IMMUNOLOGICAL CROSS-REACTION BETWEEN EUKARYOTE AND PROKARYOTE PYRUVATE KINASE

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1. Introduction

The glycolytic enzyme, pyruvate kinase (ATP: pyruvate phosphotransferase; EC 2.7.1.40), from *Escherichia coli* cross-reacts immunologically with the enzyme from man. Since the divergence of eukaryotes and prokaryotes is estimated to have occurred 1.4 billion years ago [1], this demonstrates remarkable stability of the immunological determinants and the responsible structural features in this enzyme during evolution. The detection of similar molecular structures in *E. coli* and man provides additional support for the concept of unity of life at the molecular level [2].

2. Materials and methods

Antisera against the human muscle isozyme [3] the human erythrocyte isozyme [4] and the bovine muscle isozyme [5] were used as probes for immunological similarities. We have previously shown that the human muscle and human erythrocyte isozymes do not cross-react using either anti-human erythrocyte or anti-human muscle isozyme antisera [6]. The anti-human muscle isozyme antiserum cross-reacts with the bovine muscle isozyme while the erythrocyte isozyme antiserum does not. The probes used can distinguish differences between mammalian pyruvate kinase isozymes.

The human erythrocyte pyruvate kinase used was a partially purified preparation [7]. The purification of the human muscle isozyme has been described elsewhere [3].

E. coli cells were prepared with the growth medium described by Waygood and Sanwal [8] from the stock culture collection of the Department of Microbiology and Immunology. The cells were harvested by centrifugation and then sonicated after the pellets were resuspended in cold 2.5 mM NaH_2PO_4, 2.5 mM KH_2PO_4, pH 7.5, 1 mM K_2EDTA and 10 mM 2-mercaptoethanol. The two pyruvate kinase isozymes in *E. coli* were separated by DEAE-cellulose (Whatman DE52) chromatography [9]. The isozyme eluting first is responsive to media changes and is activated by fructose-1, 6-diphosphate whereas the second isozyme is constitutive [9] and is activated by 5′-AMP and other organic monophosphates [10].

A yeast supernatant with pyruvate kinase activity was prepared as described by Hunsley and Suettler [11].

3. Results and discussion

The unfractionated *E. coli* supernatant formed precipitin lines on immunodiffusion with all three anti-mammalian enzyme antisera (fig.1).

Following separation of the two *E. coli* pyruvate kinase activities, enzyme inactivation experiments were performed. The inducible enzyme was not inactivated by any of the three antisera. The constitutive enzyme was partially inactivated by the antisera against the two human isozymes but was not inactivated by anti-bovine muscle pyruvate kinase (fig.2) which indicates that the inactivation by the two anti-human antisera is not due to unrelated factors common to all rabbit sera. The lack of inactivation by anti-bovine muscle isozyme antiserum is in accord with other data indicating that

