EFFECT OF ADENINE NUCLEOTIDE MOIETY OF COENZYME A ON PHOSPHOTRANSACETYLASE

Masao Shimizu, Tadao Suzuki, Yasuhiro Hosokawa, Osamu Nagase and Yasushi Abiko

Research Laboratories
Daiichi Seiyaku Co., Ltd.
Edogawa-ku, Tokyo 132, Japan

Received November 18, 1969

SUMMARY

Structural analogues of CoA lacking in aletheine moiety as well as in pantetheine moiety were found to compete with CoA in the phosphotransacetylase reaction. Moreover, the latter compound has induced an ability of the enzyme to acetylate pantetheine in the absence of CoA, which was inhibited by desulfo-CoA. These results suggest that adenine nucleotide moiety of CoA plays an essential role in being recognized as CoA by phosphotransacetylase.

In an effort to elucidate the structural involvement of CoA in its own coenzyme activity, we have synthesized various structural analogues of CoA and analyzed their interactions with phosphotransacetylase(Acetyl-CoA:orthophosphate acetyltransferase, EC 2.3.1.8) The enzyme from Escherichia coli B and that from Clostridium kluyveri have been employed in the analysis. The former was shown to be an allosteric enzyme activated by pyruvate and inhibited mainly by NADH, while the latter enzyme had no such allosteric character(1, 2, 3). This paper describes the discovery of an essential moiety of CoA for being recognized as CoA by the enzymes involved, in addition to the different kinetic behaviors in the enzyme reaction between the adenine nucleotide moiety of CoA and allosteric inhibitors. Details will be published in a subsequent paper.

MATERIALS AND METHODS

CoA, PTSH and P-PTSH(4) as well as de-alet-CoA-NH_2(5) were synthesized chemically in these laboratories. 3'-P,5'-ADP and 3'-P,5'-ADP-CH_3 were also synthesized in these laboratories(6). PTS-Ac was prepared by a modified method of Walton et al.(7). Desulfo-CoA was prepared according to the method of Chase et al. (8). Oxy-CoA was kindly supplied by Dr. C. J. Stewart(9). Chemical structures of these CoA analogues and the nucleotides are presented in Table I. Acetyl-P and phosphotransacetylase of Cl. kluyveri were purchased from Boehringer. Preparation of purified

TABLE I

The Simplified Chemical Structures of CoA and Its Analogues

CoA

Ado-3'-P-5'-P-P-CH_2-$C(CH_3)_2$-CH(OH)-CO-NH-CH_2-CH_2-CO-NH-CH_2-CH_2-SH

oxy-CoA

Ado-3'-P-5'-P-P-CH_2-$C(CH_3)_2$-CH(OH)-CO-NH-CH_2-CH_2-CO-NH-CH_2-CH_2-OH

desulfo-CoA

Ado-3'-P-5'-P-P-CH_2-$C(CH_3)_2$-CH(OH)-CO-NH-CH_2-CH_2-CO-NH-CH_2-CH_3

de-alet-CoA-NH_2

Ado-3'-P-5'-P-P-CH_2-$C(CH_3)_2$-CH(OH)-CO-NH_2

3'-P,5'-ADP-CH_3

Ado-3'-P-5'-P-P-CH_3

3'-P,5'-ADP

Ado-3'-P-5'-P-P

Abbreviations: acetyl-P, acetyl phosphate; PTSH, pantetheine; P-PTSH, pantetheine 4'-phosphate; PTS-Ac, S-acetyl pantetheine.

phosphotransacetylase from E. coli B in addition to the methods of measuring CoA-dependent arsenolysis reaction and S-acetyl-forming reaction, those catalyzed by this enzyme was described previously (2, 3).

RESULTS

Effects of CoA analogues on phosphotransacetylase.

Oxy-CoA, which has OH group instead of SH group of CoA, as well as desulfo-CoA which lacks in SH group were reported to compete with CoA in the acetyl-CoA-forming reaction catalyzed by phosphotransacetylase of Cl. kluyveri(8, 9). It was confirmed that these two analogues competed with CoA also in the arsenolysis reaction: Ki's of oxy-CoA were 2.6×10^{-6} M for the enzyme of E. coli B and 1.0×10^{-6} M for that of Cl. kluyveri; Ki's of desulfo-CoA were 3.4×10^{-6} M and 4.0×10^{-6} M for the former and for the latter enzyme, respectively.

