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# SPECIFIC ACTIVITIES OF 6-PHOSPHOGLUCONATE DEHYDROGENASE, GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND GLUCOSE-6-PHOSPHATE ISOMERASE DURING *BUFO BUFO* DEVELOPMENT

MICHELE MIRANDA

*Istituto di Biologia Generale, Università dell'Aquila, Via Assergi 6, 67100 L'Aquila (Italy)*

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## Summary

It has been suggested by some authors that during amphibian development, due to the higher glucose-6-phosphate dehydrogenase (EC 1 1 1.49) activity compared to that of 6-phosphogluconate dehydrogenase (EC 1.1.1 43), 6-phosphogluconate could accumulate in the embryo tissues and regulate the channelling of glucose-6-phosphate into glycolysis Here, on the base of the specific activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glucose-6-phosphate isomerase (EC 5 3.1.9) found in the embryos of *Bufo bufo* during development, it is discussed whether 6-phosphogluconate can accumulate and play a regulative role on glucose-6-phosphate metabolism in the anuran embryo

## Introduction

The egg and the embryo of amphibia carry out an active glycolysis [1,2] and show detectable activities of glucose-6-phosphate dehydrogenase (EC 1 1 1 49) and 6-phosphogluconate dehydrogenase (EC 1 1 1 43) [3—5]

The homogenates of *Bufo bufo* and *Rana esculenta* oocytes metabolize 6-phosphogluconate and ribulose-5-phosphate to lactate [2], which would indicate that the whole pentose phosphate cycle is carried out by such biological materials

The pentose phosphate cycle plays a major role during embryonic development, where active syntheses of nucleic acids, lipids and proteins occur, by furnishing pentoses, intermediates for aminoacid biosynthesis and NADPH for reductive processes

During the embryonic development of *Rana pipiens* [3,4], of the sea urchin [6,7] and of *Ascaris lumbricoides* [8], after fertilization, there is an increase in glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase

activity, moreover, during chick development, the fluctuations of glucose-6-phosphate dehydrogenase activity have been related to cell proliferation and to the DNA content of the embryo [9]

During the development of chick brain [10], from stage 4 to stage 20, the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase fluctuate and the former is about twice the latter

Wallace [3] found that homogenates of embryos of developping *Rana pipiens* showed increasing glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity, to a ratio of 10 to 1, respectively. The same author [3] suggested that, due to the lower 6-phosphogluconate dehydrogenase activity, as compared to the glucose-6-phosphate dehydrogenase activity in the embryo, 6-phosphogluconate, which is a powerful inhibitor of glucose-6-phosphate isomerase [11,12], would accumulate and regulate glucose-6-phosphate partitioning by modulating glucose-6-phosphate isomerase activity But in the cytosol of *Bufo bufo* and *Rana esculenta* oocytes [2], it has been shown that 6-phosphogluconate does not inhibit lactate production from glucose-6-phosphate, in rat kidney [13], the administration of 6-amino-nicotinamide, an inhibitor of 6-phosphogluconate dehydrogenase, does not lower lactate yield from glycolysis

Since the assay conditions used by Wallace [3] to detect 6-phosphogluconate dehydrogenase were somewhat, different from those used by more recent authors [14—16] and, moreover, it seemed antifinalistic that cells in active proliferation were under glycolytic block, it was decided by the author of the present work to detect 6-phosphogluconate dehydrogenase activity during *Bufo bufo* development Glucose-6-phosphate isomerase and glucose-6-phosphate dehydrogenase activities also were assayed, due to the close relationship with glucose-6-phosphate metabolism and 6-phosphogluconate dehydrogenase

The assays of 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase were carried out on the cytosols prepared from *Bufo bufo* embryos, as described in Materials and Methods, to avoid NADPH reoxidation by respiratory chain or microsomes In Wallace's work [3], where embryo homogenates were used, no mention is made about the addition of inhibitors of mitochondrial respiratory chain to the reaction mixtures to prevent NADPH reoxidation