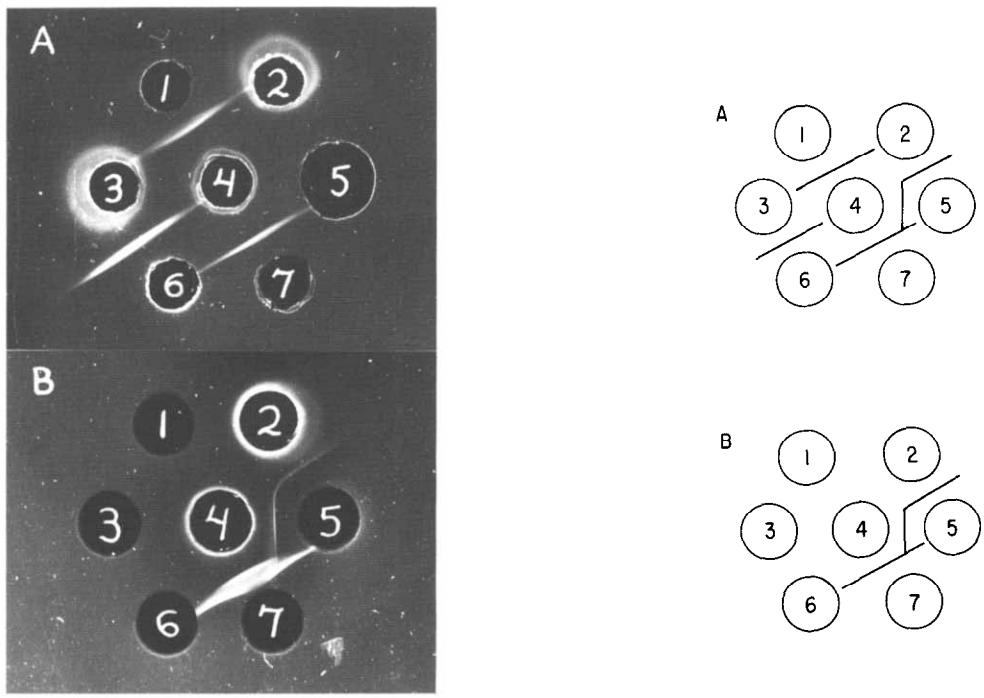

Fig.1. Gel immunodiffusion analysis of *E. coli* supernatant with the three antisera. The method and conditions were as described by Campbell et al. [17]. (A) The wells contained: 1 and 7, human muscle pyruvate kinase, 32 units/ml; 2 and 3, anti-human erythrocyte pyruvate kinase; 4, anti-human muscle pyruvate kinase; 5, *E. coli* supernatant, 0.04 units/ml; 6, human erythrocyte pyruvate kinase, 3.0 units/ml. (B) The wells contained; 1, 3 and 6, empty; 2, anti-bovine muscle pyruvate kinase; 4, anti-human muscle pyruvate kinase; 5, *E. coli* supernatant, 2.52 units/ml; 7, human muscle pyruvate kinase, 32 units/ml.

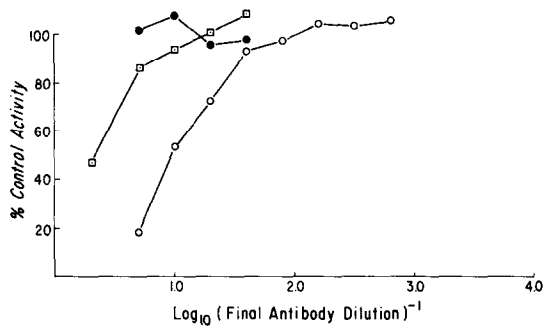

Fig.2. Each of the two *E. coli* pyruvate kinase isozymes was tested for antibody inactivation by the three antisera [6]. For the constitutive enzyme present in the second peak from the DEAE-cellulose column, 1 mM AMP was added to the assay mix and the activity was adjusted to 0.26 units/ml. The figure shows the constitutive enzyme with: anti-human muscle pyruvate kinase (○); anti-human erythrocyte pyruvate kinase (□); anti-bovine muscle pyruvate kinase (●).

this reagent detects some antigenic determinants undetectable by anti-human muscle isozyme antiserum and vice versa (unpublished).

Similar experiments with the yeast supernatant showed no interaction with any of the antisera.

The amino acid sequence studies on D-glyceraldehyde 3-phosphate dehydrogenase have permitted sequence comparisons of a prokaryote and eukaryote glycolytic enzymes [12]. The lobster and pig enzymes show 70% residue identity, lobster and yeast 68% while there is 51% identity between the sequences of the lobster and *Bacillus stearothermophilus*.

Immunological studies of cytochrome *c* [13] and lysozyme [14] from different species indicate that immunological cross-reactivity disappears when amino acid sequences differ by 30–40%. The immunological cross-reactivity between *E. coli* and mammalian pyruvate kinase, thus, implies considerable sequence homology between the isozymes with the homology likely to be

in the same range as that observed for D-glyceraldehyde 3-phosphate dehydrogenase. The human erythrocyte and muscle isozymes have evolved to gene products sufficiently different so that the proteins do not present readily detectable cross-reaction, however, each apparently retains some of the ancestral immunological determinants present on the constitutive *E. coli* isozyme.

Cohen et al. [15] have proposed that the central role of the glycolytic pathway in the metabolism of the mature red cell has restricted the evolutionary process acting on erythrocyte glycolytic enzymes. Mutations are highly likely to be detrimental and, therefore, selected against. It is interesting to note that it is the constitutive isozyme in *E. coli* which retains determinants recognized by both human antisera. By similar reasoning, the basic necessity for a functional constitutive enzyme is likely to restrict acceptable mutations and cause a slow rate of evolution. From comparisons of amino acid compositions we have suggested that the rate of divergent evolution of the pyruvate kinase isozymes has been slower than that observed for cytochrome *c* [16]. The immunological evidence presented here is consistent with that idea. Convergent evolution to give the same results seem less likely. DNA exchange between eukaryote and prokaryote, while topical, would be a rather unlikely explanation.

Acknowledgements

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