Short Communications

Guinea-Pig Liver Polynucleotide Phosphorylase

By P. S. FITT and YEW PHEW SEE

Department of Biochemistry, University of Ottawa, Ottawa 2, Ont., Canada

(Received 3 November 1969)

Polynucleotide phosphorylase has been isolated from a wide variety of bacteria (Grunberg-Manago, 1963). Similar activities have also been detected in guinea-pig liver nuclei (Hilmoe & Heppel, 1957), *Ascaris lumbricoides* (Entner & Gonzalez, 1959), human sperm and urine (Hakim, 1959), rat epithelioma (Yagi, Ozawa & Konogi, 1959), HeLa-cell nuclei (Harris, 1963), wheat seedlings (Kessler & Chen, 1964), rat liver nuclei (Siebert *et al.* 1966) and extracts of rat liver and brain (Murthy, Bharucha & Raynaud-Jammet, 1969). However, the results could have been due to combinations of other enzymes (Smellie, 1963), and no unequivocal evidence exists for the presence of polynucleotide phosphorylase in animal or plant cells. We now describe the partial purification and proof of specificity of a polynucleotide phosphorylase from guinea-pig liver nuclei.

Crude liver nuclei from fed male guinea pigs were prepared in 2.2M-sucrose–10mM-tris–HCl buffer, pH 7.4 (Chauveau, Moulé & Rouiller, 1956). Three extractions with 0.25M-sucrose–1mM- β -mercaptoethanol–10mM-tris–HCl buffer, pH 7.4, then two with 0.14M-NaCl–1mM- β -mercaptoethanol, pH 7, yielded active sucrose and saline extracts. These extracts were dialysed against 1mM- β -mercaptoethanol–2mM-EDTA, pH 7 ('BME-EDTA'). After removal of insoluble material by centrifugation, the sucrose extract contained 3494 units (specific activity 3 units/mg) and the saline extract 1933 units (specific activity 19.4 units/mg). The sucrose extract was purified by (i) precipitation with acetone (48%, v/v) and (ii) pH 5.7 precipitation. The precipitates were dissolved in 2mM-EDTA–1mM- β -mercaptoethanol–10mM-imidazole–HCl buffer, pH 7.2 ('imidazole buffer'). After (i), the solution was dialysed overnight against BME-EDTA. After (ii), the enzyme was adsorbed on a DEAE-cellulose column, which was washed with imidazole buffer until no more protein emerged. Stepwise elution with NaCl in imidazole buffer, followed by dialysis of the eluates against BME-EDTA, gave two active fractions eluted by 0.1M- and 0.2M-NaCl. During dialysis, fine granular precipitates containing all

the activity formed. They were both collected, washed three times with BME-EDTA and resuspended in BME-EDTA (1ml) to give the final fractions, E₁ and E₂. For protein and nucleic acid determinations, the precipitates were dissolved by addition of sodium dodecyl sulphate (final concn. 50mM). Enzymic activity was determined with the suspensions, because the detergent interfered with the assays. Fraction E₁ contained 132 units (specific activity 710 units/mg). Fraction E₂ contained 19 units (specific activity 290 units/mg). The purification has been repeated successfully several times.

The phosphorolysis assay medium (0.1ml) contained: 0.1M-imidazole–HCl buffer, pH 7.2; 10mM-MgCl₂; 1mM- β -mercaptoethanol; 10mM-K₂H³²PO₄ (about 30000 c.p.m./ μ mol); poly A, 1.5mg/ml; enzyme, up to 2.7 units. After 3 h at 37°C the reaction was stopped with 1% (w/v) charcoal in 2.3% (w/v) HClO₄ (1ml). The charcoal was collected, washed and dried, and its radioactivity was determined (Fitt & Fitt, 1967). A unit was defined as the amount of enzyme catalysing incorporation of 1nmol of [³²P]P_i into charcoal-adsorbable material/h.

Fractions E₁ and E₂ contained over 20% (w/w) of nucleic acid and had similar enzymic properties. They required Mg²⁺ and catalysed phosphorolysis of polyribonucleotides at rates that decreased with the polymer in the order poly A > poly C > poly U, poly G and RNA. DNA was not phosphorolysed. Both fractions were inhibited 55% by 50mM-NaCl and 100% by 0.2M-NaCl.

