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Catechol as a Nucleophilic Catalyst of Peptide Bond Formation

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Abstract: The aminolysis of a mildly activated aminoacid ester, benzyloxycarbonyl-L-phenylalanine cyanomethyl ester, by glycine esters in the presence of catechol has been studied as a model of catalysis by RNA *cis*-vicinal-diol systems in protein biosynthesis. Catechol accelerated the aminolysis, especially in the presence of bases, probably by nucleophilic catalysis. Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: catalysis; enzyme mimetics; neighbouring group effect; peptides; ribozymes

Although the *cis*-1,2-diol group is an integral part of the RNA structure, our understanding of the contribution of this functionality in RNA catalysis is still fragmentary. The *cis*-2′,3′-diol system of bonded or internal guanosine is a powerful nucleophile or good leaving group in RNA splicing [1]. Similar enhanced reactivity is observed for the *cis*-2′,3′-diol of tRNA 3′-terminal adenosine in ribosomal peptide bond synthesis [2]. The nucleophilic or electrophilic participation of the adjacent to the *cis*-1,2-diol phosphodiester bond 2′-OH in RNA is a chemical determinant for the catalytic activity of both large and small ribozymes [3].

In 1949 Gordon *et al.* [4] found that alkylene glycols markedly increased the rate of the ammonolysis of carboxylic acid esters in aprotic organic media. Much more mechanistic information, however, is available for the reactivity of catechol. Its monoanion has been reported to be a much better nucleophile than the phenoxide ion towards a number of carboxylic acid derivatives [5–8] and towards phosphonofluoridates such as the nerve gases sarin and soman [9–11]. On the other hand, catechol monoesters of protected amino acids are known to aminolyse abnormally rapidly without racemization [12, 13]. It appears, therefore, that an acceleration of the aminolysis of protected amino acid esters could be achieved by their catecholysis followed by *in situ* aminolysis of the resulting catechol esters. Here we report on such a nucleophilic catalysis by catechol of peptide bond synthesis and comment on its pertinence to the catalysis of peptide bond biosynthesis.

The cyanomethyl esters of *N*-protected amino acids are known from classical peptide synthesis to be mildly activated, requiring perhaps 24 h for complete coupling [14, 15]. Figure 1 shows that Z-Phe-OH cyanomethyl ester reacts sluggishly in the presence of triethylamine in chloroform with H–Gly–OR (R = Me, Et, Bn) to give the peptide Z–Phe–Gly–OR. Previous studies have shown that such aminolysis reactions are accelerated by bifunctional acid-base catalysts, the best known being 1-hydroxybenzotriazole (HOBt) [16, 17]. The catalytic effect of HOBt, however, like that of pentafluorophenol or phenol, is small (Table 1). A more distinct acceleration is observed with catechol. Such a pronounced rate acceleration is not seen

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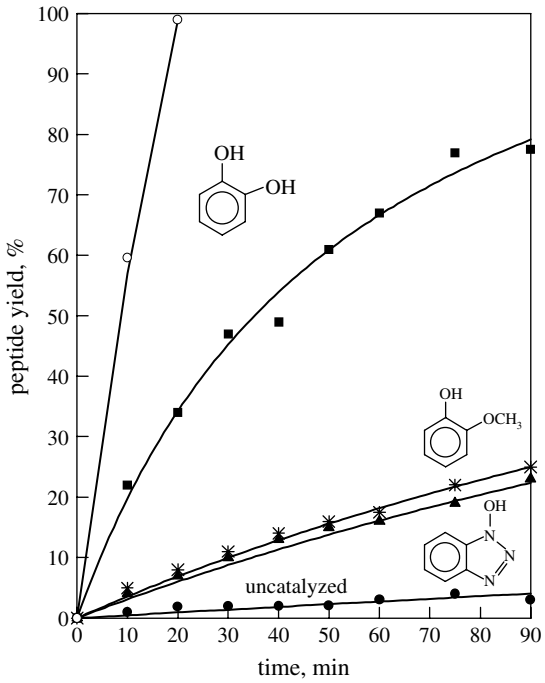


Figure 1 Acceleration of Z–Phe–OCH_2CN and H–Gly–OR (R = Me, Et, Bn) coupling in chloroform (●) by HOBt (▲), guiacol (✳) in the presence of Et_3N, and catechol in the presence of Et_3N (■) or DBU (○).

with o-methoxyphenol (guiacol) (Table 1) suggesting the crucial role of the 1,2-dihydroxy system for catalysis.

