rapid exchange of cell populations in this organ. The results suggest that the functional state of the lysosomal proteolytic system is indissolubly connected with all stages of intracellular protein metabolism, and the decline in lysosomal cathepsin activity during inhibition of protein biosynthesis may be a regulatory mechanism, aimed at preserving the protein reserves of the cell. Since cathepsins have the shortest half-life of all the lysosomal hydrolases [9], there is reason to suppose that participation of lysosomal proteinases in the maintenance of stable equilibrium between protein biosynthesis and catabolism is a genetically determined mechanism.

LITERATURE CITED

1. F. M. Baccino, M. Messina, M. Musi, et al., in: Structure and Functions of Lysosomes [Russian translation], Novosibirsk (1980), p. 19.
2. A. V. Vasil'ev, T. A. Kapelevich, and V. A. Tutel'yan, Vopr. Med. Khimii, No. 3, 127 (1983).
3. L. A. Lokshina, Usp. Biol. Khim., 18, 162 (1977).
4. A. A. Pokrovskii and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1968), p. 5.
5. A. A. Pokrovskii and V. A. Tutel'yan, Biokhimiya, 33, 809 (1968).
6. A. A. Pokrovskii, A. I. Archakov, and T. V. Alenicheva, Tsitologiya, 10, 1467 (1968).
7. A. A. Pokrovskii, L. G. Ponomareva, and V. A. Tutel'yan, in: Enzymic Methods of Analysis [in Russian], Moscow (1969), p. 471.
8. J. S. Amenta, M. J. Sargus, and F. M. Baccino, Biochim. Biophys. Acta, 476, 253 (1977).
9. J. S. Amenta, M. J. Sargus, and F. M. Baccino, J. Cell. Physiol., 97, 267 (1978).
10. A. J. Barrett, in: Proteases and Biological Control, New York (1975), p. 457.
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. T. G. Petters, M. Muller, and C. de Duve, J. Exp. Med., 136, 1117 (1972).

ISOLATION OF TISSUE-SPECIFIC INHIBITORS OF DNA SYNTHESIS (CHALONES) FROM RAT LIVER

E. V. Parfenova and S. A. Ketlinskii

UDC 612.35.014.3018:612.6/-088.1

KEY WORDS: liver chalones; partial hepatectomy; control of proliferation; gel filtration.

Several methods of obtaining and purifying chalones from mammalian liver homogenate have been described [6-9], but none of them is universally suitable and can yield the inhibitor in a pure form. The molecular weight of such preparations as have been obtained varies within wide limits (from 1000 to 40,000 daltons), and their preliminary characteristics are not identical. In the investigation described below an attempt was made to isolate and purify G_1-chalone from an aqueous extract of normal rat liver, possessing chalone activity, and partially purified by alcoholic fractionation. The writers showed previously [2] that such an extract is heterogeneous in composition and contains several inhibitors of DNA synthesis and mitosis whose action is exhibited in an in vivo system.

EXPERIMENTAL METHOD

Water-soluble liver proteins from noninbred albino rats obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR, precipitated with ethanol in a saturation of between 55 and 81%, were used as the original material [2, 3]. After lyophilization the resulting chalone-containing liver extract was fractionated on Sephadex G-75 (column 19 × 460 mm), and the sample applied contained 100-300 mg protein in 4 ml water.

Department of Morphology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 41-43, July, 1983. Original article submitted November 17, 1982.

0007-4888/83/9607-0926 $07.50

TABLE 1. Effect of Fractions of Chalone-Containing Extract of Rat Liver Obtained by Gel Filtration on Sephadex G-75 on Incorporation of [^{3}H]Thymidine into DNA of Regenerating Rat Liver in Vitro (M ± m)

Fraction of extract	Number of animals	Protein concentration in sample, mg/ml	Specific incorporation of [^{3}H]thymidine into liver DNA, %	Multiplicity of purification	Content in whole extract, %
Control	23	—	100	—	—
Whole extract	14	1,0	50±10*	—	—
I	5	0,5	100±30	—	
	4	1,0	80±20	—	45
	5	0,05	100±10	—	
Ia	5	0,10	90±20	—	
	5	0,25	100±10	—	10
	5	0,50	120±20	—	
	3	0,05	80±10	—	
II	3	0,25	50±20*		
	3	0,50	50±10*	4	40
	3	1,0	40±10*		
	3	0,005	60±10*		
	3	0,025	40±10*	200	5
III	3	0,05	50±20*		
	3	0,10	60±20*		

Legend. Multiplicity of purification defined as ratio of minimal concentration of whole extract exhibiting inhibitory effect (1 mg/ml) to concentration of the particular fraction exhibiting the same effect. *P < 0.05 compared with control.