Table 1 (Expts. a, b and c) shows that NDP is the major radioactive product formed during incubation of either fraction E₁ or fraction E₂ with polyribonucleotides and [³²P]P_i in phosphorolysis conditions. Little NTP is formed. The amount of radioactive NMP produced is low, and probably less than shown: some hydrolysis of [³²P]NDP to NMP and [³²P]P_i occurs during evaporation of the final ammoniacal solution, and the t.l.c. system cannot separate them.

Four reactions might lead to formation of NDP

Table 1. *Specificity of guinea-pig liver polynucleotide phosphorylase*

In method A the reaction mixture (0.3 ml) contained: 0.1 M-imidazole-HCl buffer, pH 7.2; 10 mM-MgCl₂; 1 mM-β-mercaptoethanol; 10 mM-K₂H³²PO₄ (about 30000 c.p.m./μmol); poly A or poly C, 1.5 mg/ml; fraction E₁, 92.5 μg/ml, or fraction E₂, 65 μg/ml (in one case fraction E₁, 46 μg/ml, and fraction E₂, 32.5 μg/ml, together), or saline extract (0.74 mg/ml). Toluene was added to prevent microbial contamination. Incubation was at 37°C for 12 h. Charcoal (1%, w/v) in 2.3% (w/v) HClO₄ (1 ml) was added. The mixture was kept at 0°C for 10 min, and 1.25 mM-KH₂PO₄ in 1% (w/v) HClO₄ (2 ml) then added. The charcoal was collected and washed five times with 1.25 mM-KH₂PO₄ in 1% (w/v) HClO₄ (3 ml) and three times with water (3 ml). It was transferred with water (1 ml) to a Pasteur pipette fitted with a cotton-wool plug covered with cellulose powder. The nucleotides were eluted with 2% (w/v) NH₃ in 50% (v/v) ethanol. The eluate was evaporated to about 50 μl under partially reduced pressure at room temperature and applied, together with non-radioactive NMP, NDP and NTP (0.01 μmol of each), to a t.l.c. plate coated with Serva TLC-DEAE-cellulose. The chromatogram was developed with 4% (w/v) formic acid (Fahn, Albers & Koval, 1965). The nucleotides were located with u.v. light, and the spots were scraped off and the radioactivities counted (liquid scintillation). A control without enzyme was run exactly as above and the results were corrected accordingly. In method B the reaction and control mixtures (0.1 ml) had the same composition as in method A except that they contained (i) non-radioactive K₂HPO₄ and (ii) 1 mM-[¹⁴C]AMP, [¹⁴C]ADP or [¹⁴C]ATP instead of polyribonucleotide. After 12 h at 37°C samples (50 μl) were applied directly to t.l.c. plates, and the spots were separated and the radioactivities counted as above. The table shows the radioactivities (c.p.m.) found in NMP, NDP and NTP after incubation in the indicated conditions. The values in parentheses represent the radioactivities present in the compounds as percentages of the total radioactivity applied to the chromatogram.

Expt.	Enzyme	Substrate	P _i	Radioactivity products [c.p.m. (%)]		
				NTP	NDP	NMP
Method A						
<i>a</i>	E ₁	Poly A	[³² P]P _i	317 (2.2)	13168 (93)	565 (4)
<i>b</i>	E ₂	Poly A	[³² P]P _i	186 (1.2)	13240 (88)	1509 (10)
<i>c</i>	E ₁ + E ₂	Poly C	[³² P]P _i	114 (1.1)	8923 (88)	1112 (11)
<i>d</i>	Saline extract	Poly A	[³² P]P _i	83 (4.8)	1605 (92)	55 (3.2)
Method B						
<i>e</i>	E ₁	[¹⁴ C]ATP	P _i	15686 (83)	2192 (12)	987 (5)
<i>f</i>	E ₂	[¹⁴ C]ATP	P _i	12929 (86)	1153 (8)	917 (6)
<i>g</i>	E ₁	[¹⁴ C]ADP	P _i	3828 (13)	14624 (50)	11067 (37)
<i>h</i>	E ₂	[¹⁴ C]ADP	P _i	3203 (13)	14064 (61)	6503 (26)
<i>i</i>	E ₁	[¹⁴ C]AMP	P _i	0 (0)	682 (4)	16588 (96)
<i>j</i>	E ₂	[¹⁴ C]AMP	P _i	1 (0)	70 (0.5)	12422 (99.5)