The catalytic effect of catechol is observed in the presence of sterically hindered tertiary amines (Table 1) that excludes direct nucleophilic attack of these organic bases. The rate increase closely parallels their acid dissociation constants (pKa) (Table 1). The calculated concentration of catechol monoanion using the pKa value measured in acetonitrile for 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (pKa 23.9) is 70 mM; for triethylamine (pKa 18.5) – 0.1 mM and for N-ethylmorpholine (pKa 15.7) – 0.006 mM. The difference in pKa values of catechol and catechol monoanion in water is ca. 3.5 pKa units [20] suggesting that the concentration of catechol dianion is negligible. This implies that the catechol monoanion is the catalytic species in the observed catecholic peptide bond synthesis.

In the absence of the amine component H-Gly-OR, Z–Phe–OCH_2CN and catechol react with the formation of a compound that is stable under the reaction conditions used (anhydrous aprotic organic solvent and organic base) (Figure 2a). It has been isolated by preparative HPLC and ^1H NMR analysis identifies the compound as the

Table 1 Effects of Different Effectors on the Second Order Rate Constant *k* for the Coupling of Z–Phe–OCH_2CN and H–Gly–OMe at 25 °C

Effector	pKa		ϵ[a]	$k \times 10^2$ ($M^{-1}.min^{-1}$)
	in water	in acetonitrile		
A. Catalyst + triethylamine in chloroform				
None				0.4
Guiacol	9.9			1.3
HOBt	4.0			1.2
Phenol	9.9	26.6[b]		1.4
Pentafluorophenol	5.5			1.2
Catechol	9.5	26.2[d]		6.7
B. Base + catechol in chloroform				
N-ethylmorpholine	7.7	15.7[c]		2.7
Triethylamine	10.6	18.5[b]		6.7
DBU	11.6	23.9[c]		19.3
C. Solvent + triethylamine + catechol				
Acetonitrile			26.5	9.3
Chloroform			4.4	6.7
DMF			36.7	2.2
DMSO			49.0	0.9

[a] dielectric constant.
[b] Reference [18].
[c] Reference [19].
[d] Estimated using ΔpKa = 16.6 found for phenol [18].

transesterification product *N*-benzyloxycarbonyl-L-phenylalanine catechol ester (Z–Phe–OCat). In the presence of the amine component H–Gly–OR, the concentration of this ester is kinetically controlled (Figure 2b) implying it to be an intermediate during the peptide bond synthesis. When subjected to aminolysis by H–Gly-OR separately, this catechol ester yields neatly the peptide Z–Phe–Gly-OR in accordance with the reported enhanced aminolytic reactivity of amino acid catechol esters [12, 13]. No intermediate formation is detected when the rest of the nucleophiles (HOBt, phenol, pentafluorphenol or guiacol) are used instead of catechol.

The results described so far are consistent with a nucleophilic catalytic mechanism of action of the catechol monoanion in peptide bond synthesis by aminolysis of the mildly activated Z–Phe–OH cyanomethyl esters by H–Gly–OR (Scheme 1). Actually, the observed catechol monoanion catalysis meets the three main requirements for nucleophilic covalent catalysis [21, 22]. (1) The catechol monoanion reacts faster with Z–Phe–OCH_2CN than the