On the other hand, even the CoA analogue lacking in aletheine moiety of the CoA structure, de-alet-CoA-NH_2(chemical structure: see Table I), was found to compete with CoA. Ki values of this compound were 4.4×10^{-4} M for the enzyme of E. coli B and 1.2×10^{-3} M for that of Cl.kluyveri. The result suggests that this compound could bind to a CoA-site of phosphotransacetylase.

Effects of adenine nucleotides on phosphotransacetylase.

Phosphotransacetylase of E. coli B was found to be inhibited by various nucleotides shown in Table II. The enzyme of this source was an allosteric enzyme which was inhibited strongly by NADH and to a lesser extent by ADP and ATP, all in noncompetitive fashion with respect to CoA(1, 3). As shown in the table, 3'-P,5'-AMP also

TABLE II

Inhibitory Effects of Adenine Nucleotides on Phosphotransacetylase

Nucleotides	Concn.	Inhibition			
		E. coli B enzyme		Cl. kluyveri enzyme	
	(mM)	(%)	type*	(%)	type*
5'-AMP	1.0	2	——	0	——
5'-ADP	1.0	48	noncompetitive	0	——
5'-ATP	1.0	37	noncompetitive	0	——
3'-P,5'-AMP	1.0	36	noncompetitive	2	——
3'-P,5'-ADP	1.0	62	mixed	33	competitive
3'-P,5'-ADP-CH_3	1.0	33	competitive	22	competitive

* represents inhibition type with respect to CoA.

Inhibition rate was estimated from the CoA-dependent arsenolysis reaction. A reaction mixture containing 6 μmoles of acetyl-P, 15.8 mμmoles of CoA, 5 μmoles of cysteine, 20 μmoles of Tris-HCl (pH 7.8), the nucleotide indicated, 3.0 units of the enzyme and 50 μmoles of K-arsenate(pH 7.8) in a final volume of 1 ml was incubated at 25°C for 15 min. Acetyl-P was measured by hydroxamic acid method I(2). Inhibition type was determined in 0.5 ml of the reaction mixture being incubated at 25°C for 10 min in the presence and the absence of the nucleotide. The reaction mixture contained 2 μmoles of acetyl-P, 10 μmoles of cysteine, 10 μmoles of Tris-HCl (pH 7.8), varied concentrations of CoA ranging from 35 to 200 mμmoles, 0.3 - 0.4 unit of the enzyme and 25 μmoles of K-arsenate (pH 7.8). Acetyl-P was measured by hydroxamic acid method II (2).

noncompetitively inhibited the enzyme reaction, while inhibition by 3'-P,5'-ADP was a mixed type and that by 3'-P,5'-ADP-CH_3 was competitive in respect of CoA with a Ki value of 1.1×10^{-3} M. On the other hand, ADP, ATP and 3'-P,5'-AMP had no inhibitory effect on the enzyme of Cl. kluyveri which was not a regulatory enzyme(1, 3), whereas both of 3'-P,5'-ADP and 3'-P,5'-ADP-CH_3 inhibited this enzyme competitively with CoA: Ki's were 9.1×10^{-4} M and 2.0×10^{-3} M, respectively. These results suggest that 3'-P,5'-ADP might be essential in the CoA structure for being recognized as CoA by

phosphotransacetylase.

Acetylation of PTSH in the presence of adenine nucleotides.

