BBA 66512

ISOENZYMES OF ADENYLATE KINASE IN HUMAN TISSUE

JOHN C. KHOO AND PERCY J. RUSSELL
Department of Biology, University of California, San Diego La Jolla, Calif. 92037 (U.S.A.)

(Received October 18th, 1971)

SUMMARY

A study of the isoenzymes of adenylate kinases (ATP:AMP phosphotransferases EC 2.7.4.3) from several tissues of human and rabbit indicated that there is a minimum of two sets of isoenzymes within an individual. Isoenzyme sets were distinguished by differences in inhibitions by $AgNO_3$ and an antiserum against rabbit muscle adenylate kinase. The adenylate kinases from muscle, erythrocytes and brain were similar and form one set of isoenzymes. The adenylate kinases of liver, kidney, spleen and heart were similar and form another set of isoenzymes.

The discovery of the isoenzymes of adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) in human erythrocytes and muscle[1,2] was shown to occur in three polymorphic forms, AK 1, AK 2, and AK 2-1 (ref. 2). Based upon the similarity of the isoenzyme electrophoretic patterns in erythrocytes and muscle, it has been

TABLE I

INHIBITION OF ADENYLATE KINASE FROM VARIOUS TISSUES BY ANTI-RABBIT MUSCLE ADENYLATE KINASE

The assay system for adenylate kinase was that of Adams[7]. Human tissues were homogenized in 50 mM Tris–HCl buffer (pH 7.5) in 1 mM mercaptoethanol and then sonicated for 2 min at 20 kcycles. Debris was removed by centrifguation at 600 000 $g \cdot$min. The preparation of anti-rabbit muscle adenylate kinase from guinea pigs and the inhibition assay were previously given[8,9].

Animal	*Tissue*	*Percent inhibition*
Rabbit	Skeletal muscle	95
	Erythrocytes	93
	Brain	88
	Liver	0
	Kidney	0
	Eye lens	0
Human	Skeletal muscle	94
	Erythrocytes	94
	Brain	83
	Heart	35
	Liver	22
	Spleen	54

suggested that the isoenzymes of adenylate kinase from various tissues within an individual were identical[1-3]. This paper shows that the adenylate kinase from various human tissues occurs as at least two sets of adenylate kinase isoenzyme patterns from a single individual. Table I shows the degree of inhibition of adenylate kinase from various tissues by guinea pig antiserum against rabbit muscle adenylate kinase. The dilution of the antiserum was adjusted so that just less than 100% inhibition was obtained for the homologous system. The adenylate kinase from rabbit liver, rabbit kidney and rabbit eye lens were not inhibited by the antiserum. The adenylate kinase from human heart, liver and spleen were not inhibited completely even in antiserum excess and were further investigated by starch gel electrophoresis. Fig. 1 shows the pattern of adenylate kinase isoenzymes from human tissues. The pattern from brain (d) is similar to that of erythrocytes (a) and muscle (not shown). The patterns of liver (b), kidney (c) and spleen (e) are similar to one another but differ from (a) and (d). Fig. 2 shows the pattern of adenylate kinase isoenzymes from erythrocytes (a)

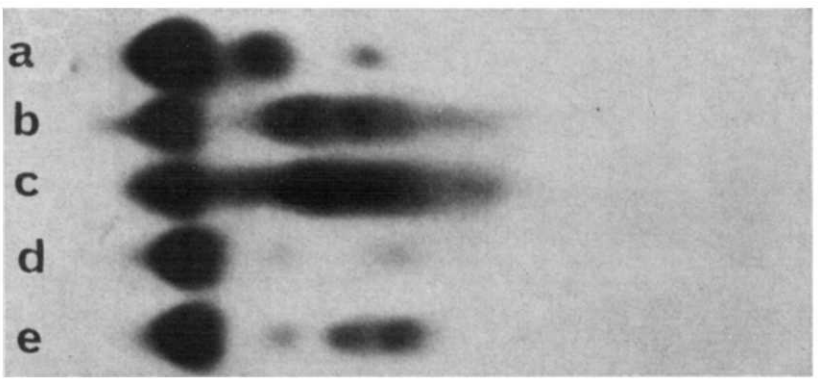

Fig. 1. Starch gel electrophoretic patterns of adenylate kinase isoenzymes from human tissue, phenotype AK 1 (ref. 2); a, erythrocytes; b, liver; c, kidney; d, brain and e, spleen. The bridge buffer was 0.1 M Tris–citrate, pH 8.6, and the gel buffer was 0.02 M Tris–citrate, pH 8.6. Electrophoresis was carried out at 250 V, 12.5 mA and 4 °C for 16 h. The staining solution for detecting the adenylate kinase activity was the same as that of Fildes and Harris[1].

and the inhibitions by a 5-fold excess of anti-rabbit muscle adenylate kinase (b). Some of the adenylate kinase isoenzymes of liver (c) and heart (e) were not inhibited by excess antiserum, as shown by (d) and (f) in Fig. 2. The two most cathodal isoenzymes of heart adenylate kinase from another individual of the phenotype AK 2-1 (ref. 2) were the only isoenzymes inhibited by the antiserum. The patterns and reactions of kidney adenylate kinase (not shown) were similar in all respects to heart adenylate kinase and liver adenylate kinase.

