THE BINDING OF ALDOLASE TO ISOLATED NUCLEI

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Although there is considerable evidence suggesting that the nuclear membrane is freely permeable to macro-molecules, in particular proteins, one of the difficulties standing in the way of such a view is the work of HOGEBOOM AND SCHNEIDER[1] showing that even after isolation in aqueous media mouse liver nuclei still carried nearly all the soluble DPN synthesizing enzyme of the homogenate and that disruption of the nuclear membranes by sonic vibrations resulted in the release into solution of 74% of the enzyme and 65% of the total nitrogen of the nuclear fraction. This was interpreted as one case of a soluble enzyme (KORNBERG[2]) being retained within the nucleus by the nuclear membrane (see also HOGEBOOM AND SCHNEIDER[3]). As reported previously (ROODYN[4,5]), rat liver nuclei prepared by the method of HOGEBOOM, SCHNEIDER AND STRIEBICH[6] carried significant amounts of aldolase, a freely soluble enzyme (see for example HERBERT, GORDON, SUBRAHMANYAN AND GREEN[7]). The following work was done in order to obtain information as to how the enzyme was bound to the nuclei.

Nuclei for the experiments in Tables I and III were prepared by the method of HOGEBOOM *et al.*[6] and finally suspended in 0.25 *M* sucrose/0.00018 *M* $CaCl_2$. Aldolase was estimated by the liberation of alkali labile P from hexose diphosphate. The methods used are described in detail in the previous publications (ROODYN[4,5]).

The effect of treating the nuclei with ultrasonic vibrations for one minute is shown in Table I.

TABLE I

EFFECT OF ULTRASONIC VIBRATIONS ON ALDOLASE OF ISOLATED NUCLEI

Nuclei were prepared from 40 ml of a 20% rat liver homogenate and finally suspended in 0.25 *M* sucrose/0.00018 *M* $CaCl_2$. They were subjected to ultrasonic waves for one minute in a 500 watt Mullard Magnetostriction Ultrasonic Generator at 25 kc/sec and 3.8 amp. Suitable precautions were taken to keep the temperature near 0° during the treatment. Disrupted nuclei were spun ta 100,000 *g* for 30'. Values in columns 2 and 3 are expressed per mg of original nuclear nitrogen. Figures in parentheses percentage of value for disrupted nuclei.

Fractions	*Nitrogen*	*Aldolase (μmoles§ HDP/h)*	*Aldolase per mg nitrogen*	*Specific activity / Specific activity of disrupted nuclei*
Intact nuclei	1.00	62.9		0.86
Disrupted nuclei	1.00	74.0		1.00
Supernatant	0.077	2.46 (3.3)	32.0	0.43
Sediment	0.875	71.4 (96.5)	81.5	1.10
Recovery	0.952	(99.8)		

§ HDP: hexose diphosphate.

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This treatment resulted in the complete destruction of all the nuclei and only pieces of torn nuclear membrane, nucleoli and fine debris were visible under the microscope. It can be seen that only 3.3% of the total enzyme was released into solution, together with 7.7% of the total nitrogen.

Nuclei were then isolated in a hypotonic medium as shown in Table II.

TABLE II

ISOLATION OF NUCLEI IN A HYPOTONIC MEDIUM

A 5 % w/v homogenate of perfused rat liver was prepared in 0.06 *M* sucrose/0.00018 *M* $CaCl_2$. It was sedimented at 600 × *g* for 10 min. The pellet was resuspended in 0.00018 *M* $CaCl_2$ and sedimented at 600 *g* for 10 min. The resuspensions and sedimentations were repeated twice more. Results of fractionation expressed for the amount of liver containing one mg of total nitrogen or for the amount of fraction derived from this. Values in parentheses are percentage of value for original homogenate.

