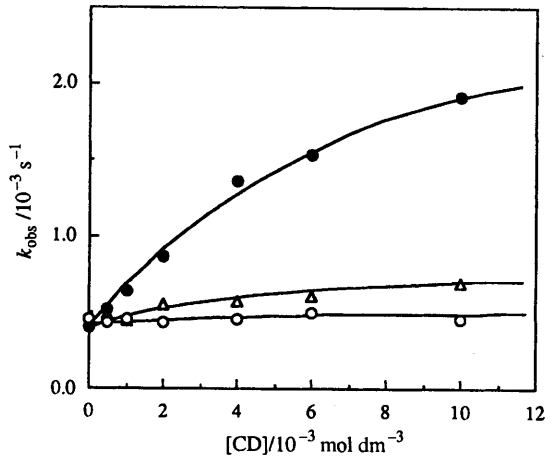


Fig. 1 Dependence of k_{obs} on [CD] at pH = 4.64, 25 °C; [OA] = 6 × 10^{-5} mol dm^{-3}, [DTN] = 6 × 10^{-4} mol dm^{-3}; ○: α-CD, ●: β-CD, △: γ-CD

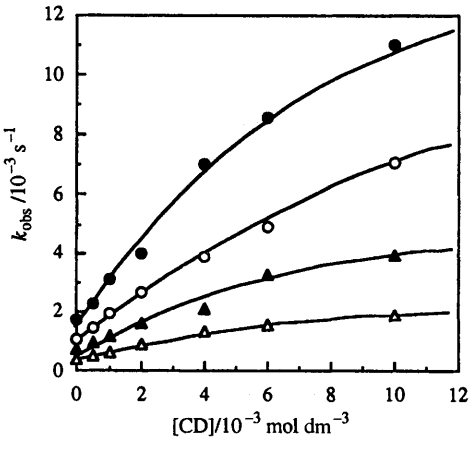


Fig. 2 Dependence of k_{obs} on [DTN] and [β-CD] at pH = 4.65, 25 °C; [OA] = 6 × 10^{-5} mol dm^{-3}; [DTN] × 10^3 mol dm^{-3} are 4.8, 2.4, 1.2 and 0.6 from the top

Fluorescence spectroscopy of the inclusions of DTN by CDs

It is likely that the above enhancement of the catalytic activity of DTN is due to its inclusion into the cavity of β-CD. To confirm this possibility, the competitive inclusion between 1-anilino-naphthalene-8-sulfuric acid ammonium salt (ANS) and DTN by CDs was examined by the fluorescence spectroscopy. As shown in Fig. 3, in the absence of DTN the fluorescence intensities of ANS increased with increasing concentration of CDs, giving saturation curves with β- and γ-CDs, but there was only a slight increase of the intensity with α-CD. Such data are well known in the literature and rationalized as to indicate the cavity sizes of β- and γ-CD being large enough for the inclusion of ANS, but not in the case of α-CD.[12,13] It can be seen more clearly in Fig. 4 that the inclusion behaviour of the three CDs is quite different. Fig. 4 shows that under conditions such that ANS is well complexed with β- and γ-CDs, the

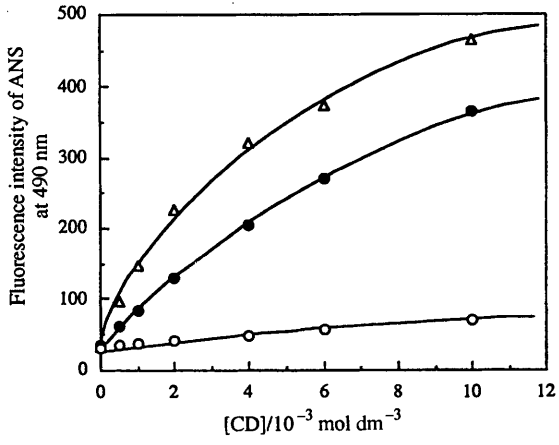


Fig. 3 Dependence of fluorescence intensity of ANS on the CD concentration: [ANS] = 1 × 10^{-4} mol dm^{-3}; pH 4.64, 25 °C; ○: α-CD, ●: β-CD, △: γ-CD

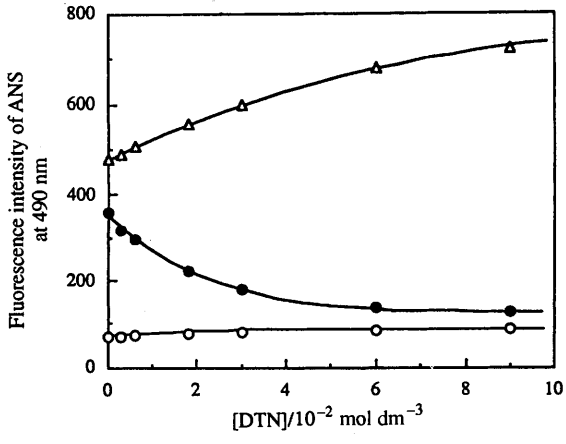
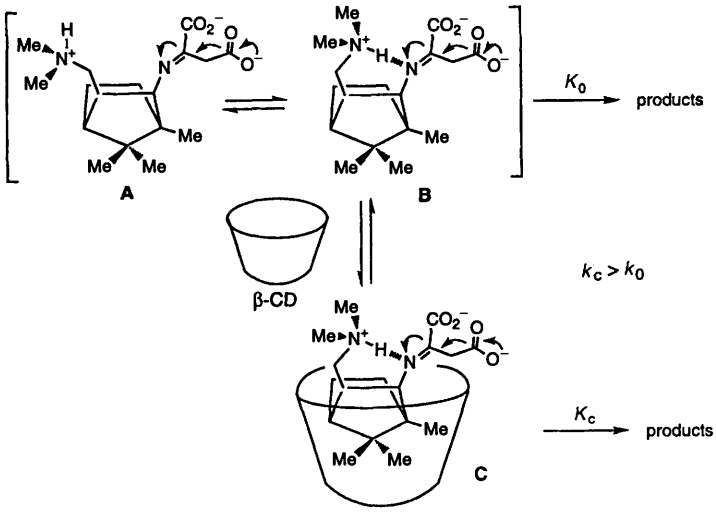


