Localization of acid phosphatases in the cell fractions of chick liver

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Summary. 3 molecular forms (P1, P2 and P3) of acid phosphatase (E.C.3.1.3.2) have been detected in chicken liver homogenate. The different intracellular localization of these molecules has been demonstrated by cellular fractionation and electrophoretic analysis. P1 and P2 phosphatases are both present in the particulate fraction. P3 is present in a pure form in the soluble fraction. The difference between the enzyme molecules present in the particulate fraction and that in the soluble one is confirmed by the different activation-inhibition effect of various ions and substances on the enzymatic activity of subcellular fractions.

The heterogeneity of acid phosphatase in the liver of mammals has been demonstrated by electrophoresis[1-5] and by chromatography[6-8]. Cellular fractionation studies, effect of activators and inhibitors and specificities towards some substrates indicate that the particulate and the soluble fractions of homogenates of guinea-pig and mouse liver contain different molecular forms of acid phosphatase[8-10]. Similar findings have been reported also for the liver of developing chicken embryos[11-13].

We have recently described an electrophoretic analysis which led to the identification of three different molecular forms of acid phosphatase in the liver of the adult chick[14]; we describe now their intracellular localization.

Materials and methods. Adult white Leghorn chicks *(Gallus gallus)* were used for our studies. Liver preparation and cellular fractionation were performed as described by Strittmatter[12] to give nuclear (N), particulate (P) and soluble (S) fractions.

Acid phosphatase activities were measured at 37 °C for 30 min in 1 ml (final volume) of a solution containing an appropriate amount of enzyme suspension, acetate buffer 50 mM, pH 5, and p-nitrophenylphosphate 5 mM. The reaction was stopped by adding 5 ml of 0.05 M NaOH and then the concentration of p-nitrophenol determined spectrophotometrically at 410 nm. Protein concentration was determined according to Lowry et al.[15]. Phosphatase activities were expressed as μmoles of p-nitrophenol per h/mg of protein.

The effects of magnesium, zinc and fluoride ions and of urea on the enzyme activity were investigated by adding to the enzyme solution $MgCl_2$, $ZnCl_2$, NaF (5 mM, final concentration) and urea (4 M, final concentration), and by determining the enzyme activity after standing for 20 min at 37 °C.

In order to solubilize the acid phosphatase molecules before the electrophoretic analysis, each liver fraction was homogenized in 5% Triton X-100. Samples so obtained were left standing for 30 min at 4 °C and then centrifuged at 100,000 × g for 60 min. Clear supernatants were pooled separately and analyzed by electrophoresis as previously described[14].

Electrophoretic patterns of acid phosphatases from chicken liver homogenate (H) and from nuclear (N), particulate (P) and soluble (S) fractions.

Results and discussion. Acid p-nitrophenylphosphatase activity shown by the liver cells of the adult chicks is distributed in the nuclear, particulate and soluble fractions as summarized in table 1. The higher percentage amounts of enzyme activity are associated with the particulate and soluble fractions, being 59% and 24%, respectively, of the total. These findings confirm those reported by Wang[11] and by Strittmatter[12,13] concerning liver cells of newborn chickens. The same authors demonstrated the presence of an acid phosphatase activity, strongly activated by Zn^{++}, in the soluble fraction. This ion, however, showed an inhibitory action on the enzymatic activity associated with the particulate fraction. Inhibition effects of L(+) tartrate, cysteine and fluoride ion also suggested that different enzyme molecules are responsible for these 2 enzymatic activities[11,13].

Similar tests have been carried out by us on the subcellular fractions of the adult chick liver homogenate. Mg^{++}, Zn^{++}, F^- and urea were used as modifier ions. The results obtained are reported in table 2. A strong inhibition on the nuclear and on the particulate fractions is exerted by fluoride. In the soluble fraction, however, a relatively high enzymatic activity is maintained even in the presence of NaF. Treatment of both cellular fractions with 4 M urea leads to similar conclusions. As shown in table 2, the denaturing effect of urea is more marked on the acid phosphatase of the soluble fraction than on the phosphatase of the particulate fraction. A more significant effect on the enzyme activity of the soluble and of the particulate fractions is shown by Zn^{++} ion. While an enzyme activa-

Table 1. Acid phosphatase activities and their distribution in the subcellular fractions of the adult chick liver

Cellular fraction	Acid phosphatase Specific activity*	Distribution (%)
Homogenate	2.80 ± 0.27	
Nuclear	1.58 ± 0.10	12
Particulate	3.90 ± 0.29	59
Soluble	1.90 ± 0.12	24
Recovery		95

* μmoles of p-nitrophenol/h/mg of proteins ± SD.

Table 2. Effect of some ions on acid phosphatase activity of the subcellular fractions of the adult chick liver

Cellular fraction	Specific activity (%)*			
	Mg^{++}	Zn^{++}	F^-	Urea
Nuclear	108	90	36	48
Particulate	110	75	30	60
Soluble	120	135	60	40

* The percent values of the enzymatic activities are calculated by assuming as reference an enzyme solution to which are added amounts of buffer instead of the modifier substances.

tion is evident in the case of soluble fraction, an inhibition is clearly shown by the particulate and the nuclear ones. Treatment of the enzyme with Mg^{++} leads to a small activation in all the cases.