## Materials and Methods

### *Embryos*

*Bufo bufo* adults in amplexus were captured near Rome Ovulation and fertilization occurred in the laboratory The eggs were cultured at 12° C in tap water Jelly was removed from early embryonic stages by treatment with 4% sodium-thioglycolate at pH 8 60 in a ratio of 1 1 (v/v) The embryos of *Bufo bufo* were staged by reference to Rugh [17]

### *Cytosol preparation*

At each selected stage, 200 embryos, after three washes in 0 10 M triethanolamine at pH 7 60, were suspended in the same medium up to a volume of 5 ml and homogenized with a Potter homogenizer at 4° C The homogenate was then

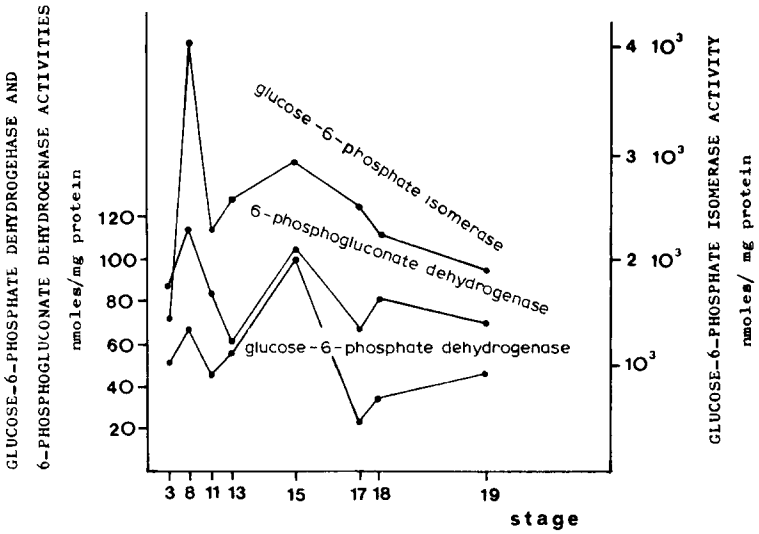


Fig 1 Specific activities of 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase in the cytosol from *Bufo bufo* embryos during development At each selected stage, 200 embryos were washed three times with 0 10 M triethanolamine at pH 7 60, then suspended in the same medium up to a volume of 5 ml and homogenized Homogenate was centrifuged at 110 000 × *g* for 120 min Supernatant collected by a syringe was used for enzymatic assays Enzymatic assays were carried out spectrophotometrically at 30°C For reaction mixtures, see the text Proteins were detected by biuret method Values plotted are means of duplicate, triplicate or quadruplicate assays of the same cytosol S E never exceeded 5% of the mean

centrifuged at 110 000 × *g* for 120 min in a SW 50 rotor with a Spinco Beckman L-50 centrifuge After the centrifugation the cytosol (supernatant) was collected from the tube with a syringe, to avoid contamination by the pellet and upper lipidic layer

*Enzymatic assays*

Enzymatic activities were detected by a Perkin Elmer Model 356 recording spectrophotometer, at 30° C, following the $NADP^+$ reduction at 340 nm Kinetics were of zero order with respect to substrate, cofactor and ion concentrations The reaction mixtures for 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase were according to Bergmeyer [16]

*Protein assay*

The protein content of the cytosols was detected by the method of biuret, treating samples with 1% sodium-deoxycholate in a ratio of 1 1 (v/v)

*Reagents*

Reagents were purchased from Sigma and Boehringer.

## Results and Discussion

As it is shown in Fig 1 the specific activities of glucose-6-phosphate isomerase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogen-

ase gradually increase from stage 3 to stage 8, when a maximum is reached After stage 8, glucose-6-phosphate isomerase and glucose-6-phosphate dehydrogenase specific activities decrease very fast until stage 11, while 6-phosphogluconate dehydrogenase reaches a minimum at stage 13 After the respective minima have been reached, the three enzymatic activities increase again up to stage 15, where new maxima are located, and then again decrease From Fig 1 a close parallelism is also evident, between the developmental curves of the three specific activities, which would suggest a coordinate expression of the three enzymes studied here Also in rat uterus [18], during pregnancy, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glycolytic enzymes are parallelly expressed

