Phosphoglycerate mutase isozymes in the lower vertebrates

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Summary. The phosphoglycerate mutase isozyme patterns from cyclostomata, cartilaginous and bony fish were analyzed. The observed patterns indicate that a duplication of a single ancestral gene coding for phosphoglycerate mutase took place during the evolution of the gnathostome vertebrates.
Key words. Phosphoglycerate mutase isozymes; evolution; gene duplication.

Phosphoglycerate mutase (PGAM EC 2.7.5.3) catalyzes the reversible reaction: 3-phosphoglycerate+2,3-bisphosphoglycerate $\rightleftharpoons$2-phosphoglycerate+2,3-bisphosphoglycerate. Three PGAM isozymes exist in mammalian tissues[1-3] which result from homodimeric and heterodimeric aggregation[4] of two subunits encoded by two distinct genes[5,6]. In most tissues, including brain, liver, spleen and erythrocytes a fast-migrating isozyme (AA) is found, composed of two identical subunits produced by the gene PGAMA. In muscle a slow-moving isozyme (BB, gene PGAMB) occurs and only in heart muscle and developing skeletal muscle is the heterodimer AB observed. The isozymes and genes are named in accordance with the guidelines for Human Gene Nomenclature[6]. An extensive survey of PGAM isozyme patterns in other vertebrate classes showed that the tissue-specific distribution is quite conservative during vertebrate evolution[7]; birds, however, have only one enzyme band in all tissues[7,8]. The only vertebrate class where so far no data have been reported is the cyclostomata.

Materials and methods. The following species were examined: Lamprey *(Lampetra planeri, Lampetra fluviatilis)*, slime eel *(Myxine glutinosa)*, shark *(Scyliorhinus caniculus, Squalus acanthias)*, ray *(Raja batoides)*, eel *(Anguilla anguilla)*, trout *(Salmo gairdneri)*, and bream *(Abramis brama)*. After removal of heart, liver, kidney, spleen, brain and muscle, the tissues were homogenized in a three-fold volume of 100 mM triethanolamine buffer, pH 7.6, containing 0.4 mM dithioerythritol, 4 mM EDTA and bovine serum albumin in a concentration of 1.0 mg/ml. The tissue extracts were centrifuged for 20 min at 42,000 × g, and after appropriate dilution with extraction buffer an equal volume of 97% glyerol was added. All steps were carried out at 0–4 °C. Cellulose acetate gel electrophoresis was carried out at 200 V for 90 min at 18 °C. For PGAM-specific staining the following assay mixture was used; it contained, in a final volume of 560 µl: 89 mM triethanolamine, pH 7.6, 8.9 mM $MgSO_4$, 0.5 mM NADH, 12.6 mM 3-phosphoglycerate, 0.49 mM 2,3-bisphosphoglycerate, 0.97 mM ADP, 0.52 U enolase, 1.17 U pyruvate kinase, 3.32 U lactate dehydrogenase. For further details on cellulose acetate electrophoresis see Bücher et al.[9].

Results and discussion. Eel tissues show the conservative PGAM isozyme pattern common to most vertebrate classes, e.g. mammals, reptiles, amphibians and most bony fish[7]. In bream a more complicated pattern occurs, consisting of six isozymes, whereas in trout tissues a set of four isozymes is observed after electrophoresis (fig. 1). Three possible explanations for the occurrence of the additional isozymes might be given; namely, posttranslational modification, heterozygosity at PGAMA and/or PGAMB, or multiplication of genes combined with subsequent independent evolution of the distinct loci.

No indication exists that PGAM is a glycoprotein, so differential sialyzation can probably be excluded as being the cause for the additional isozymes. Aging of protein, often observed in erythrocytes[10], produces a typical isozyme pattern consisting of a major cathodal band accompanied by one or more minor anodal bands. Such a pattern is observed in the liver of bream, but not in other tissues such as the brain, where the most anodal band has the strongest staining intensity.