These results are in good agreement with those of Wang[11] and Strittmatter[12,13] and strongly suggest that different enzyme forms are present in different cell compartments of the chick liver. Since 3 electrophoretically different acid phosphatases are present in the whole homogenate[14], it is particularly interesting to point out which of the enzyme bands are present in each subcellular fraction. The electrophoretic analysis reported in the figure confirms that 3 enzyme activity bands, here labelled P1, P2 and P3, are detected in the whole homogenate (H). The particulate fraction shows the presence of the 2 slower bands P1 and P2, whereas the soluble one contains only the fast migrating band P3. All the enzymatic bands are present in the nuclei. The migration differences between the electrophoretic bands of samples N and P and those of H are due to the preliminary purification procedures. It has been demonstrated by Allen and Gockerman[2] that the physical and physicochemical treatments leading to the purification of the subcellular fractions of rat liver cells strongly influence the electrophoretic mobilities of the acid phosphatases. Also in the case of chick the electrophoretic behavior depends on the preliminary treatment of the samples. So, the total homogenate, which is not subjected to purification procedures like the nuclear and the particulate fractions shows an electrophoretic migration of bands lower than that of samples N and P. When the particulate and the soluble fractions are mixed together, the distribution of bands typical of the total homogenate is restored, though the migration on the gel is increased according to that of N and P samples.

These electrophoretic results confirm what was suggested by the inhibition experiments reported here and furthermore demonstrate that acid phosphatase activity in the particulate fraction is due to 2 almost electrophoretically distinct molecule types, whereas that of the soluble fraction is due to only one.

Previous work on the heterogeneity of acid phosphatase demonstrated the existence of 2 molecular forms of this enzyme in the mitochondrial-lysosomal fraction of rat liver homogenate and of only one molecular form in the soluble fraction of the liver of some mammalian species[8-10]. Our results demonstrate that a similar heterogeneity is also found in bird liver and the subcellular distribution of acid phosphatase molecular forms is similar.

1 T. Barka, J. Histochem. Cytochem. *9*, 542 (1961).
2 J.M. Allen and J. Gockerman, Ann. N.Y. Acad. Sci. *121*, 616 (1964).
3 A. Kaneko, T. Ikeda and T. Onóe, Biochim. biophys. Acta *222*, 218 (1970).
4 H.D. Campbell, N.P.B. Dudman and B. Zerner, FEBS Lett. *31*, 123 (1973).
5 F. Panara and C. Cirotto, IRCS Med. Sci. *5*, 235 (1977).
6 B.W. Moore and P.U. Angeletti, Ann. N.Y. Acad. Sci. *94*, 659 (1961).
7 T. Barka, J. Histochem. Cytochem. *9*, 564 (1961).
8 S. Shibko and A.L. Tappel, Biochim. biophys. Acta *73*, 76 (1963).
9 M.W. Neil and M.W. Horner, Biochem. J. *92*, 217 (1964).
10 M.W. Neil and M.W. Horner, Biochem. J. *93*, 220 (1964).
11 K.M. Wang, Biochem. J. *115*, 191 (1969).
12 C.F. Strittmatter, Biochim. biophys. Acta *284*, 183 (1972).
13 C.F. Strittmatter, Biochim. biophys. Acta *284*, 196 (1972).
14 F. Panara, C. Cirotto, I. Arangi and L. Barberini, Experientia *34*, 983 (1978).
15 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).

The localization of acetylcholinesterase in the optic lobe in the developing chick embryo

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Summary. The localization of acetylcholinesterase (AChE) in the optic lobe in the developing chick embryo was studied histochemically and biochemically. The histochemical reaction of AChE increased remarkably between stage 42 and 44 especially in the neuropile. The increase of the biochemical activity of the AChE in the synaptic membrane fraction occurred at a later stage than that in the microsomal fraction. These findings can be interpreted as the result of axonal transport of the enzyme from the synthetic to the synaptic site.

It is known that a high concentration of AChE is present in the optic lobe in non-mammalian vertebrates[2]. Especially, it has been suggested that the enzyme plays an important role in relation to intrinsic neuronal activity in the optic tectum[3,4]. Boell et al.[5] and Filogamo[6]studied the change in the localization of tectal AChE in the developing tadpole and the chick embryo respectively, so as to follow the relationship between the distribution of the enzyme and synapse formation. On the other hand, Ciani et al.[4] studied AChE in the quail optic tectum by electron microscopy and suggested that the enzyme was released from the site of synthesis to the neuropile. In this report, in order to relate the localization of AChE in the developing chick embryo optic lobe with the cell structure, the changes in the subcellular distribution of the enzyme activity were compared with the changes in its histochemical localization.

Materials and methods. Fertilized eggs from the White Leghorn strain of chicken were incubated at 37.5 °C (±1 °C) and approximately 80% relative humidity until used. The developmental stages were determined at the time of sacrifice according to the description of Hamburger and Hamilton[7]. Experiments were performed from stages 38 to 45+. For AChE histochemistry, the brains were fixed in buffered fixative (pH 7.4), containing 4% paraformaldehyde, at 4 °C for 16 h, and washed with Holt's Gum-Sucrose solution at 4 °C for 24 h. The fixed brains were fast-frozen and cut into 10-μm sections with a cryostat. Sections were processed according to the method of Karnovsky and Roots[8]. For the quantitative determination of AChE in subcellular fractions, both optic lobes were homogenized in ice-cold 0.32 M sucrose and fractionated according to the method of Inoue et al.[9] with some modifications.