IMMUNOLOGICAL RELATEDNESS OF TWO ISOZYMES OF 3-PHOSPHOGLYCERATE KINASE FROM THE MOUSE

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Received 1 September 1978

1. Introduction

Two isozymes of 3-phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3, PGK) are known to be present in mammalian tissues. PGK-1** is found in all somatic tissues, and its structural locus is X-linked in humans [1], kangaroos [2] and mice [3]. PGK-2 has been detected only in the sperm and testis of a number of mammals; however, there are exceptions where it has been found in somatic cells (kangaroo, dog, fox [4]). Alleles determining electrophoretic variation map to chromosome 17 in the mouse [5]. These two isozymes were purified in our laboratory, and their biochemical properties compared [6]. They were highly similar in their biochemical properties even though the two loci must have been separated for over 100 million years [7]. To further explore the relatedness of these isozymes, antisera against the two PGK isozymes were produced separately, and their immunological properties were compared. The results of these studies suggest some antigenic relatedness between these two PGK isozymes.

2. Materials and methods

Initial immunizations with PGK-1 and PGK-2 utilized 250 μg purified protein emulsified with Freund's adjuvant, complete for the first infection and incomplete thereafter. Intervals of 1–2 weeks occurred between injections and the antisera used were obtained after 4–6 injections. The serum to PGK-2 obtained by this procedure will be referred to as 'serum I'. An alternate procedure was also used, consisting of injecting single acrylamide gel slices after electrophoresis of purified PGK-2 [8], 'serum II'. Since both serum I and II showed unexpected cross-reactivity with liver and kidney homogenates, a third immunization scheme with PGK-2 obtained from a different preparation was performed. PGK-2, 60–80 μg, in Freund's adjuvants were injected on day 1, 8, 15, 22 and 29 and sera was obtained 1 week later, 'serum III'. In order to eliminate PGK activity in the sera, they were heated to 60°C for 10 min before being used for immunoinactivation experiments.

To test the specificity and cross-reactivity of the antisera for the PGK isozymes, aliquots of crude muscle homogenate (1:5, w/v, 0.1 M potassium phosphate, pH 6.5) containing PGK-1 or partially purified PGK-2 from mouse testis, both with a constant amount of PGK activity, were incubated with increasing amounts of serum in final vol. 150 μl for 30 min at room temperature, followed by 1 h at 4°C. The samples were then immediately assayed for residual PGK activity as in [6]. The incubation mixture contained varying volumes of a BSA solution (57 mg/ml, in 0.1 M potassium phosphate, pH 7.4) in order to obtain a constant protein concentration. Double immunodiffusion tests were by standard methods [9]. Immunoelectrophoresis was in barbital buffer (pH 8.6), in 1.1% agar.

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** PGK-1 and PGK-2 have also been called [6] PGK-A and PGK-B, respectively. However, the former nomenclature is preferred by mouse geneticists

3. Results

3.1. *Studies with anti-PGK-1 serum*

In double-immunodiffusion tests, the antiserum against PGK-1 showed a single precipitation band with pure PGK-1. Muscle, liver kidney and testicle homogenates and red blood cell hemolysates also gave single precipitin lines that fused with the precipitin bands obtained with the pure PGK-1, indicating total identity of the precipitating material in all the samples. This was expected, because PGK-1 is known to be present in all these tissues. No precipitation was detected with pure PGK-2.

The immunoinactivation studies revealed that anti-PGK-1 serum obtained from bleeding the rabbit at different times inactivated PGK-1 to the same degree (see fig.1). The sera did not cause total inactivation of PGK-1 activity. Anti-PGK-1 serum, when incubated with PGK-2, produced ~50% inactivation of enzyme activity (fig.2). Therefore anti-PGK-1 serum, although it does not form precipitin bands with PGK-2, does inactivate PGK-2 in the immunoactivation experiment. This observation suggests that PGK-2 cross-reacts with anti-PGK-1 serum by forming soluble complexes.