As described above, 3'-P,5'-ADP, an adenine nucleotide moiety of CoA was shown to compete with CoA in the phosphotransacetylase reaction of Cl. kluyveri. Moreover, it was found that, when 3'-P,-5'-ADP was present in the reaction mixture, the enzyme gained an ability to acetylate PTSH with acetyl-P in the absence of CoA(Curves a and b in Fig. 1). Products from both reactions of Curve

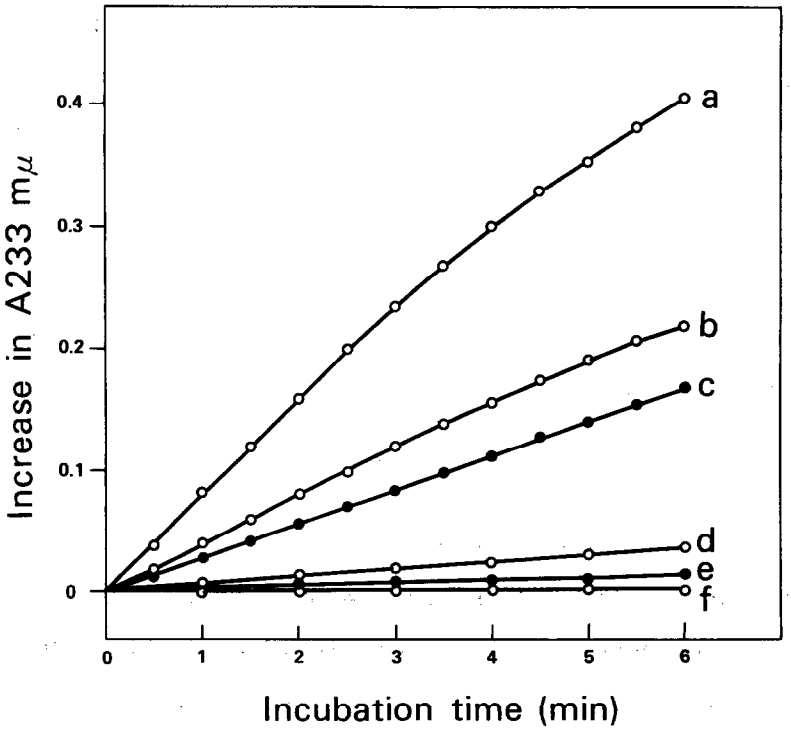

Fig. 1. Acetylation of pantetheine in the presence of adenine nucleotides. Phosphotransacetylase from Cl. kluyveri(48 units) was incubated at 22°C in 3 ml reaction mixture containing 8 μmoles of acetyl-P, 3 μmoles of dithiothreitol, 100 μmoles of NH_4Cl and 120 μmoles of Tris-HCl(pH 7.8). Increase in the absorbancy at 233 mμ was read in a cuvette of 1-cm light pass. Pantetheine(PTSH) and the nucleotide were added to the mixture accordingly to the reaction systems: Curve a, 1.0 μmole of PTSH + 0.5 μmole of 3'-P,5'-ADP; Curve b, 0.5 μmole of PTSH + 0.5 μmole of 3'-P,5'-ADP; Curve c, 0.5 μmole of PTSH + 3'-P,5'-ADP-CH_3; Curve d, 0.5 μmole of PTSH alone; Curve e, 0.5 μmole of P-PTSH + 0.5 μmole of 3'-P,5'-AMP ; Curve f, 0.5 μmole of 3'-P,5'-ADP alone.

a and Curve b were identified with PTS-Ac by paper chromatography: Rf's of the products, 0.73; authentic PTS-Ac, 0.74; solvent, water-saturated methyl ethyl ketone. From these results it was strongly suggested that 3'-P,5'-ADP has induced PTSH-acetylation as a result of its specific interaction with a CoA-site of phosphotransacetylase. This acetylating reaction was found to be inhibited by desulfo-CoA, a potent competitive inhibitor of CoA, again supporting that the CoA-site of the enzyme was responsible for this nucleotide-induced PTSH acetylation. The presence of PTS-Ac was confirmed also in the reaction mixture of Curve c, in which PTSH was incubated together with 3'-P,5'-ADP-CH_3. Little or no increase in the absorbancy at 233 mμ, however, were found with the reaction mixtures containing PTSH alone, 3'-P,5'-AMP + P-PTSH or 3'-P,5'-ADP alone, respectively.

In the presence of 3'-P,5'-ADP-CH_3, PTSH was acetylated also by the phosphotransacetylase reaction of E. coli B, while in its absence no acetylation occured.

