TISSUE-SPECIFIC ISOENZYME PATTERNS OF CREATINE KINASE (2.7.3.2.) IN TROUT*

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Received 31 March 1971

1. Introduction

A comparative examination of the electrophoretically separable isoenzymes of creatine kinase (CPK) showed an extended heterogeneity of the isoenzyme pattern from certain fish species compaired to higher vertebrates.

The additional isoenzyme bands may at least in part be due to the tetraploidy of certain fish species and the result of mutations at the two known CPK-gene loci. However, the biochemical behaviour of some of the additional protein bands point to an epigenetic event as the more likely cause of their formation.

The present communication reports on the isolation, characterization and modification, by different treatments, of skeletal muscle CPK from trout tissue.

2. Methods

Male rainbow and brown trout from a local hatchery were used in these experiments. The animals were sacrificed, most of the skeletal muscle tissue excised and a 20% homogenate was prepared in a blendor. After a 2 hr extraction in 0.02 M tris, 0.001 M EDTA at pH 8.0 and centrifugation at about 50,000 *g*, the supernatant was placed on a DE-52 (Whatman) - Ion exchange column (2.5 cm X 30 cm). Three pools with CPK activity were eluted by a linear NaCl gradient ranging from 0 in 0.01 M tris to 0.25 M in 0.01 M tris, pH 8.0. They correspond to the three isoenzymes observed in muscle tissue. Each pool was run through a G-150 Sephadex column (2.5 X 100 cm). After rechromatography of the three active pools on DE-52 (1 X 12 cm), the three isoenzymes were separated in a rather pure form as judged by electrophoresis. Enzyme activity was determined either by a spectrophotometric method or by a histochemical method described elsewhere [1, 2]. The fractions were subjected to zone electrophoresis either on vertical starch gels for activity determination or on polyacrylamide disc gel electrophoresis for protein determination; for conditions see figure legends. Sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis was performed with slight modifications according to the procedures described by Weber and Osborn [3] and Dunkert and Rueckert [4].

In vitro dissociation and reassociation experiments were performed by either freezing and thawing, lyophilisation or by incubation in 8 M urea + 0.1 M 2-mercaptoethanol followed by either dilution or dialysis [5].

3. Results

Fig. 1 demonstrates the difference in the CPK-isoenzyme pattern between trout and rat tissues. Whereas the three isoenzymes in rat tissues have

* Supported by grants no. 3.165.69 and 3.247.69 of the Swiss National Foundation; grant no. 113 of the F. Hoffmann-La-Roche Stiftung and by a grant of the Muscular Dystrophy Association of America, Inc.

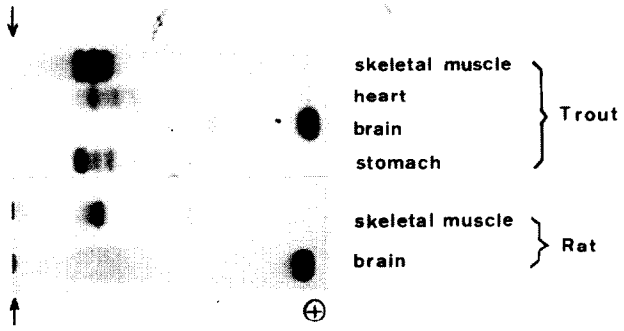

Fig. 1. Vertical starch gel electrophoresis of several high speed supernatants of tissue extracts from rainbow trout and rat. Specific staining for CPK according to [2]. The arrow marks the origin. Continuous buffer system: EDTA–tris–boric acid. pH 8.6, 16 hr, 15 mA, 300 V.

been described and characterised as homo- and heterodimers of two genetically different types of subunits [1] a different explanation appears to apply for the three bands in trout muscle tissue.

Fig. 2 shows the results of reversible dissociation experiments of purified isoenzymes I, II and III. It is seen that upon dissociation and reaggregation of individual isoenzymes not only are all the naturally occurring isoenzymes generated, but eventually new, faster migrating bands may appear. When newly generated bands were eluted from the starch gels, and dissociated and reaggregated, new bands were again formed.

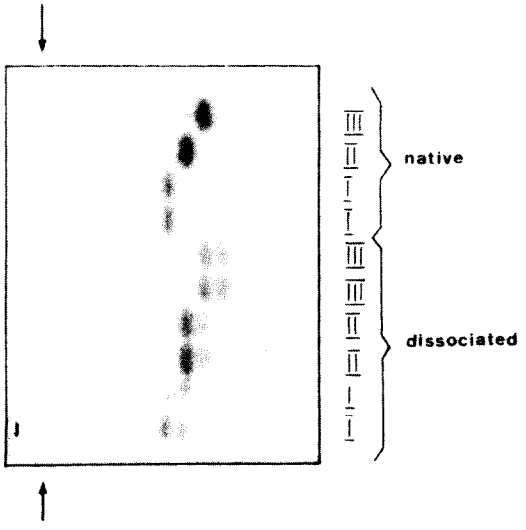

Fig. 2. Vertical starch gel electrophoresis of purified CPK-isoenzymes I, II, III from rainbow trout before and after dissociation–reaggregation. The arrow marks the origin. For conditions see fig. 1.