Fig. 4 Dependence of fluorescence intensity of ANS on the DTN concentration: [ANS] = 1 × 10^{-4} mol dm^{-3}, [CD] = 1 × 10^{-2} mol dm^{-3}, pH 4.64, 25 °C; ○: α-CD, ●: β-CD, △: γ-CD



Scheme 2 Mechanism of the β-CD effect on the enhancement of the catalytic activity of DTN for the decarboxylation of OA

addition of DTN caused a decrease of the intensity in the case of β-CD, further increase of the intensity in the case of γ-CD, but essentially no change of the intensity in the case of α-CD. These results indicate that the cavity of β-CD is not large enough to include both guests so that a competitive inclusion occurs, while the cavity of γ-CD allows further inclusion of the second guest, which enhances the apolar nature of the cavity to result in a further increase of ANS intensity.

^{1}H NMR spectra

In the ^{1}H NMR spectra DTN shows three methyl proton signals at δ 0.91 for the 1-methyl group and at δ 0.96 and 0.97 for the two 7,7-dimethyl groups in the absence of β-CD in D_2O. These signals shifted to δ 0.94, 0.98 and 0.99 in the presence of 1×10^{-3} mol dm^{-3} of β-CD, and further to δ 1.06, 1.00 and 1.01 in the presence of 1×10^{-2} mol dm^{-3} of β-CD. The shift of the 1-methyl group, $\Delta\delta$(ppm) = 0.15, was much larger than those of the other two methyl groups (0.04). The methyl protons of the dimethylamino group showed a singlet at δ 2.89 in the absence of β-CD. This signal shifted to δ 2.91 and 2.92 in the presence of 10^{-3} and 10^{-2} mol dm^{-3} of β-CD, respectively. These shifts also seem to indicate the inclusion of DTN into the cavity of β-CD, although they are not so conclusive as the above fluorescence spectroscopy.

Mechanism of activation

As previously reported, the decarboxylation of OA catalysed by DTN involves the formation of imine as an obligatory intermediate.[8] A much higher activity of DTN as compared to those of the *trans*-counterpart and the related 1,3-diamines was attributed to a *cis* structure of two amino groups. Two major factors were considered for a *cis* structure: (i) the charge–charge repulsion between the two neighbouring positive ammonium groups lowers the pK_a of the primary amino group so as to be active at the lower pH regions and (ii) the stabilization of the imine intermediate by an electrostatic and/or hydrogen bonding. Scheme 2 shows simplified mechanism for the present β-CD effect.

The pK_1 and pK_2 values of DTN (10^{-3} mol dm^{-3}) are 6.59 and 9.84, respectively,[8] in the absence of β-CD, whereas the pK_1 and pK_2 values of DTN were found to change to 5.86 and 9.46, respectively, in the presence of 10^{-2} mol dm^{-3} of β-CD. These lower pK_a of DTN and the neutralisation of charges of the complex in Scheme 2 would be expected to accelerate the reaction within the CD cavity.

In Scheme 2, DTN and OA form an imine intermediate, which must be a mixture of conformers such as **A** and **B**. The rate-determining step is considered to be C–C bond cleavage. This step requires the protonation of imine nitrogen and is favoured when the C–C bond to be cleaved is perpendicular to the plane of C=N double bond. To meet these criteria, the conformer **A** would not be suitable, since the protonated dimethylamino group is remote from both the imine nitrogen and the carboxylate anion, and thus it requires external acid catalysis for the protonation of imine nitrogen. Instead, in the conformer **B**, a model study indicates tight hydrogen bonding between the two nitrogen atoms as well as an ion-pairing between the ammonium and carboxylate groups forming a six-membered ring. Such a frozen structure would be favourable for an intramolecular proton transfer concerted with the C–C bond cleavage. Here, a similar seven-membered structure involving the other β-carboxylate group is neglected because it places the C–C bond to be cleaved in a near coplanar conformation with the C=N bond. CPK molecular models indicate the inclusion of conformer **B** to form **C** is more favourable than the conformer **A**. Here, it is assumed that the molecule is included from the side of methyl groups and the wide side of β-CD, although the conclusive evidence is lacking for the orientation of the inclusion.

In summary, the conformational 'fitness' of DTN, caused by inclusion into the hydrophilic environment of the β-CD cavity, could facilitate the catalytic decarboxylation of OA.

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