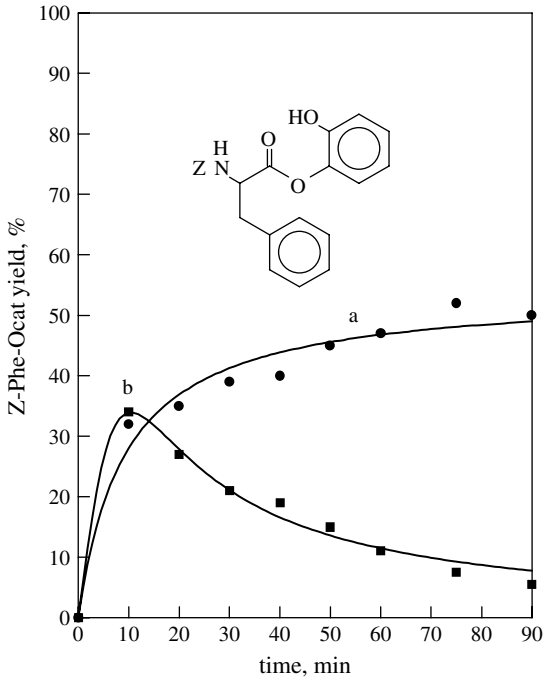
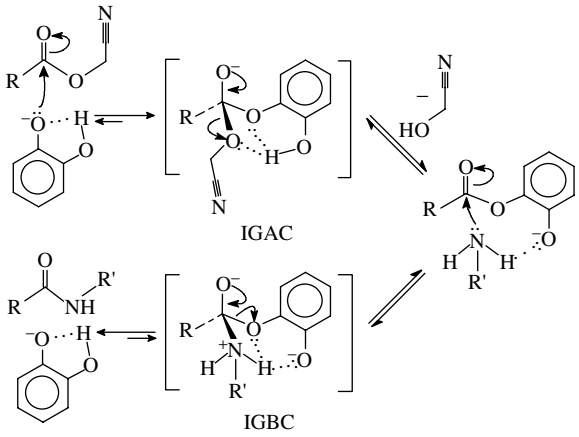


Figure 2 Kinetically controlled accumulation of an intermediate, Z–Phe–OCat, during the catechol-catalysed aminolysis of Z–Phe–OCH_2CN in chloroform in the absence (a) and in the presence (b) of the amine component H–Gly–OMe.



Scheme 1 Proposed mechanism of nucleophilic catalysis by the catechol monoanion.

latter does with the amino component H–Gly–OR since an accumulation of the intermediate occurs (Figure 2b). (2) The intermediate catechol ester is capable of faster aminolysis than the parent ester as has been known from previous publications [12, 13]. (3) The concentration of intermediate Z–Phe–OCat is kinetically controlled (Figure 2b) suggesting that the product Z–Phe–Gly–OMe is thermodynamically more stable than the intermediate catechol ester. On the other hand, the accumulation of the intermediate catechol ester (Figure 2) is consistent with a rate-limiting deacylation. This conclusion is supported by the slight solvent effect (Table 1) attributed to the intramolecular general base catalysis (IGBC) by the adjacent OH in the aminolysis of catechol amino acid esters [12, 13]. Therefore, according to the principle of microscopic reversibility, the formation of the catechol ester Z–Phe–OCat is subject to intramolecular general acid catalysis (IGAC) (Scheme 1). The slower rates observed for other nucleophiles (Figure 1, Table 1) imply that the formation of a covalent intermediate is no longer a rapid step in the case of these bifunctional catalysts or that a synchronous action of the two groups takes place as reported for 1-hydroxybenzotriazole [16]. The abnormal nucleophilicity of the catechol monoanion has been attributed to a low requirement for its solvation due to the strong intramolecular hydrogen bonding in less polar media of the diol unionized hydroxyl to the adjacent oxyanion [8, 23].

CONCLUSIONS

The observed catechol-catalysed peptide synthesis could be considered as a congruent reaction to ribosomal peptide synthesis, the catechol being a transfer RNA mimic. The 3′-terminal of the t-RNA *cis*-2′,3′-diol reacts with an activated amino acid to yield the covalent intermediate amino acyl-t-RNA that undergoes aminolysis on the ribosome by subsequent amino acid t-RNA [2]. Catechol and nucleosides, however, are structurally dissimilar since ribose vicinal hydroxyls are connected by a single C–C bond in contrast to catechol hydroxyls connected by an unsaturated C–C bond. Studies using improved bioorganic models of *cis*-vicinal diol catalysis are in progress in this laboratory and the results will be published soon elsewhere.