Fraction	*Total nitrogen (mg)*	*Aldolase μmoles HDP/h*	*Aldolase/mg nitrogen*	*Relative concentration*
Homogenate	1.00	27.3		
Combined supernatants	0.95	22.2 (81.3)		
Nuclear fraction	0.085	4.46 (16.3)	52.5	1.92
Recovery	1.035	(97.6)		

The results show that the nuclei still possessed considerable enzyme activity (the enzyme being nearly twice as concentrated in the nuclear fraction as in the original homogenate) after three sedimentations in 0.00018 *M* $CaCl_2$.

The effect of suspending isolated nuclei in saline solutions weakly buffered with sodium maleate was studied, and it was found that if the ionic strength was below 0.003 no enzyme was extracted from the nuclei over the range pH 5.5 to 6.9. Increasing the ionic strength to 0.17 resulted in the extraction of 90–95% of the enzyme after a maximum of 45 min in the presence of the extraction medium (Table III).

TABLE III

EXTRACTION OF ALDOLASE FROM ISOLATED NUCLEI

Nuclei suspended in buffered media of the following final composition 0.25 *M* sucrose: 0.00018 *M* $CaCl_2$: 0.005 *M* sodium maleate at pH 5.9 and 6.9. The ionic strength of both solutions was brought to 0.17 by addition of NaCl. 15 minutes after being suspended in the media at 0°, nuclei were centrifuged at 113,000 *g* for 30 min and supernatants and sediments assayed for aldolase. Results are given for fractions derived from the amount of nuclei that contained one mg of total nitrogen. The activity of the original nuclear fraction was 65.5 μmoles HDP split per hour per mg N. Figures in parentheses % of value for original nuclear fraction.

pH of extraction medium	*Fraction*	*Aldolase (μmoles HDP split per hour)*	
5.9	Supernatant	62.0	(95.0)
	Sediment	4.0	(6.1)
	Recovery	66.0	(101)
6.9	Supernatant	59.0	(90.0)
	Sediment	5.4	(8.2)
	Recovery	64.4	(98.2)

The results given above agree with the observations of DOUNCE[8] on the behaviour of

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the aldolase of nuclei isolated in diluted citric acid. He has reported that sonic vibrations (900 cycles per minute) resulted in little release of enzyme and that aldolase belongs to the "easily soluble protein fraction" of isolated nuclei, this fraction being dissolved by 0.9% NaCl at pH 6 to 7. This similarity of behaviour in nuclei prepared by two different methods of isolation is interesting. The results do not agree with the observation of HOGEBOOM AND SCHNEIDER[1] that sonic vibrations causes the release of 65% of the nitrogen from nuclei prepared in calcium chloride–sucrose media even though the nuclei were prepared by the same method. A possible reason is that the short ultra sonic treatment used in this work was sufficient to shatter all the nuclear membranes without causing a secondary "solubilization" of normally insoluble material, which might follow depolymerization of nucleic acid (ANDERSON[9], GOLDSTEIN AND STERN[10]).

The fact that nuclei isolated in a hypotonic medium still contain aldolase at a concentration of twice that of the original homogenate, and carry 16% of the total activity, suggests that at least this amount of enzyme is not retained within the nucleus by a semi-permeable membrane. In this connection it may be noted that ANDERSON AND WILBUR[11] demonstrated that sucrose exerts no appreciable osmotic effect on rat liver nuclei and that volume changes in isolated nuclei are considered by these workers to be due primarily to ionic effects on the nuclear colloids rather than to osmotic effects.

The above results indicate that, in the case of aldolase, salt linkages between enzyme and binding site (presumably nucleic acid), and not the integrity of the nuclear membrane, are responsible for the retention of enzyme within the nucleus.

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SUMMARY

Complete disruption by ultrasonic vibrations of rat liver nuclei isolated in calcium chloride-sucrose media does not result in release of bound aldolase. Nuclei also still retain significant amounts of aldolase even after isolation in a hypotonic medium. However, the enzyme is completely released by suspending intact nuclei in a medium of ionic strength 0.17. The results indicate that aldolase is bound to nuclei by salt linkages and is not retained by the nuclear membrane.

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