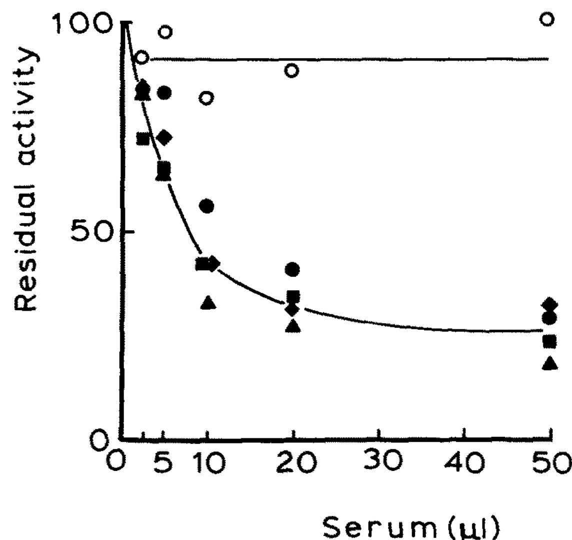

Fig.1. Immunoinactivation of PGK-1 by sera obtained from different bleedings. (○) PGK-1 + non-immune serum; (●,▲,■,◆) PGK-1 + anti-PGK-1 antisera from 4 different bleedings. PGK-1, ~60 ng, were used for the incubation.

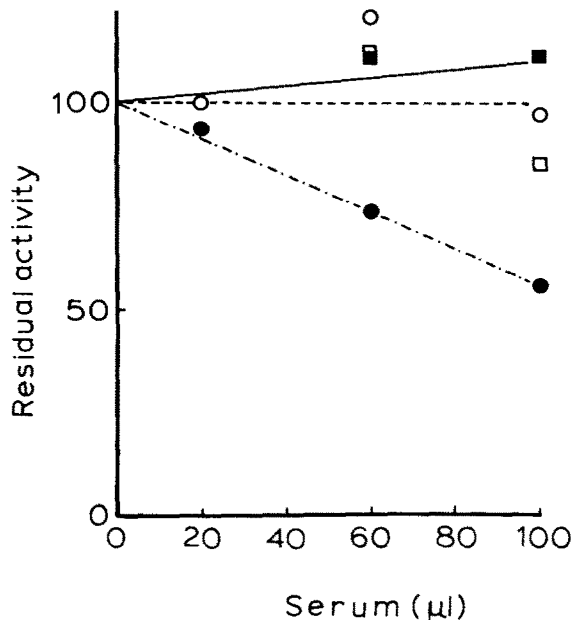

Fig.2. Cross-reactivity of anti-PGK antisera. (●) anti-PGK-1 + PGK-2; (■) anti-PGK-2 (serum II) + PGK-1; (○) PGK-1 + non-immune serum; (□) PGK-2 + non-immune serum. The values given are the average of duplicates of two simultaneous tests. The enzymes, ~60 ng, were used for the incubation.

3.2. *Studies with anti-PGK-2 sera*

All sera raised against PGK-2 gave precipitation bands with pure PGK-2 and testis homogenates. None of the anti-PGK-2 sera gave precipitin bands with pure PGK-1, mouse serum, or mouse erythrocytes. In immunoinactivation experiments, anti-PGK-2 serum II caused 50% inactivation of the enzyme activity, whereas serum I and serum III inactivated >90% of the initial PGK-2 enzyme activity (fig.3). Serum III was 30-times more effective/unit volume in the inactivation of PGK-2. Cross-reactivity studies revealed that anti-PGK-2 sera did not inactivate PGK-1, even when incubated with an excess amount of serum (fig.2).

Precipitation arcs were also detected between the anti-PGK-2 and liver and kidney homogenates (fig.4). The precipitation arcs fused with the band obtained with PGK-2, although there appeared to be a spur of PGK-2 over the liver and kidney arcs. Immunoelectrophoresis using either serum I or II disclosed a single arc of low mobility in testes. An arc of identical

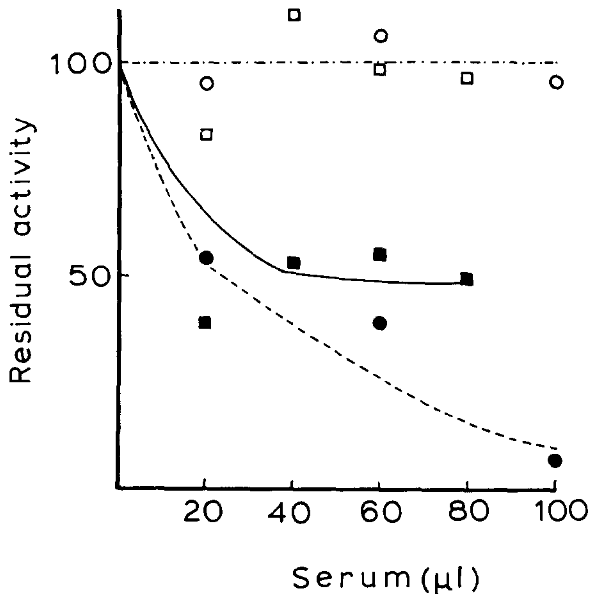

Fig.3. Immunoinactivation of PGK-2. (○,□) PGK-2 + non-immune serum; (●) PGK-2 + serum I; (■) PGK-2 + serum II. PGK-2, ~60 ng, were used for the incubation. The values given are the average of duplicates of two simultaneous tests.

