A ribonucleotide phosphorylase in chicken embryo

The ability of chicken-embryo slices or mince to incorporate certain nucleosides as enzymically formed nucleotides into the cellular DNA and the polynucleotide pyrimidines of RNA has been previously reported by FRIEDKIN *et al.*[1] and by REICHARD[2].

We have recently found a ribonucleotide-polymerizing enzyme in the homogenate from 9-day-old chicken embryos. The enzyme activity was found to be mainly in the material sedimented at 700 × *g*. The maximum activity of the crude enzyme, requiring the presence of Mg^{++}, was achieved at pH 7.2 but was still strong at pH 8.0, whereas at pH 9.4 no detectable activity could be observed. Since we performed our experiments with an unpurified fraction, no statement can be made as yet concerning the questions whether nucleotide diphosphates or triphosphates represent the only substrate for the polymerase, or whether there is need for a highly polymerized nucleotide strand as primer in the RNA-synthesizing process[3-6]. The presence of a nucleotide polymerase in embryonic extract, however, may help to elucidate its importance for cellular growth.

All experiments were performed in duplicate and measurements made against a control in which the enzyme fraction had been preheated at 100° for 3 min.

TABLE I

SPECIFIC ACTIVITIES OF THE SOLUBLE NUCLEOTIDE FRACTIONS (NORIT FRACTIONS)

The complete system contained in a total of 1.25 ml: 160.0 μmoles Tris-HCl buffer of appropriate pH, 2.0 μmoles $MgCl_2$, 0.2 μmole EDTA, 10.0 μmoles K^+, 5.0 μmoles ADP, AMP or ATP, 0.1 μmole KH_2PO_4, 20.0 μC ^{32}P ($3.1 \cdot 10^6$ counts/min), 0.25 ml corresponding to 660–725 μg protein of the homogenate or of the homogenate fraction in 0.25 *M* sucrose.

	Counts/min/μg P		
	pH 7.2	*pH 8.2*	*pH 9.5*
Complete system	1260	600	60
omit Mg	120	10	3
omit enzyme	0	0	0
omit ADP		negligible	
replace ADP by:			
ATP*	1050	265	8
AMP	18	10	2
Control	3	2	2

* The sample contained chromatographically detectable amounts of ADP.

After the reaction had been completed (60 min at 36.5°), the acid-insoluble material was precipitated by cold 0.3 *M* $HClO_4$. An aliquot of the supernatant after centrifugation—usually 0.1–0.2 ml—was adsorbed by partly deactivated Norit. Removal of the non-adsorbed material was accomplished by several washings with water initially containing traces of inorganic phosphorus. The adsorbed nucleotides were eluted with 50% ethanol containing NH_3 (pH 10.2). Table I shows the specific activity of the eluate at various pH's.

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid (dinatrium salt); ADP, adenosine diphosphate; AMP, adenylic acid; ATP, adenosine triphosphate; IMP, inosine monophosphate; IDP, inosine diphosphate.

In order to determine the localization of the polymerizing enzyme in the cell as well as that of the radioactivity of the compounds formed during enzymic action, a chromatographic separation of the Norit eluate from an experiment at pH 7.2 with ADP as substrate was performed with various homogenate fractions. After

TABLE II

LOCALIZATION OF POLYMERIZING ENZYME IN CHICKEN-EMBRYO CELL

	AMP	*ADP*	*ATP*	*IMP*	*IDP*
Whole homogenate	none*	2205	2630	—	
	50*	25	22**	120**	
Nuclear fraction	none	700	1640	negligible	—
(700 × *g* sediment)	170	220	155	20	traces
Mitochondrial fraction	none	60	430	negligible	—
(10,000 × *g* sediment)	145	215	90	not det.	—
Cytoplasmic fraction	none	45	80	—	60
(10,000 × *g* supernatant)	76	125	130**	98**	98**

* Top figure indicates specific activity (counts/min/μg P); bottom figure indicates mμmoles nucleotide present.

** Chromatographically not separated, calculated from adsorption data.

concentration *in vacuo*, the Norit eluates were applied to paper (Whatman No. 1) for chromatography in the butyric acid–NH_3 system. Spots were developed by u.v. light, cut out, and eluted with 0.02 *N* HCl. The respective adsorption data in the 250–290 mμ region as well as the phosphorus content were determined, and revealed the presence of AMP, ADP, ATP, IMP, and IDP. Correspondings samples of reference substances were run simultaneously.

From the appearance of radioactive ATP in nearly equimolar amounts with inactive AMP (Table II), it can be concluded that a strong myokinase in addition to the nucleotide phosphorylase is present in the nuclear fraction, whereas in the cytoplasmic supernatant, where no polymerase activity is recorded, the deaminase action leading to inosine nucleotides becomes dominant.

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