Cooperation of Imidazolyl and Carboxylate Residues for Efficient Cleavage of Bis(nitrophenyl) Hydrogenphosphates

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Synopsis. 4-Imidazoleacetate efficiently catalyzes the cleavage of bis(2,4-dinitrophenyl) hydrogenphosphate and bis(4-nitrophenyl) hydrogenphosphate (1600 and 15000 fold acceleration at pH 8.0 at 1.0 mol dm^{-3}). The effective catalysis is ascribed to the intramolecular cooperation of the imidazolyl and the carboxylate residues.

Currently considerable interest has been focusing to the preparation of artificial nucleases.[1] There, small catalytic molecules are attached to the moieties showing specific interaction with the nucleic acids.

Virtually all of the systems previously studied employ metal complexes as the catalytic sites, mainly because no organic molecule has shown sufficient catalysis. Thus, the enhancement of the activity of organic catalysts for the cleavage of phosphodiesters, the most stable form of phosphoesters,[2] is highly required for the further development of the field.

Breslow reported that imidazole catalyzes the cleavage of uridylyl(3′-5′)uridine.[3] The catalysis of cyclodextrins bringing two imidazolyl groups for the cleavage of cyclic phosphate of 1,2-benzenediol was also shown.[4] The present paper reports that 4-imidazoleacetate exhibits significantly large catalytic activity for the cleavage of bis(2,4-dinitrophenyl) hydrogenphosphate (**1**) and bis(4-nitrophenyl) hydrogenphosphate (**2**), due to the cooperation of the imidazolyl and the carboxylate residues.

Experimental

The substrate **1** was prepared from phosphoryl chloride and 2,4-dinitrophenol according to the literature.[5] The cleavage of **1** and **2** was carried out at 50 °C, and was followed by the increase of the absorbance at 400 nm (due to the appearance of the nitrophenolates). The second-order catalytic rate constant k_{cat} was determined from the slope of the linear plot of the pseudo first-order rate constant vs. the concentration of the corresponding species. The pK_a values of the imidazoles were evaluated by the titration.

Results and Discussion

4-Imidazoleacetate exhibited quite an efficient catalysis for the cleavage of **1** and **2**: at the concentration 1.0 mol dm^{-3} and pH 8.0, the acceleration was 1600 and 15000 fold. The k_{cat} value for the cleavage of **1** is 4.6 times as large as the corresponding value of unsubstituted imidazole. The pH-rate constant profile showed that the active species involves both neutral imidazolyl residues and carboxylate ion.

Figure 1 shows the Brönsted plot for the cleavage of **1** catalyzed by various imidazoles. The points for all the imidazoles, except for 4-imidazoleacetate (number 1), satisfactorily fit the straight line of the slope 0.12. The basicity of the imidazolyl residue governs the

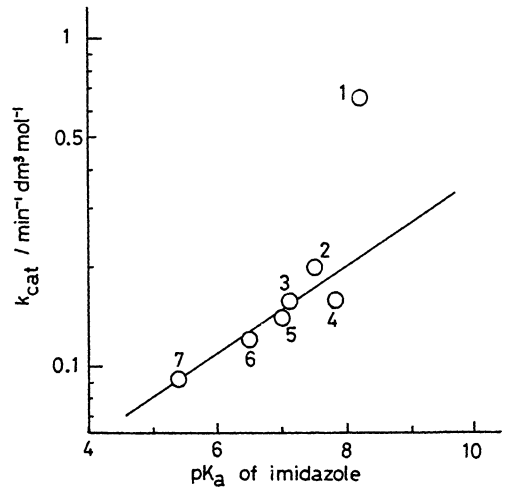

Fig. 1. Brönsted plot for the imidazole-catalyzed cleavage of **1** at 50 °C: The numbers near the circles refer to the substituents in imidazoles. (1) 4-CH_2COO^-; (2) 4-CH_3; (3) *N*-CH_3; (4) 2-CH_3; (5) H (unsubstituted imidazole); (6) 4-CH_2OH; (7) 4-$(CH_2)_2NH_3^+$.

efficiency of the catalysis.

However, the point for 4-imidazoleacetate significantly deviates from the straight line: the k_{cat} value is about 3 times as large as the value estimated from the straight line. This enhancement is ascribed to the cooperation of the imidazolyl residue and the adjacent carboxylate. The intramolecular cooperation is definitely confirmed by no measurable cooperation of unsubstituted imidazole and acetate ion for the cleavage of **1**. Furthermore, the 4-imidazoleacetate-catalyzed cleavage of **1** exhibits a significant D_2O solvent isotope effect (k_{cat}(in H_2O)/k_{cat}(in D_2O)=2.3), showing proton-transfer in the rate-determining step.[6]

In the transition state for the present catalysis, formed by the nucleophilic attack of the nitrogen atom of the imidazolyl residue to **1**, the adjacent carboxylate partially abstracts a proton from the other nitrogen atom of the imidazolyl residue. This suppresses the formation of a positive charge in the imidazolyl residue, promoting the catalysis (Fig. 2). The mechanism is associated with the "charge-relay" system, proposed for serine proteases[7] and further supported by the model systems,[8] although the imidazolyl residue in the "charge-relay" system functions as general-base catalyst rather than nucleophilic catalyst.

The above argument has been supported by the fact that no D_2O solvent isotope effect is observed for the catalysis by the imidazoles other than 4-imidazoleacetate. Here the catalysis proceeds via

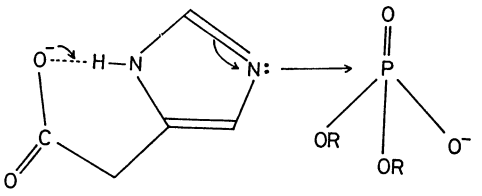

Fig. 2. Proposed mechanism for the hydrolysis of **1** and **2** catalyzed by 4-imidazoleacetate: R shows 2,4-dinitrophenyl or 4-nitrophenyl residue.

nucleophilic attack by the imidazolyl residues without rate-determining proton transfer.

In conclusion, 4-imidazoleacetate is quite efficient for the cleavage of phosphodiesters **1** and **2**, due to the cooperation of the catalytic residues. This finding indicates a strong possibility of the development of the artificial nucleases involving organic moieties as the catalytic sites.

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References

1) P. B. Dervan, *Science*, **232**, 464 (1986); J. K. Barton, *Science*, **233**, 727 (1986); D. S. Sigman, *Acc. Chem. Res.*, **19**, 180 (1986); L. A. Basile, A. L. Raphael, and J. K. Barton, *J. Am. Chem. Soc.*, **109**, 7550 (1987).

2) R. Singleton, Jr., *J. Chem. Educ.*, **50**, 538 (1973).

3) R. Breslow and M. LaBelle, *J. Am. Chem. Soc.*, **108**, 2655 (1986).

4) R. Breslow, J. B. Doherty, G. Guillot, and C. Lipsey, *J. Am. Chem. Soc.*, **100**, 3227 (1978).

5) C. A. Bunton and S. J. Farber, *J. Org. Chem.*, **34**, 767 (1969).

6) M. L. Bender, R. J. Bergeron, and M. Komiyama, "The Bioorganic Chemistry of Enzymatic Catalysis," John Wiley & Sons, New York (1984).

7) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature (London)*, **221**, 337 (1969).

8) M. Komiyama and M. L. Bender, *Bioorg. Chem.*, **6**, 13 (1977); M. Komiyama, M. L. Bender, M. Utaka, and A. Takeda, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 2634 (1977).