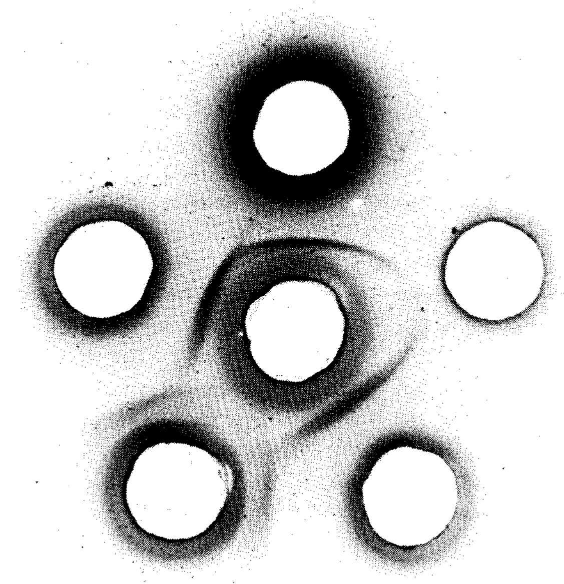

Fig.4. Stained immunodiffusion plate of the reaction of anti-PGK-2 with tissue extracts. Center well: anti-PGK-2 III; 12:00 o'clock: liver extracts; 2:00 o'clock: kidney extract; 5:00 o'clock: testes extract; 7:00 o'clock: anti-PGK-2 I; 10:00 o'clock: testes extract.

mobility was present in liver and kidney on immunoelectrophoresis; arcs of higher mobility which fused with the low mobility arc (wings) were sometimes seen. Spurs of the secondary arc found in liver and kidney over the primary arc could be seen with serum II. Serum III only revealed a PGK-2-like arc on immunoelectrophoresis of liver. When both serum I and serum II were incubated with liver homogenate, no inactivation of PGK activity was observed, even at high serum concentrations. Nonetheless, massive adsorption of both serum I and serum II with liver extracts removed the ability of these antisera to precipitate PGK-2.

4. Discussion

The high degree of similarity in biochemical properties between PGK-1 and PGK-2 suggests that their protein structures are similar and antigenic cross-reactivity might be found. However, PGK-2 is controlled by an autosomal locus, while PGK-1 is X-linked. Since the mammalian X-chromosome has been highly stable in evolution [7], the duplication of an hypothetical ancestral locus must have occurred early in mammalian evolution. Thus, the two loci might have acquired many changes in base sequence while maintaining the same function. Our immunological results which reveal a weak (detectable by enzyme inactivation but not by precipitation) reaction of antiserum to PGK-1 with PGK-2 but no reaction of the antisera to PGK-2 with PGK-1 support the notion of considerable sequence diversity in these two proteins but, also, the presence of definite cross-reactivity. An antibody to human PGK-1 did not, cross-react with human PGK-2 in immunoinactivation [10].

We were surprised to discover a cross-reaction of the antisera to PGK-2 with tissue extracts other than testis. This seemed not to be due to a contaminating antibody as:

(1) Function of the precipitation arcs between the antisera and the two extracts occurred;
(2) Antiserum to more highly purified (disc gel slices) PGK-2 gave the same result.

Immunoelectrophoretic analyses of the tissue extracts from other organs were different with the different sera but disclosed a band migrating similarly to PGK-2. This possibly represents PGK-2 since antiserum I inhibits a small amount of total kidney PGK. The simplest explanation is that PGK-2 is present in other organs in mice as in other species, immunological methods being more sensitive than electrophoresis for detecting small amounts of a protein. Immunoelectrophoresis with some antisera detected another band with electrophoretic mobility different than that of PGK-2. The arcs formed by the two immunoelectrophoretic bands fuse with variable spurring. This result suggests that the antisera detect a similar antigenic determinant on a different molecule. The electrophoretically distinct, cross-reacting component could represent different multimers of the same monomer, a proenzyme or a functionally unrelated protein.

Acknowledgements

This work was supported in part by grant GM-15419 from NIH. We thank Ken Harper for excellent technical assistance.

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