Histone Hydrolase in Tadpole Liver

During the last several years, histones have attracted a great deal of attention because of their close spacial relationship to DNA in the cell and their inhibitory effect on the function of DNA[1]. It is, therefore, of great importance to examine the degree of heterogeneity and turnover of various fractions of histones. However, ample evidence has indicated that studies of this type have been complicated by the fact that histones are easily hydrolyzed even during the most careful isolation conditions[2-4]. We have recently reported the presence of a proteolytic enzyme in rat kidney microsomes which hydrolyzes basic proteins such as histones and protamine[5]. In the course of a search for a good starting material for further purification of the enzyme we have observed in the tadpole liver an enzyme which was somewhat different from the rat kidney enzyme: the tadpole enzyme had an optimum pH around 6–8 while the rat enzyme had 9.0, and the former was located in the cytosol while the latter was in the microsomal fraction. Both enzyme preparations were specific toward basic proteins such as polylysine, protamine and histone. However, the tadpole enzyme was most active on polylysine while the rat enzyme was on protamine. Furthermore, addition of some other proteins such as albumin, globulin and polyglutamic acid inhibited the activity of the enzyme from tadpole liver while these proteins were without effect on the enzyme from rat kidney microsomes. Some of the implication of the role of these enzymes in the control of the amount of histone have been discussed.

Various proteins used in Table II were obtained from Sigma Chemical Co. (St. Louis, Mo.) and *Rana catesbeiana* tadpoles from Connecticut Valley Biological Supply Co., Southampton, Mass. Various histones were purified from type II-A of Sigma by the method described[6].

Detailed procedures for the enzymatic assay were described previously[5]; for each assay, livers from 3 tadpoles were pooled. The livers were homogenized in 0.25 *M* sucrose solution as 20% with an electrically driven teflon homogenizer. The homogenate was passed through a double layer of cheese-cloth. In the case of tadpole tail, the tail was cut into small pieces, the pieces were homogenized and the supernatant after centrifuging the homogenate at 1000 *g* in clinical centrifuge for 1 min was taken for assay. $^1/_{10}$ and 0.2 ml (2 concentrations) of the homogenate, 0.2 ml of substrate protein suspension (0.6 mg), 0.1 ml of 0.5 *M* phosphate buffer at pH 6.0 in a total volume of 0.5 ml were incubated at 37 °C for 20 min. The reaction was terminated by addition of 0.5 ml of 30% trichloracetic acid (TCA). For a control, the substrate protein suspension was added after the reaction was stopped by TCA. The mixture was centrifuged at 39,000 *g* for 10 min, and a portion of the clear supernatant (usually 0.05–0.2 ml) was transferred to a Coleman spectrophotometer cuvette (19 × 105 mm) which contained a predetermined amount of NaOH to neutralize the pH of the solution to about 5. Water was then added to bring the total volume to 1.0 ml and the ninhydrin color was developed according to the method described[7]. The values in the following experiments are the average of duplicate determinations for each two enzyme concentrations, and have been corrected for the control. The enzyme activity is expressed as specific activity which corresponds to A_{580}/20 min/mg enzyme protein. One unit of A_{580} corresponds to 0.24 μmoles of leucine. The enzyme activity, therefore, is an increase of ninhydrin color in 15% TCA-soluble fraction after reacting the substrate protein with the enzyme preparation. Protein concentration was determined by the method of LOWRY et al.[8], and subcellular fractionation of tadpole liver was carried out according to the method of SCHNEIDER[9].

The majority of the enzyme has been found in the cytosol of the tadpole liver (Table I). This is quite in contrast to the histone hydrolase found in rat kidney which was located in microsomes[5]. Furthermore, this pattern of distribution of the enzyme distinguishes itself from cathepsins which have been reported to be in lysosome fraction[10]. Figure 1 A illustrates that approximately 0.5 mg of histone type II-A suspension saturates

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Table I. Distribution of histone hydrolase among various subcellular fractions of tadpole liver

Fraction	Volume (ml)	Protein (mg/ml)	Total protein (mg)	Enzyme activity (A_{580}/20 min/mg protein)	Total enzyme activity (A_{580}/20 min)	Recovery Protein (%)	Recovery Enzyme (%)
Whole homogenate	4.0	20.7	82.8	0.821	67.9	100.0	100.0
Nuclear	5.0	3.17	15.9	0.843	13.4	19.2	19.7
Mitochondrial	3.0	3.08	9.2	1.194	11.0	11.1	16.2
Microsomal	4.0	3.92	15.7	0.589	9.25	19.0	13.6
Soluble[a]	3.6	9.90	35.6	1.111	39.6	43.1	58.3

[a] Soluble fraction was obtained by centrifuging 4.0 ml of whole homogenate at 105,000 *g* for 1 h. The rest of the conditions are the same as described under methods.