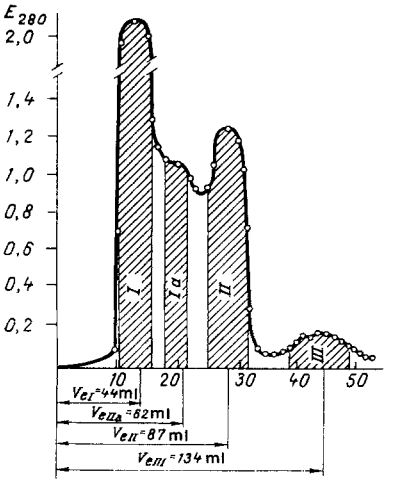

Fig. 1. Gel-filtration of chalone-containing extract of rat liver on Sephadex G-75. Abscissa, Nos. of samples; ordinate, absorption at 280 nm. I, Ia, II, and III) Individual fractions: I) $V_e/V_o = 1.0$, $K_{av} = 0$; Ia) $V_e/V_o = 1.4$, $K_{av} = 0.2$; II) $V_e/V_o = 2.0$, $K_{av} = 0.4$; III) $V_e/V_o = 3.0$, $K_{av} = 1$.

Proteins were eluted with water at the rate of 40 ml/h, and the volume of the samples was 3 ml. The protein content in the eluate was determined spectrophotometrically from absorption at 280 nm. The fractions obtained were lyophilized and kept at −4°C. The biological activity of the fractions was determined by the method in [8] in an in vitro system, according to their effect on incorporation of [^{3}H]thymidine into slices (1 mm) of rat liver

TABLE 2. Incorporation of [^{3}H]Thymidine (in cpm/optical density unit at 260 nm) into DNA of Rat Kidney and Testis Slices in Vitro under the Influences of Fractions of Chalone-Containing Liver Extract (M ± m)

Fraction of extract	Number of animals	Protein concentration in sample, mg/ml	Specific incorporation of [^{3}H]thymidine	
			into DNA of kidneys	into DNA of testis
Control	10	—	40±10	40±10
II	5	0,5	30±5	30±10
III	5	0,1	20±10	30±10

regenerating 24 h after partial hepatectomy, during short-term culture (for 120 min) at 37°C in medium 199, which contained 20 μCi [^{3}H]thymidine (specific activity 4 Ci/mmole) in 1 ml. After the end of incubation the slices were washed to remove excess of isotope with a large volume of cold physiological saline and, after homogenization, DNA was isolated from them by the method of Schmidt and Thannhauser in Georgiev's modification [1] and its specific radioactivity was determined by liquid scintillation counting [4]. The DNA concentration was determined spectrophotometrically by measuring absorption of the solution at 260 nm. The in vitro system described above was adequate for determination of the intensity of DNA synthesis in the liver tissue, for incorporation of [^{3}H]thymidine into DNA was a linear function of time during incubation of the slices for 30-120 min. DNA synthesis was determined similarly in slices of the kidneys and testis of adult rats.

The protein spectrum and molecular weights of the fractions obtained by gel filtration were determined by electrophoresis in 10% polyacrylamide gel in the presence of sodium dodecylsulfate (SDS), in the generally accepted manner [5], using bovine serum albumin (67,000 daltons) and trypsin (22,000 daltons) as standards.

EXPERIMENTAL RESULTS

The chalone-containing extract of rat liver, as the writers showed previously [2], is heterogeneous in composition and consists of ten protein components detectable by polyacrylamide gel electrophoresis. Investigation of this extract in the presence of SDS showed that the molecular weights of these components ranged from 17,000 to 100,000 daltons.

Gel filtration of the chalone-containing rat liver extract on a Sephadex G-75 column yielded four fractions (Fig. 1). Fraction I, the elution volume of which corresponded to the outer bed volume of the column V_o, was equivalent in protein content to 45% of the whole extract and included the four components of the extract with highest molecular weights, between 40,000 and 100,000 daltons. Determination of the biological activity of this fraction showed that in the in vitro system in concentrations of 0.5-1 mg/ml it had no effect on the level of [^{3}H]thymidine incorporation into sections of the regenerating liver (Table 1). Fraction Ia which, in protein content, was equivalent to 10% of the whole extract, likewise had no effect on DNA synthesis in concentrations of 0.05-0.5 mg/ml (Table 1). Fractions I and Ia of chalone-containing extract of normal rat liver thus contain ballast proteins, which account for more than half of the total protein of the extract.

