Short Communications

Acid Deoxyribonuclease Activity in Purified Calf Thymus Nuclei

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(Received 11 May 1971)

Acid deoxyribonuclease (deoxyribonuclease II, EC 3.1.4.6) is found in most mammalian cells (Laskowski, 1961, 1967; Bernardi, 1968; Lehman, 1967; Allfrey & Mirsky, 1952), where its highest activity is usually observed in actively dividing cells (Lehman, 1967). The literature contains various conflicting reports on the localization of acid deoxyribonuclease. Webb (1953) described deoxyribonuclease II as a cytoplasmic enzyme, Brown, Jacobs & Laskowski (1952) reported its presence in the nucleus, Schneider & Hogeboom (1952) and Sinsheimer (1957) found it in the mitochondria, and Bowers (1964) and de Duve, Wattiaux & Baudhuin (1962) described it as a lysosomal enzyme, and discounted the presence of acid deoxyribonuclease in nuclei and mitochondria on the grounds that the deoxyribonuclease activity in them was caused by contamination with lysosomes, and therefore that acid deoxyribonuclease is located only in the lysosomes.

The purpose of this paper is to report that, in addition to its presence in the lysosomes, deoxyribonuclease II is also found in the nucleus, where it may have a role in DNA replication.

Materials and methods. Deoxyribonuclease II was assayed at least in duplicate by modification of the 'acid-soluble nucleotides' method (Laskowski, 1961) as follows. Enzyme preparation (5–100 μ l) was added to 3 ml of calf thymus DNA (E_{260} 1.0) prepared by the method of Kay, Simmons & Dounce (1951) in 0.175 M-sodium acetate buffer, pH 4.5, and was incubated at 37°C for 10–20 min. Incubation was terminated by cooling in ice and adding 0.3 ml of 60% (w/v) perchloric acid. After a further 10 min in the ice the tubes were centrifuged at 5000g for 10 min at 4°C and the E_{260} of the supernatant was read against an appropriate blank. One deoxyribonuclease II unit is the amount of enzyme causing a change of 0.001 E_{260} unit/min under the above conditions (Slor, 1970a,b; Slor & Hodes, 1970).

Acid phosphatase activity was assayed with *p*-nitrophenyl phosphate (Fluka A.-G., Buchs, Switzerland) as substrate by following its hydrolysis to *p*-nitrophenol. Enzyme preparation (10–

50 μ l) was added to 2.5 ml of 2.2 mM substrate in 0.15 M-sodium acetate buffer, pH 4.5, and was incubated for 10–20 min at 37°C. Incubation was terminated by addition of 0.5 ml of 2 M-NH₃, which also developed the yellow colour of the *p*-nitrophenol. The yellow colour was read against an appropriate blank at 400 nm. One unit of acid phosphatase is the amount of enzyme hydrolysing 1 μ mol of substrate/min under the above conditions: ϵ_{400} of *p*-nitrophenol is 12000.

Nuclei were purified from calf thymus by the method of Allfrey, Littau & Mirsky (1964).

Results. Our approach in clarifying whether acid deoxyribonuclease is exclusively lysosomal or also located in nuclei involved the use of acid phosphatase as a reference enzyme. Acid phosphatase is a lysosomal enzyme (de Duve, 1958) and, if deoxyribonuclease II activity found in preparations of purified nuclei is due to contamination with lysosomes (de Duve, 1958; de Duve *et al.* 1962), then there should be a similar contamination by acid phosphatase, and the ratio of deoxyribonuclease II to acid phosphatase should remain constant. If, however, the deoxyribonuclease II activity found in the nuclei is not due to lysosomal contamination, the ratio of deoxyribonuclease II to acid phosphatase should increase markedly. The purification steps of calf thymus nuclei, their total activities of deoxyribonuclease II and acid phosphatase, and the ratio of deoxyribonuclease II to acid phosphatase in each step are summarized in Table 1.