Heterozygosity at PGAMA or PGAMB probably does not explain the additional isozymes, since their occurrence and relative staining intensities are tissue-specific. In the case of heterozygosity, additional isozymes showing identical staining intensity in all tissues would have to be expected. So, the aberrant isozyme pattern in bream and trout is probably best explained as resulting from tetraploidization events in the evolution of both cyprinoid and clupeoid fish species[11,12]. In bream this has led to the existence of four PGAM genes, PGAMA, PGAMA', PGAMB and PGAMB', which in turn results in the aggregation of six isozymes, AA, A'A, A'A', BB, B'B and B'B'. It is remarkable that no heterodimers consisting of A or A' on the one hand and B or B' on the other hand are formed. From the observed isozyme it may be concluded that only one set of genes is expressed in a given tissue. In trout probably one of the duplicated PGAMB genes was lost or is no longer expressed, causing the formation of only one B type isozyme. Again, no heterodimeric aggregation of B and A/A' subunits seems to occur, although a PGAMA gene and PGAMB are expressed in heart muscle.

It is not clear whether the absence of an AB isozyme is due to decreased stability of the heterodimer or to expression of A and B type genes in different cells. The isozyme patterns observed in

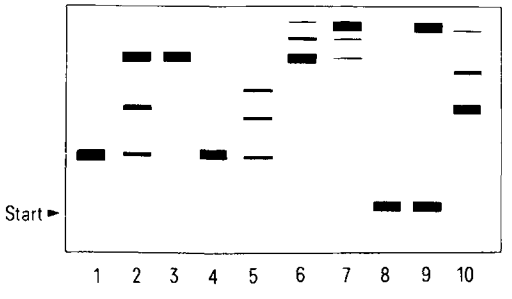

Figure 1. PGAM isozyme patterns in tissues of eel, bream and trout. *1–3* eel muscle, heart, brain; *4–7* bream muscle, heart, liver, brain; *8–10* trout muscle, heart, brain.

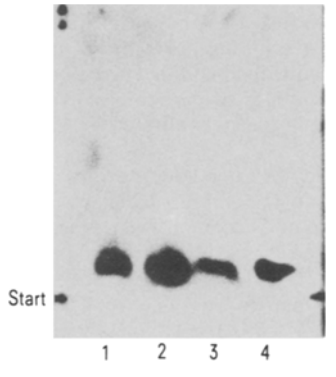

Figure 2. PGAM activity bands from tissues of the slime eel. *1* muscle, *2* heart, *3* kidney, *4* brain.

three species of cartilaginous fish correspond to those described by Mezquita et al. for the dog shark *(S. caniculus)*[7]. All of the three species belonging to the orders petromyzoniformes and myxiniformes (class cyclostomata) have only one PGAM band in all tissues (fig. 2)., comparable to the single-banded pattern reported for birds. However, whereas the loss of isozymes in birds is secondary, the single-banded pattern in the cyclostomata may be caused by the existence of only one gene coding for PGAM, reflecting a primitive ancestral stage of vertebrate evolution. This hypothesis implies that the existence of two distinct PGAM loci is due to a duplication of the ancestral gene during the evolution of vertebrates possessing jaws. This fits well with data reported on lactate dehydrogenase and hemoglobin genes[13,14].

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1 Omenn, G.S., and Cheung, S.C.Y., Am. J. hum. Genet. *26* (1974) 393.
2 Chen, S.H., Anderson, J., Giblett, E.R., and Lewis, M., Am. J. hum. Genet. *26* (1974) 73.
3 Adamson, E.D., J. Embryol. exp. Morph. *35* (1976) 355.
4 Bartrons, R., and Carreras, J., BBA *708* (1982) 167.
5 Fundele, R., Bücher, Th., Gropp, A., and Winking, H., Devl Genet. *2* (1981) 291.
6 Junien, C., Despoisse, S., Turleau, C., Grouchy, J. de, Bücher, Th., and Fundele, R., Ann. Génét. *25* (1982) 25.
7 Mezquita, J., and Carreras, J., Comp. Biochem. Physiol. *70B* (1981) 237.
8 Omenn, G.S., and Hermodson, M.A., in: Isozymes III. Developmental Biology, p. 1005. Ed. C.L. Markert. Academic Press, New York 1975.
9 Bücher, Th., Bender, W., Fundele, R., Hofner, H., and Linke, I., FEBS Lett. *115* (1980) 319.
10 Yuan, P.N., Talent, J.M., and Grazy, R.W., Mech. Ageing Dev. *17* (1981) 151.
11 Ohno, S., Wolf, V., and Atkin, N.B., Hereditas *59* (1968) 169.
12 Hinegardner, R., and Rosen, D.E., Am. Nat. 106 (1972) 621.
13 Whitt, G.S., Shaklee, J.P., and Markert, C.L., in: Isozymes IV. Genetics and Evolution, p. 381. Ed. C.L. Markert. Academic Press, New York 1975.
14 Goodman, M., and Moore, G.W., Syst. Zool. *22* (1973) 508.