Muscle adenylate kinase is inhibited by sulfhydryl reagents[4] and liver adenylate kinase is not[5,6]. Thus, $AgNO_3$ inhibits muscle adenylate kinase, but was used as a purification step for liver adenylate kinase[5]. Fig. 3 shows the results of treatment of human adenylate kinase with 1 mM $AgNO_3$. All of the muscle (a) isoenzymes were inhibited, whereas only the most cathodal adenylate kinase isoenzymes of heart (b), liver (c) and kidney (d) were inhibited. The inhibition patterns were similar to those with the antiserum.

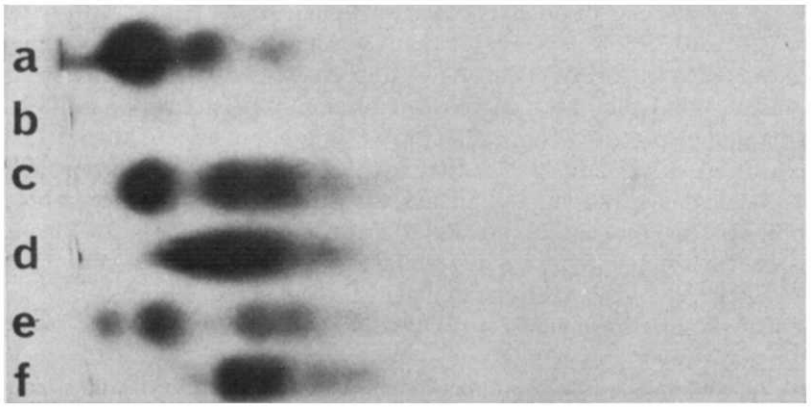

Fig. 2. Lack of inhibition of some adenylate kinase isoenzymes from human liver and heart by excess antiserum. The conditions were the same as in Fig. 1. a, Erythrocytes (phenotype AK 1); b, erythrocytes (phenotype AK 1) *plus* antiserum; c, liver (phenotype AK 1); d, liver (phenotype AK 1) *plus* antiserum; e, heart (phenotype AK 2-1); f, heart (phenotype AK 2-1) *plus* antiserum.

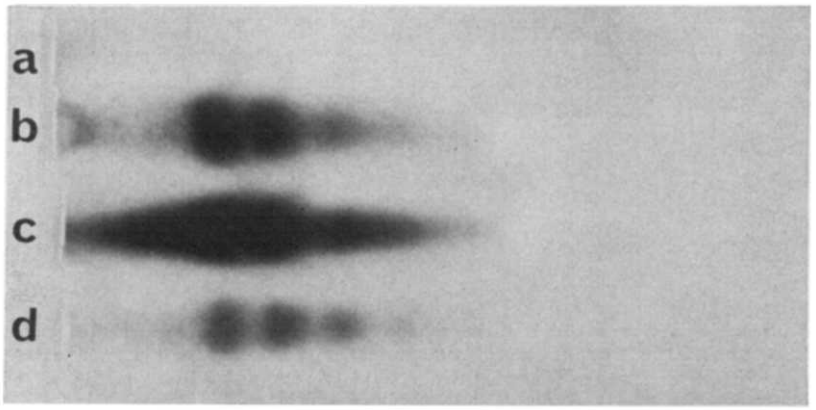

Fig. 3. Lack of inhibition of some adenylate kinase isoenzymes of human adenylate kinase by $AgNO_3$. The tissue extracts were incubated for 10 min at 0°C in 1 mM $AgNO_3$ and then subjected to electrophoresis. The extracts shown are a, muscle *plus* $AgNO_3$; b, heart *plus* $AgNO_3$; c, liver *plus* $AgNO_3$ and d, kidney *plus* $AgNO_3$.

The view for the identity of adenylate kinase isoenzyme sets in all tissues within an individual[1–3] does not appear to be correct. A minimum of two types of adenylate kinase isoenzyme patterns were distinguished by electrophoresis, by inhibitions with antiserum and by inhibitions with $AgNO_3$. The importance of the buffer systems used in the starch gel electrophoresis cannot be overemphasized. The different sets of adenylate kinase isoenzymes may have direct application in the detection of organ specific dysfunctions and in studies of tissue differentiation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr N. O. Kaplan for his criticisms of this work.

This work was supported in part by the American Cancer Society Institutional Grant No. IN-93.

REFERENCES

1 R. A. Fildes and H. Harris, *Nature*, 209 (1966) 261.
2 H. Harris, *Cancer Res.*, 26 (1966) 2054.
3 D. J. H. Brock, *Biochem. Genet.*, 4 (1970) 617.
4 T. A. Mahowald, E. A. Noltmann and S. A. Kuby, *J. Biol. Chem.*, 237 (1962) 1535.
5 M. Chiga and G. W. E. Plaut, *J. Biol. Chem.*, 235 (1960) 3260.
6 F. S. Markland and C. L. Wadkins, *J. Biol. Chem.*, 241 (1965) 4124.
7 H. Adams, in H. U. Bergmeyer, *Methods of Enzymatic Analysis*, Academic Press, New York, 1965, p. 539.
8 S. Minatogawa and P. J. Russell, *Biochem. Biophys. Res. Commun.*, 31 (1968) 48.
9 J. C. Khoo and P. J. Russell, *Biochim. Biophys. Acta*, 220 (1970) 239.