Fraction II, equivalent to 40% of the whole extract in protein content, was highly heterogeneous and was found to be particularly rich in proteins with molecular weights of 17,000-30,000 daltons, although high-molecular-weight components also were present. Proteins of this fraction inhibit incorporation of [^{3}H]thymidine into DNA of regenerating liver slices (Table 1). The minimal dose causing 50% inhibition was 0.25 mg/ml, only one-quarter of the dose of the whole extract causing the same effect. Fraction II was not cytotoxic, as shown by morphological analysis of spleen cells after injection of this fraction into C57BL mice intraperitoneally in a dose of 1.2 mg. No cytotoxic effect likewise was exhibited on treatment of cells of a transplanted hemocytoblastoma with proteins of fraction II (in a dose of 0.5 mg/ml). The inhibitory effect of fraction II on DNA synthesis in the liver is evidently tissue-specific in character, for it did not change the level of incorporation of [^{3}H]thymidine into slices of adult rat kidney and testis (Table 2).

Fraction III, equivalent to about 5% of the whole extract, according to the results of gel electrophoresis in the presence of SDS contains one major component with molecular weight of about 17,000 daltons. It may per-

haps also contain components with lower molecular weights, not detectable in the electrophoretic system used, for the elution volume of this fraction corresponds to the total volume of the column V_t, and the lower limit of fractionation on Sephadex G-75 is 3000 daltons. Fraction III inhibited DNA synthesis in slices of regenerating liver; 50% inhibition was reached with this fraction in a concentration of only 0.05 mg/ml (lower concentrations were not tested), which is 200 times less than the dose of whole extract causing the same effect. Considering that, as regards its protein content, fraction III is equivalent to 5% of the extract it can be concluded that in the course of isolation of the active principle from chalone-containing liver extract not only is it purified, but also activated, evidently on account of removal of components preventing manifestation of inhibitor activity. Fraction III, unlike the rest, is very hygroscopic and "melts" on keeping, but it completely retains its biological activity in the process. Dialysis of this fraction for 24 h against water likewise did not change its activity. Morphological analysis of the spleen of the C57BL mice after intraperitoneal injection of fraction III in a dose of 0.15 mg per mouse did not reveal any cytotoxic action, and this was confirmed also by a study of the viability of transplanted hemocytoblastoma cells after incubation for 1 h at 37°C in the presence of 0.25 mg/ml of this fraction. Fraction III did not affect incorporation of [^{3}H]thymidine into slices of rat kidney and testis, probably indicating that its biological action is tissue-specific in character (Table 2).

On gel-filtration of an ethanol precipitate of the aqueous extract of rat liver on Sephadex G-75 two fractions were obtained, which were noncytotoxic and which inhibited incorporation of [^{3}H]thymidine tissue-specifically into DNA of rat liver regenerating after partial hepatectomy in vitro (i.e., exhibited a G_1-chalone effect). One of these fractions ($V_e/V_o = 2.0$; $K_{av} = 0.4$) was heterogeneous, and rich in proteins with molecular weight of 17,000-30,000 daltons; its biological activity was exhibited in concentrations $\geq$0.25 mg/ml. Activity of the other fraction ($V_e/V_o = 0.3$; $K_{av} = 1$) was found in concentrations of under 0.005 mg/ml; its chief component was a protein with molecular weight of 17,000 daltons.

LITERATURE CITED

1. I. B. Zbarskii and S. S. Debov (editors), Chemistry and Biochemistry of Nucleic Acids [in Russian], Leningrad (1968).
2. S. A. Ketlinskii and E. V. Parfenova, Byull. Éksp. Biol. Med., No. 7, 96 (1981).
3. V. B. Okulov and L. I. Chekulaeva, Arkh. Anat., No. 7, 106 (1976).
4. O. A. Rys'ev, A. K. Zharkov, and E. I. Dolgirev, A Scintillation Method of Measuring Tritium in Biology and Medicine [in Russian], Moscow (1978).
5. U. K. Laemmli, Nature, 227, 680 (1970).
6. M. Lenfant, L. Kren-Proschek, W. G. Verly, et al., Can. J. Biochem., 51, 654 (1973).
7. G. Secas, W. G. Owen, and R. T. Cook, Exp. Cell Res., 122, 47 (1979).
8. W. G. Verly, Nat. Cancer Inst. Monogr., 38, 175 (1973).
9. B. Vinet and W. G. Verly, Eur. J. Cancer., 12, 211 (1976).