EXPERIMENTAL

General Procedures, Methods and Materials

Reverse phase HPLC analyses were performed on a Waters Liquid Chromatograph equipped with an absorbance detector model 441 set at 280 nm and

a column of Nucleosil 100-5C_{18} (12.5 cm × 4.6 mm) for analytical runs, or Nucleosil 100-5C_{18} (25 cm × 10 mm) for semi-preparative runs. ^{1}H spectra were taken on a Bruker Avance-DRX 250 spectrometer at 300 K with tetramethylsilane as internal standard. Chemical shifts are reported in δ (ppm).

General Procedure for the Typical Aminolysis Experiment

A solution of 0.12 mmol H–Gly–OR (R = Me, Et, Bn) hydrochloride and 0.12 mmol organic base in 0.2 ml organic solvent was added to 0.04 mmol Z–Phe–OCH_2CN [24] in 0.1 ml of the same solvent. The reaction was started by the addition of 0.40 mmol of a bifunctional catalyst and 0.40 mmol base in 0.1 ml of the same organic solvent. Both the disappearance of starting substrate (Z–Phe–OCH_2CN) and the appearance of products were followed by analytical RP-HPLC. When the reaction was over, the reaction mixture was evaporated to dryness under reduced pressure, dissolved in the mobile phase (55% CH_3CN in 20 mM phosphate buffer, pH 7.0) and applied on a semi-preparative HPLC column. Appropriate fractions were pooled, evaporated to dryness and the residue subjected to structural analysis.

***N*-benzyloxycarbonylphenylalanyl-glycine methyl ester (Z–Phe–Gly–OMe).** ^{1}H NMR ($CDCl_3$): δ = 3.01 (d, 2H, J = 6.7 Hz, Cβ–H of Phe), 3.11 (s, 3H, CH_3), 3.87 (dd, 1H, J = 5.0, 18.3 Hz, Cα–H of Gly), 3.99 (dd, 1H, J = 5.4, 18.3 Hz, Cα–H of Gly), 4.40 (m, 1H, Cα–H of Phe), 5.08 (s, 2H, CH_2Ph), 5.22 (1H, NH of Phe), 6.27 (1H, NH of Gly), 7.08-7.31 (m, 10H, C_6H_5).

Analytical RP-HPLC (50% CH_3CN in 20 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.0; flow 0.8, 298.2 K) t_R = 3 min.

Anal. Calcd: C 64.86, H 5.95, N 7.57. Found: C 64.80, H 6.05, N 7.49.

m.p. 119 °C (lit. [25] m.p. 120 °C)

***N*-benzyloxycarbonylphenylalanine catechol ester (Z–Phe–OCat).** ^{1}H NMR ($CDCl_3$): δ = 3.19 (dd, 1H, J = 8.0, 13.9 Hz, Cβ–H), 3.28 (dd, 1H, J = 6.6, 13.9 Hz, Cβ–H), 4.59 (m, 1H, Cα–H), 5.13 (s, 2H, CH_2Ph), 5.29 (d, 1H, J = 5.2 Hz, NH), 6.79–6.99 (m, 4H, C_6H_4), 7.23–7.41 (m, 10H, C_6H_5).

Analytical RP-HPLC (50% CH_3CN in 20 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.0; flow 0.8, 298.2 K) t_R = 5 min.

Anal. Calcd: C 70.53, H 5.37, N 3.58. Found: C 70.49, H 5.39, N 3.49.

RP-HPLC Kinetic Studies

Aliquots were withdrawn at appropriate time intervals, diluted with the mobile phase and subjected to RP-HPLC analysis with isocratic elution (50% CH_3CN in 20 mM phosphate buffer, pH 7.0), 0.8 ml/min flow rate at 298.2 K. The concentration of the reaction product was calculated from its peak area. The second order rate constant k was calculated from initial rate data.

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