# 大腸菌および哺乳類アデニレートキナーゼ(AK)間の免疫学的交差反応


| 誌名 | Japanese journal of veterinary science |
|---|---|
| ISSN | 00215295 |
| 著者 | 渡辺, 清隆<br>北垣, 貴央<br>山本, 晋二 |
| 巻/号 | 49巻6号 |
| 掲載ページ | p. 1180-1182 |
| 発行年月 | 1987年12月 |



農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

AgriKnowledge

# Immunological Cross-reaction between *Escherichia coli* and Mammalian Adenylate Kinases

Kiyotaka WATANABE, Takahisa KITAGAKI, and Shinji YAMAMOTO
*Laboratory of Biochemistry, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada 034, Japan*

(Received 15 April 1987/Accepted 3 September 1987)

*Jpn. J. Vet. Sci.* 49(6): 1180–1182, 1987

KEY WORDS: adenylate kinase, cross reaction, *Escherichia coli.*

Adenylate kinases (ATP+AMP⇌2ADP) are ubiquitous and monomeric proteins [11]. In mammals, there are two isoenzymes: cytosolic adenylate kinase isoenzyme 1 (AK1) and mitochondrial isoenzyme 2 (AK2) [3, 4, 17]. *Escherichia coli* adenylate kinase (AKe) has been purified and characterized [1, 5, 7], and its kinetic properties and amino acid sequence deduced from the nucleotide sequence [2] have been compared with those of mammalian enzymes [13, 15]. The immunological relationship between AKe and mammalian enzymes, however, has not been elucidated so far. Thus we examined here the immunological cross-reactivity of AKe with AK1 or AK2 by enzyme immunoinactivation test and enzyme-linked immunosorbent assay(ELISA).

Adenylate kinase activity was measured in the reverse direction using a coupled colorimetric assay [18]. Protein was determined by the method of Lowry *et al.* [10], using bovine serum albumin as a standard.

AKe was purified essentially according to the method of Goelz and Cronan [5] from *E. coli* strain B (ATCC 11303), which was obtained from Miles Laboratories, Inc., USA. Purified AKe had a specific activity of 1600 units/mg protein and one free sulfhydryl group, whose reaction with Ellman's reagent led to loss of the enzymic activities to about 50% in 10min. AK1 and AK2 were purified from the rat skeletal muscle and liver, respectively, by the method described previously [18]. Rabbit antisera against AKe, AK1 and AK2 were prepared by the method described before [17], and they inhibited completely the enzymic activities of respective homologous antigens (Fig. 1).

AKe was not inactivated at all by either of antisera against AK1 and AK2 and *vice versa* (Fig. 1). However, definite cross-reactions between AKe and rat enzymes were detected by ELISA (Fig. 2). Control sera from rabbits injected with *Mycobacterium tubercu-*

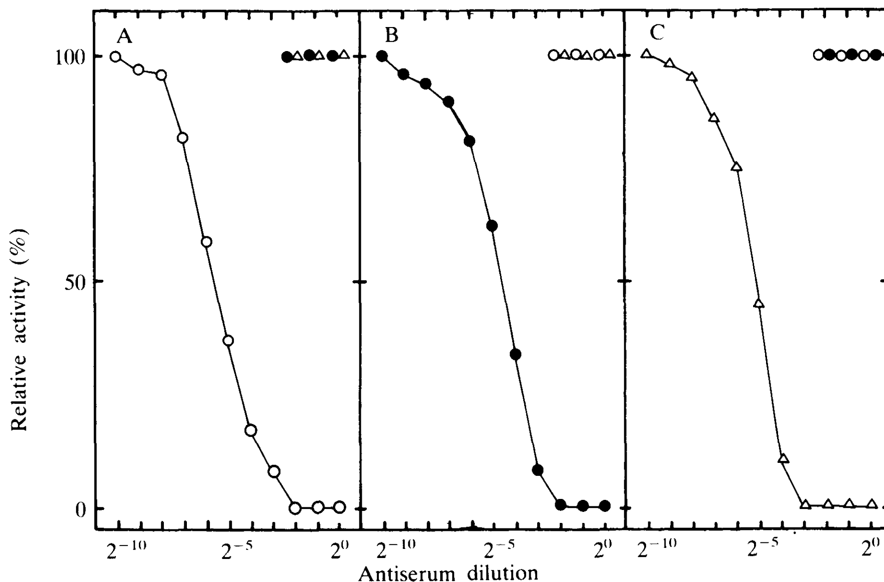


Fig. 1 Enzyme immunoinactivation test. To 0.5 m*l* aliquots of antisera (anti-AK1, A; anti-AK2, B; anti-AKe, C) diluted serially (2-fold) with 20 mM sodium phosphate buffer (pH 7.0) containing 150 mM NaCl and 0.1% bovine serum albumin, the same volumes of 2 units/m*l* enzymes (AK1, ○; AK2, ●; AKe, △) were added. The mixtures were allowed to stand for 24 hr at 4°C. The results are expressed as the percentage of the remaining activity.