DISCUSSION

The structural analogue of CoA which lacks in aletheine was shown to compete with CoA in the phosphotransacetylase reaction. Further studies have revealed that 3'-P,5'-ADP and 3'-P,5'-ADP-CH_3, those corresponding to the adenine nucleotide moiety of CoA have also competed with CoA. Moreover, these nucleotides were found to induce an ability of the enzyme to acetylate PTSH, which was inhibited by desulfo-CoA, a potent competitive inhibitor of CoA. These results strongly suggest that 3'-P,5'-ADP affects specifically a CoA-site of the enzyme, indicating that the adenine nucleotide moiety of CoA plays a central role in being recognized as CoA by phosphotransacetylase. The induced-fit specificity theory of

Koshland(10) might be prefered to interpret the nucleotide-induced PTSH acetylation. Both of 3'-phosphate and 5'-pyrophosphate in the CoA structure were necessary for the specificity of phosphotransacetylase, since 3'-P,5'-AMP and 5'-ADP did not compete with CoA.

3'-P,5'-ADP was found to inhibit phosphotransacetylase of Cl. kluyveri competitively with CoA, while it inhibited the enzyme of E. coli B in a mixed manner. The latter was an allosteric enzyme which was activated by pyruvate and inhibited by NADH, ADP and ATP. These inhibitors affected the enzyme of E. coli B noncompetitively with CoA and gave sigmoidality of the reaction with respect to the acetyl-P concentration(3). Inhibition of 3'-P,5'-AMP was also noncompetitive, while that of 3'-P,5'-ADP-CH_3 was competitive with respect to CoA. It appears, therefore, that the enzyme of E. coli B recognized 3'-P,5'-ADP in part as CoA and in another part as an allosteric inhibitor. On the other hand, the enzyme of Cl. kluyveri had no such allosteric character(3) so that this enzyme should recognize the nucleotide only as CoA.

In these laboratories, several other CoA analogues were synthesized. These involve analogues modified on cysteamine moiety of CoA(11), those modified on pantoyl moiety(5) and on adenine nucleotide moiety(12). Kinetic analysis of the interaction between these analogues and phosphotransacetylase will be reported in a subsequent paper.

ACKNOWLEDGEMENTS

The authors wish to express their deep gratitude to Prof. Dr. F. Lynen, Max-Plank-Institut für Zellchemie, München, for his interest, and to Dr. M. Koike, professor of Nagasaki University, for his valuable advice and discussion. The authors are also indebted to Dr. T. Ishiguro, president of this company, for his encouragement and support.

REFERENCES

1. T. Suzuki, Y. Abiko and M. Shimizu, Biochem. Biophys. Res. Commun., 35, 102, 1969.
2. M. Shimizu, T. Suzuki, K. Kameda and Y. Abiko, Biochim. Biophys. Acta, in the press.
3. T. Suzuki, Biochim. Biophys. Acta, in the press.
4. M. Shimizu, O. Nagase, S. Okada, Y. Hosokawa, H. Tagawa, T. Suzuki and Y. Abiko, Chem. Pharm. Bull.(Tokyo), 15, 655, 1967.
5. M. Shimizu, O. Nagase, Y. Hosokawa, H. Tagawa and Y. Yotsui, Chem. Pharm. Bull.(Tokyo), submitted.
6. Y. Hosokawa, O. Nagase and M. Shimizu, Chem. Pharm. Bull. (Tokyo), submitted.
7. E. Walton, A. N. Wilson, F. W. Holly and K. Folkers, J. Am. Chem. Soc., 76, 1146, 1954.
8. J. F. A. Chase, B. Middleton and P. K. Tubbs, Biochem. Biophys. Res. Commun., 23, 208, 1966.
9. T. L. Miller, G. L. Rowley and C. J. Stewart, J. Am. Chem. Soc., 88, 2299, 1966.
10. D. E. Koshland, Jr. and K. E. Neet, Ann. Rev. Biochem., 37, 359, 1968.
11. M. Shimizu, O. Nagase, Y. Hosokawa and H. Tagawa, Tetrahedron, 24, 5241, 1968.
12. M. Shimizu, O. Nagase, S. Okada and Y. Hosokawa, Chem. Pharm. Bull.(Tokyo), 14, 683, 1966; ibid., in the press.