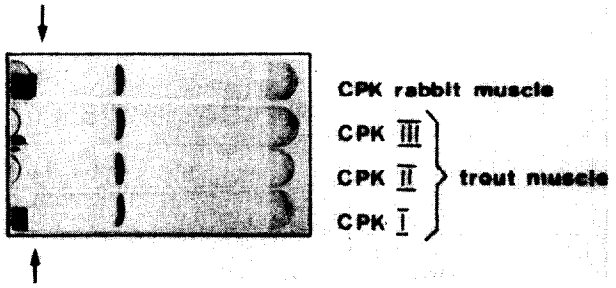

Fig. 3. SDS-disc electrophoresis of purified CPK I, II, III from trout muscle. Rabbit muscle CPK of known subunit MW (42,000) as a control. Na-phosphate buffer, pH 6.95, 2.5 hr, 5 mA per gel. Staining for protein by Coomassie brilliant blue.

In order to examine the possibility of the existence of larger multimers than dimers or of the formation of aggregates the molecular weights of the isolated isoenzymes were determined. Filtration on a calibrated Sephadex G-150 column revealed a MW of around 82,000 for all fractions, comparable to the MWs of the CPK's that are known so far [6]. SDS-electrophoresis, as shown in figs. 3 and 4, confirmed these results giving subunit-MWs of approximately 42,000 for all the observed protein bands.

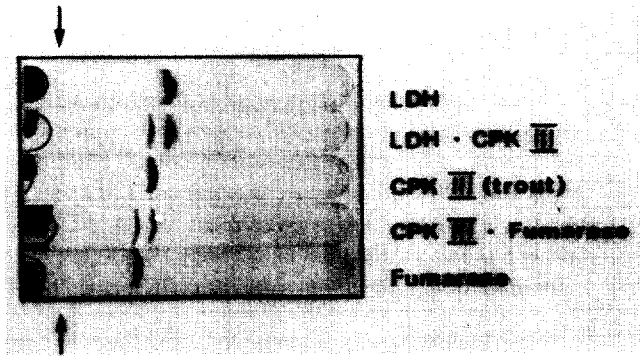

Fig. 4. SDS-disc electrophoresis of several enzyme subunits. For conditions see fig. 3. Subunit MW: lactic dehydrogenase (LDH) 35,000; CPK III 42,000; fumarase 48,000.

4. Discussion

We observed a far greater number of CPK isoenzymes compared to higher vertebrates both in tetraploid- (trout, carp) and diploid- (sword tail) fish. In addition a considerable change in the isoenzyme pattern upon storage of trout tissue in various ways is observed. Therefore we believe that this heterogeneity cannot be exclusively related to the polyploidisation of the trout genome.

With the possible exception of band I, each of the native isoenzymes from skeletal-muscle can be formed in vitro from another one as shown in fig. 2. In addition, new forms are often generated that are not present in the original extracts. This observation is particularly interesting when related to the tissue-specific isoenzyme patterns. Whereas in skeletal muscle three isoenzymes seem to be normal, in other tissues such as stomach or small intestine up to seven active bands in the native state are observed in addition to the brain and brain muscle hybrid CPKs [7]. The newly formed isoenzymes reported in the present paper always correpond in their electrophoretic migration with one or more of the additional bands observed in stomach and small intestine. Thus, the additional skeletal muscle CPKs generated by dissociation and reaggregation in trout are not mere preparation artifacts but reveal the presence in muscle tissue of an epigenetic control system that prevents the expression of the full repertory of CPK isoenzymes as found in other tissues.

The molecular weights of all native and purified isoenzymes point to a dimeric structure of the active enzyme as generally observed for CPK; none of the bands represent larger aggregates. So far we have no indications of ligand binding nor an involvement of reactive SH-groups as a possible explanation for the different electrophoretic behaviours.

We believe that the great heterogeneity of the CPK-isoenzyme patterns observed in many fish species is not only the result of a possible polyploidic genome or of allelic polymorphisms but also reflects the ability to form alternate conformative enzyme dimers. This possibility has been previously discussed for other species [8]. There may be an epigenetic factor which selectively favours the formation of conformative forms (conformers) in different cell types and tissues in vivo, a situation which can be simulated in vitro.

References

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