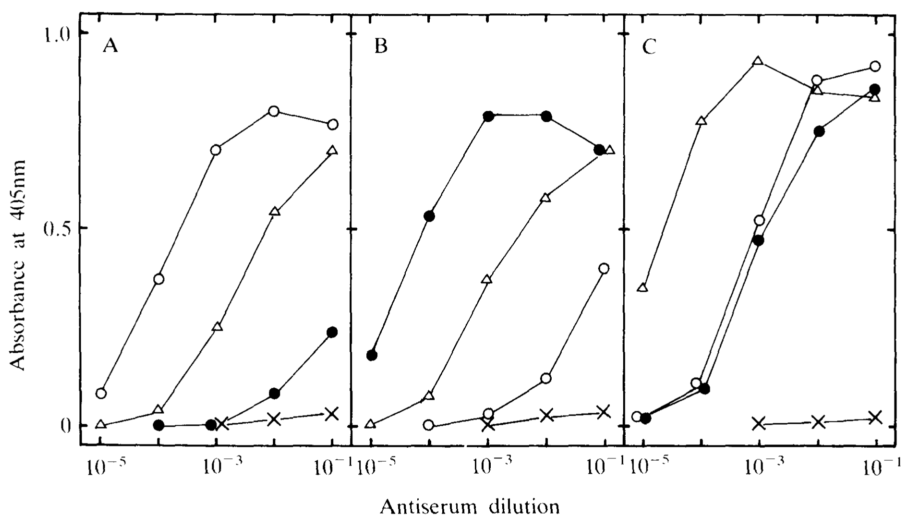


Fig. 2. Immunological cross-reactions between AKe, AK1, and AK2 by ELISA. 1.0 μg antigen (AKe, △; AK1, ○; AK2, ●; bovine serum albumin, ×) in 50 mM sodium carbonate buffer (pH 9.0) was added to each well of Immuno Plates II-96F (Nunc, Denmark) and kept at 4°C for 15 hr. After washing the wells with ELISA buffer (20 mM sodium phosphate, 150 mM NaCl, 0.1% Tween 20, 0.1% bovine serum albumin, pH 7.0), 100 μl of antiserum (anti-AK1, A; anti-AK2, B; anti-AKe, C) diluted serially (10-fold) with ELISA buffer was added to each well and the plates were incubated at 37°C for 2 hr. After washing with ELISA buffer again, 100 μl of 50 ng/ml alkaline phosphatase-labeled goat antibody to rabbit IgG (Kirkegaard & Perry Laboratories Inc., USA) was added and the plates were incubated at 37°C for 2 hr. After washing, 200 μl of 3.0 mM p-nitrophenyl phosphate dissolved in 0.1 M sodium glycine buffer (pH 10.0) containing 1.0 mM $MgSO_4$ and 1.0 mM $ZnSO_4$ was added to each well. The absorbance of p-nitrophenol released during incubation at 37°C for 30 min was determined at 405 nm with a Titertek Multiskan MC (Flow Laboratories Inc., USA).

*losis* used as an adjuvant in the other experiments did not react with AKe. No immunological cross-reaction between mammalian AK1 and AK2 was detected so far by Ouchterlony's double diffusion test or by the enzyme immunoinactivation test [17, 18]. Only slight cross-reactions were detected by highly sensitive ELISA (Fig. 2-A, B). These results indicate that the epitope(s) common to AKe and AK1 is different from that common to AKe and AK2.