The ratio of deoxyribonuclease II to acid phosphatase (Table 1) was increased from 0.26 to 7.6 in the final preparation of nuclei. This ratio could have been increased to 10.8 when nuclei were further purified on two layers to 1.6 M- and 2 M-sucrose (Allfrey *et al.* 1964). However, this step caused considerable breakage of the nuclei. The total increase in the ratio of deoxyribonuclease to acid phosphatase was about 30–40-fold. This increase by itself is very great, but it represents only the minimal value, assuming that there is no acid phosphatase in the nucleus at all, which is probably not true. If there is some acid phosphatase present

Table 1. *Purification of calf thymus nuclei, deoxyribonuclease II and acid phosphatase activities*

Enzymic assays and definition of units are described under 'Materials and Methods' in the text. The results are for a purification starting from 35 g of homogenate.

Step	Total deoxyribo- nuclease II activity (units)	Total acid phosphatase activity (units)	Deoxyribonuclease II/ acid phosphatase activity ratio	Increase in deoxyribonuclease II/ acid phosphatase activity ratio
1. Homogenate	158 000	607 000	0.26	1
2. Sediment of centrifuged homogenate	210 000	49 400	0.66	2.54
3. Washed sediment from step 2	23 600	10 900	2.16	8.3
4. Sediment after 2M-sucrose step (purified nuclei)	28 500	4 040	7.6	29.9

in the nucleus, than our value of 30–40-fold should have been higher.

Discussion. There is increasing evidence for the assumption that some deoxyribonucleases are involved in the replication of DNA. Deoxyribonuclease II activity is higher in regenerating liver than in non-regenerating liver (Allfrey & Mirsky, 1952). Usually, although not invariably, cancer cells show higher deoxyribonuclease II activity than do normal control cells (Cordonnier & Bernardi, 1968). A correlation between the capacity of certain tissues to divide and the activity of acid deoxyribonuclease was suggested on the basis of a comparative study on acid deoxyribonuclease from various sources (Allfrey & Mirsky, 1952; Cordonnier & Bernardi, 1968). Using mice, Kurnick & Kernen (1962) studied the fluctuation in deoxyribonucleases with aging, rate of cell reproduction and anabolism. They observed an increase in acid deoxyribonuclease activity of 5–6-fold in thymus and 3–4-fold in spleen during maturation. Slor (1970b) has found a peak of acid deoxyribonuclease that coincides with the peak of DNA synthesis in synchronized HeLa cells. Specific virus-induced deoxyribonucleases, both in mammalian cells (Hanafusa, 1961, 1962; McAuslan, 1965a,b; Eron & McAuslan, 1966) and in bacteria (Kunkee & Pardee, 1965; Stone & Burton, 1962; Oleson & Koerner, 1964; Bose & Nossal, 1964), have been observed. Stern (1960, 1961) found that there are periodic changes in the activities of deoxyribonucleases in lily anthers and concluded that 'the periodic induction of deoxyribonuclease activity is a mechanism for the morphogenetic development of the pollen mother cell'. Goutier & Goutier-Pirotte (1961) found that acid deoxyribonuclease activity in germinating pea seeds is highest in the root tips, where mitotic index is highest. Slor, Beaty & Hodes (1966) found that certain purine and pyrimidine derivatives, both naturally oc-

curing and base analogues, that are able to inhibit cell division or mitosis, are competitive inhibitors of acid deoxyribonuclease.

The above arguments, as well as others (Lehman, 1967; Lesca, 1971), supported by our finding that deoxyribonuclease II is not exclusively a lysosomal enzyme but is also found in the nucleus, suggest that deoxyribonucleases may play an important role in cell division, probably in the replication of DNA.

This research was supported in part by the Israel Cancer Association and by the Bat-Sheva De Rothschild Fund for the Advancement of Science and Technology, Jerusalem, Israel.

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