Fig 1 shows that glucose-6-phosphate isomerase activity is enormously higher than glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities and the latter is always higher than the former during all the early development

Assays of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities have been carried, at some developmental stages, on the in toto homogenate by the author (unpublished results) to rule out differences with respect to the cytosol, and the 6-phosphogluconate dehydrogenase/glucose-6-phosphate dehydrogenase ratios were substantially the same both in cytosols and in in toto homogenates in the presence of KCN, rotenone or antimycin A

On the basis of the findings shown in Fig 1, it is suggested that the 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase activities found by Wallace [3] during *Rana pipiens* development could be explained by species differences or by the different experimental conditions used Moreover Broyles and Strittmatter [4] found that during *Rana pipiens* development

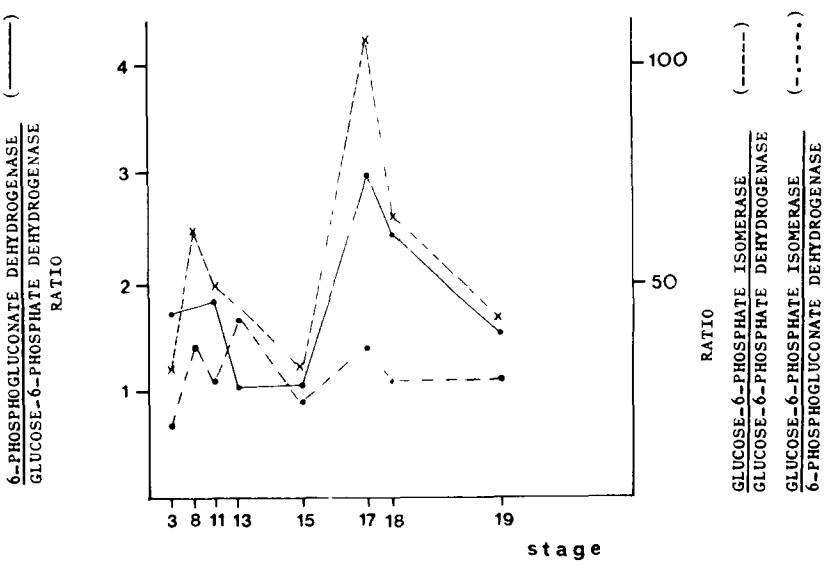


Fig 2 Ratios of glucose-6-phosphate isomerase, 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase specific activities in the cytosol from *Bufo bufo* embryos during development

glucose-6-phosphate dehydrogenase/6-phosphogluconate dehydrogenase ratios differed from those found by Wallace [3].

The maximal activities of the three enzymes shown in Fig. 1 could be related to two maxima of cell proliferation and DNA synthesis [9], the first at the blastula stage, the second at rotation. Tentatively, at stages 8 and 15, there would be maximal expression of the genes codifying for glucose-6-phosphate isomerase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase

Fig 2 shows that the findings in *Rana pipiens* by Wallace [3] and Broyles and Strittmatter [4] differ from ours during *Bufo bufo* development, the 6-phosphogluconate dehydrogenase/glucose-6-phosphate dehydrogenase ratio being always higher than 1 and becoming about 3 at stage 17 Then, since 6-phosphogluconate dehydrogenase activity is, during *Bufo bufo* development, higher than that of glucose-6-phosphate dehydrogenase, it seems unlikely that 6-phosphogluconate be able to accumulate and inhibit glucose-6-phosphate isomerase, so lowering glucose-6-phosphate channelling into glycolysis

As it is shown in Fig 2 glucose-6-phosphate isomerase activity is so many times higher than the 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase activities that, tentatively, the high glucose-6-phosphate isomerase activity in *Bufo bufo* embryo and in almost all animal and plant tissues could be a physiological defence against glucose-6-phosphate isomerase inhibition

The author, on the basis of the findings discussed in the present work, suggests that it is unlikely that 6-phosphogluconate can accumulate and regulate glucose-6-phosphate metabolism during anurans development where glycolysis, like in almost all the other biological materials, is responsive to more sophisticated regulatory mechanism based on energy charge and citrate level [2].

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