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Effects of parathyroid hormone and calcitonin on carbonic anhydrase location in osteoclasts of cultured embryonic chick bone[1]

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Summary. The ultrastructural location of carbonic anhydrase has been examined in osteoclasts of cultured embryonic chick metatarsi. In untreated cultures and in those to which parathyroid hormone (PTH) was added, the enzyme was found in close association with the plasma membrane of the ruffled border. After brief calcitonin treatment (10 min) the ruffled border disappeared and the association of carbonic anhydrase with the plasma membrane was diminished. The results indicate that the hormones employed act directly on embryonic bone to alter osteoclast structure and the intracellular location of carbonic anhydrase.
Key words. Osteoclasts; cultured bones; carbonic anhydrase; calcitonin; parathyroid hormone.

Evidence continues to be obtained that indicates that carbonic anhydrase is important in the function of osteoclasts. Recently, the ultrastructural localization of the enzyme was observed to change with different states of cellular activity[2]. A comparison of calcitonin-treated and untreated chicks showed that calcitonin inactivation was accompanied by a reduction of enzyme staining on the plasma membrane and endosteal surface of the bone beneath the osteoclast. Enzyme localization was accomplished in osteoclasts of chick long bones by an immunocytochemical method[3] combined with a modified preembedding staining procedure[4]. The occurence of carbonic anhydrase in osteoclasts from rat calvaria has been confirmed by Väänänen and Parvinen[5].

In the present study we extend our previous observations to an in vitro system employing embryonic chicks. An improvement in ultrastructure was also accomplished by the addition of osmium post-fixation to the immunocytochemical procedure.

Metatarsi from 18-day-old chick embryos were cut longitudinally and the marrow was removed. The pieces of bone were incubated (37 °C, 5% CO_2) on stainless steel grids in 2 ml BGJ_b medium (Gibco, Grand Island, New York) which contained 1 mg/ml bovine serum albumin, 100 µg/ml penicillin and 100 µg/ml streptomycin. The bones were cultured with salmon calcitonin (Sigma, St. Louis, Missouri) for 10 min or 1 h (150 µU/ml) or with bovine parathyroid hormone (PTH; see 'acknowledgments') for 12 h (400 µU/ml). Parallel cultures not treated with hormones served as controls. Bones from three to six embryos were examined in each treatment group. The times of exposure to hormones were selected on the basis of the ultrastructural preservation of the tissues which were fixed with phosphate-buffered glutaraldehyde (2.5%) and OsO_4 (1%). The osteoclasts were noticeably more fragile after calcitonin than after PTH treatment. For this reason and because the response of osteoclasts is known to be rapid[6], shorter exposure times were selected for calcitonin treatment.

Carbonic anhydrase was localized by peroxidase-antiperoxidase (PAP) immunocytochemistry as described by Anderson et al.[2]. The antibody directed against carbonic anhydrase was produced in rabbits injected with highly purified chicken carbonic anhydrase isoenzyme C, the only isoenzyme present in chicken red blood cells[7]. Antiserum specificity was reconfirmed and immunoglobulin G (IgG) was fractionated from whole antiserum by ammonium sulfate precipitation and DEAE cellulose chromatography as previously described[8]. For controls, the primary antibody was replaced with saline or a 1:50 dilution of normal rabbit immunogammaglobulin. Between 15 and 30 osteoclasts were