Table II. Specificity of histone hydrolase

Substrate protein added (0.6 mg)	Enzyme activity[a]	Increased or decreased by added protein[a]
None[b]	0.63	
Polylysine	3.33	+2.67
Protamine	2.02	+1.39
Histone type II-A[c]	1.56	+0.93
CM-cellulose-column purified:		
Slightly lysine-rich histone	2.74	+2.11
Lysine-rich histone	2.30	+1.67
Arginine-rich histone	1.30	+0.67
Polyarginine	0.52	—0.11
Albumin, bovine serum	0.45	—0.18
Polyglutamic acid	0.41	—0.22
Ribonuclease, pancreatic	0.40	—0.23
Globulin, egg white	0.27	—0.36
Lysozyme	0.19	—0.44

[a] Enzyme activity is expressed as $A_{580}/20$ min/mg enzyme protein. + sign indicates increase and — sign for decrease by the addition of proteins. [b] This value indicates the enzyme activity due to endogenous substrate protein. When the enzyme preparation was boiled for 5 min, this endogenous enzyme activity was completely abolished. [c] Commercial histone type II-A of Sigma Chemical Co. is a mixture of various histones.

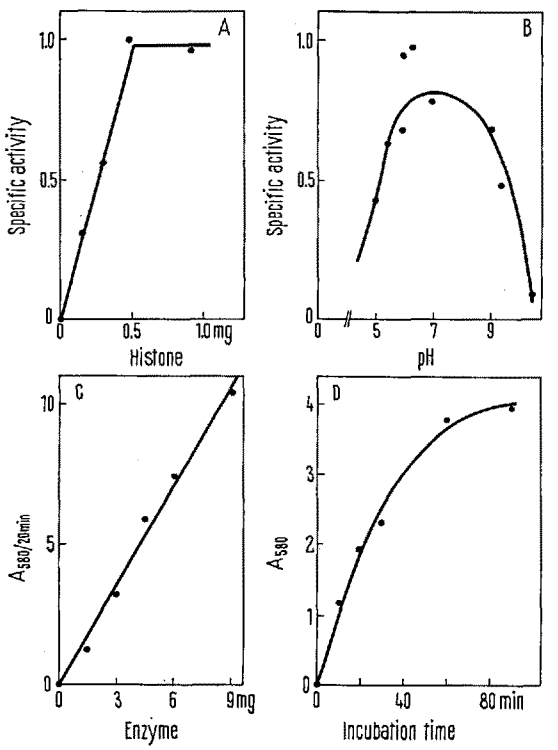

Fig. A) Effect of histone concentration. In order to show clearly the effect of histone concentration, the values in the figure were corrected for the enzyme activity with endogenous substrate protein (without added histone), which was 0.32 $A_{580}/20$ min/mg enzyme protein. Detailed experimental procedures are described in text. B) pH-curve. Phosphate buffer was used for the pH range of 5–7, and *Tris*-HCl buffer for 9–10.5. Experimental conditions are described in text. C) Enzyme concentration. Experimental conditions are described in text. D) Effect of incubation period. 2 mg of enzyme protein was used.

the hydrolysis system under the conditions used. Furthermore, the pH optimum was found to be between 6 to 8 (Figure 1 B) which is somewhat lower than the histone hydrolase from rat kidney[5] and higher than that of cathepsin which has the optimum pH around 5. Under the conditions used the concentration of the enzyme preparation is linear to the amount of product formed (Figure 1 C), and the enzyme activity is proportional to the period of incubation up to about 40 min (Figure 1 D). The enzyme is highly specific toward basic proteins such as polylysine, protamine and various histones (Table II). Among the basic proteins, polylysine is the most susceptible to the hydrolysis by the enzyme. However, in the case of histone hydrolase from rat kidney, this polypeptide was a poor substrate[5]. It is of interest to note that all the proteins except the ones mentioned above rather inhibited the enzyme activity on the endogenous substrate protein (without added protein). The significance of this observation is not clear at present. However, this might suggest that the various proteins protect the basic proteins from the hydrolytic action of the enzyme in vivo. Such a finding was not observed with the enzyme isolated from rat kidney microsomes[5].

From the foregoing results it is evident that the presently described proteolytic enzyme in tadpole liver is not similar to the enzyme from rat kidney nor to cathepsins. Furthermore, it was found that the enzyme activity in tadpole liver and tail did not significantly change during thyroxine-induced metamorphosis (unpublished data). Since catheptic activity in tadpole tail has been reported to increase extensively during metamorphosis[11], the above observation again support the contention that the present hydrolase is a hither-to-unreported proteolytic enzyme.

Since the amount of histone which is conjugated with DNA in the cell nucleus is in approximate unity and the histone is synthesized in the cytoplasm[12,13], there must be a tightly controlled feed-back control mechanism operating to control the amount eventually delivered into the nucleus. This could be achieved by either controlled synthesis or controlled degradation of an excess amount of histone. It is quite possible that the presently discussed proteolytic enzyme in the cytosol in the tadpole liver might have an important role in this context[14].

Résumé. Le cytoplasme du foie de têtard contient une enzyme qui hydrolyse spécifiquement les protéines basiques telle que la polylysine, la protamine et les histones. L'addition d'autres protéines telles que l'albumine, la globuline et l'acide polyglutamique inhibe l'activité de cette enzyme. Les résultats obtenus montrent que cette hydrolysase extraite du foie de têtard diffère des cathépsines ou de l'histone-hydrolase du rein de rat.

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