The epitopes of porcine AK1 were identified by Koyama *et al.* [8]. The 3 epitopes corresponding to the chain segments Glu-Glu-Lys-Leu-Lys-Lys (2–7), Glu-Glu-Phe-Glu-Arg-Lys (103–108) and Glu-Glu-Thr-Ile-Lys-Lys (143–148) contain common amino acid sequences, Glu-Glu-X-X′-Lys (or Arg)-Lys, and are located on the surface of the molecule [14]. None of these sequences is conserved in AK2 [3], being responsible for little cross-reaction between AK1 and AK2. The primary structures of AK1s from porcine [6], human [16], rabbit[9] and calf[9] have been determined, and their homology is strikingly high (about 93%). Russell *et al.* [12] reported that antiserum against rabbit AK1 produced in guinea pig almost completely inhibited AK1s from mammals including human, rat, dog, and guinea pig as well as rabbit, indicating high conservation of antigenicity of AK1 in mammalian species. AKe has the sequence Glu-Glu-Thr-Val-Arg-Lys (161–166) corresponding to the sequence 143–148 of porcine AK1 [2, 6, 15], which has antigenic activity [8] and is conserved in AK1s from other mammalian species [9, 16]. It is therefore probable that the sequence 161–166 of AKe is involved in the cross-reaction with AK1. Antigenic sites of AKe and AK2 have not yet been determined. Elucidation of antigenic determinants in AKe and AK2 will lead to identification of the epitope(s) common to AKe and AK2.

## REFERENCES


1. Bârzu, O., and Michelson, S. 1983. *FEBS Lett.* 153: 280–284.
2. Brune, M., Schumann, R., and Wittinghofer, F. 1985. *Nucleic Acids Res.* 13: 7139–7151.
3. Frank, R., Trosin, M., Tomasselli, A. G., Noda, L., Krauth-Siegel, R. L., and Schirmer, R. H.

1986. *Eur. J. Biochem.* 154: 205–211.
4. Frank, R., Trosin, M., Tomasselli, A. G., Schulz, G. E., and Schirmer, R. H. 1984. *Eur. J. Biochem.* 141: 629–636.
5. Goelz, S. E., and Cronan, J. E. Jr. 1982. *Biochemistry* 21: 189–195.
6. Heil, A., Müller, G., Noda, L., Pinder, T., Schirmer, H., Schirmer, I., and Von Zabern, I. 1974. *Eur. J. Biochem.* 43: 131–144.
7. Holmes, R. K., and Singer, M. F. 1973. *J. Biol. Chem.* 248: 2014–2021.
8. Koyama, Y., Endo, S., Seki, T., Sato, N., and Shiokawa, H. 1983. *Mol. Immunol.* 20: 851–856.
9. Kuby, S. A., Palmieri, R. H., Frischat, A., Fischer, A. H., Wu, L. H., Maland, L., and Manship, M. 1984. *Biochemistry* 23: 2393–2399.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. *J. Biol. Chem.* 193: 265–275.
11. Noda, L. 1973. Adenylate kinase. pp. 279–305. *In*: The Enzymes, 3rd ed. vol. 8 (Boyer, P. D. ed.), Academic Press, New York.
12. Russell, P. J., Horenstein, J. M., Goins, L., Jones, D., and Laver, M. 1974. *J. Biol. Chem.* 249: 1874–1879.
13. Saint Girons, I., Gilles, A. M., Margarita, D., Michelson, S., Monnot, M., Fermandjian, S., Danchin, A., and Bârzu, O. 1987. *J. Biol. Chem.* 262: 622–629.
14. Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H. 1974. *Nature (Lond.)* 250: 120–123.
15. Schulz, G. E., Schiltz, E., Tomasselli, A. G., Frank, R., Brune, M., Wittinghofer, F., and Schirmer, R. H. 1986. *Eur. J. Biochem.* 161: 127–132.
16. Von Zabern, I., Wittmann-Liebold, B., Untucht-Grau, R., Schirmer, R. H., and Pai, E. F. 1976. *Eur. J. Biochem.* 68: 281–290.
17. Watanabe, K., Itakura, T., and Kubo, S. 1979. *J. Biochem. (Tokyo)* 85: 799–805.
18. Watanabe, K., Kinoshita, T., Kawai, N., Tashiro, N., Mori, T., Kubo, S., and Yamamoto, S. 1985. *Jpn. J. Vet. Sci.* 47: 63–72.

## 要　約

大腸菌および哺乳類アデニレートキナーゼ（AK）間の免疫学的交差反応（短報）：渡辺清隆・北垣貴央・山本晋二（北里大学獣医畜産学部獣医生理化学教室）——大腸菌 AK（AKe）とラットシトソール AK アイソザイム 1（AK1），または，ミトコンドリア AK アイソザイム 2（AK2）との間に，免疫学的交差反応が ELISA で検出されたが，酵素免疫阻害試験では検出されなかった．AK1 と AK2 との間の交差反応は，ELISA においてもほとんど検出されなかったことから、AKe と AK1 の共通抗原決定基は，AKe と AK2 のそれとは異なると考えられた．