from polyribonucleotides or their conceivable breakdown products in these conditions: (i) hydrolysis of NTP; (ii) phosphorylation of NMP; (iii) the action of an adenylate kinase-like enzyme on a mixture of NMP and NTP; (iv) phosphorolysis catalysed by polynucleotide phosphorylase. Reactions (i) and (ii) are excluded by the results in Table 1, which show that the amount of [¹⁴C]ADP formed from [¹⁴C]ATP (Expts. *e* and *f*) or [¹⁴C]AMP (Expts. *i* and *j*) was insufficient to account for the phosphorolysis results (Expts. *a*, *b* and *c*). The amount of [¹⁴C]ATP formed from [¹⁴C]ADP (Table 1, Expts. *g* and *h*) indicates that although some adenylate kinase was present it was insufficient to account for the phosphorolysis results even if both substrates could be formed. Reaction (iii) is therefore excluded. It can also be seen (Table 1, Expts. *g* and *h*) that a phosphatase able to hydrolyse [¹⁴C]ADP to [¹⁴C]AMP was still present. It would be expected to destroy part of the [³²P]NDP formed during phosphorolysis of polyribonucleotides, so that the measured amount of NDP produced

(Table 1, Expts. *a*, *b* and *c*) was certainly lower than the true value. Thus the results in Table 1 prove that fractions E₁ and E₂ contained a polynucleotide phosphorylase.

The particulate enzymes catalysed ADP-P_i and ATP-P_i exchange in the conditions of the phosphorolysis assay. However, the ratio of exchange to phosphorolytic activity fell tenfold during purification, so a separate enzyme may be responsible for the exchange activity. Both exchange activities were strongly inhibited by ApA. It should be noted that when poly A, ADP and ATP were all omitted from the phosphorolysis assay no [³²P]P_i was converted into a charcoal-adsorbable form. We have so far been unable to detect NDP-incorporation activity in any of the fractions, even though both fraction E₁ and fraction E₂ contained little or no nucleases. A bacterial polynucleotide phosphorylase with phosphorolytic activity only has been described (Thang, Thang & Grunberg-Manago, 1967, 1969).

It can be seen (Table 1, Expt. *d*) that the crude

saline extract contained an enzyme similar to fractions E₁ and E₂. We have also isolated similar enzyme fractions from sucrose and saline extracts of rat liver nuclei.

Although the guinea-pig enzyme is probably in the nucleus, some activity occurred in other fractions, and further studies will be needed to localize it precisely. However, the demonstration that certain animal cells do contain polynucleotide phosphorylase is of great interest because of the suggestion (Harris, 1963) that such an enzyme may be responsible for intranuclear degradation of rapidly labelled nuclear RNA (Harris, Fisher, Rodgers, Spenser & Watts, 1963; Harris, 1968).

We thank the Medical Research Council of Canada for an operating grant (to P.S.F.).

- Chauveau, J., Moulé, Y. & Rouiller, C. (1956). *Expl Cell Res.* **11**, 317.
- Entner, N. & Gonzalez, C. (1959). *Biochem. biophys. Res. Commun.* **1**, 333.
- Fahn, S., Albers, R. W. & Koval, G. J. (1965). *Analyt. Biochem.* **10**, 468.
- Fitt, P. S. & Fitt, E. A. (1967). *Biochem. J.* **105**, 99.
- Grunberg-Manago, M. (1963). *Prog. nucleic Acid Res.* **1**, 93.
- Hakim, A. A. (1959). *Enzymologia*, **21**, 81.
- Harris, H. (1963). *Proc. R. Soc. B*, **158**, 79.
- Harris, H. (1968). *Nucleus and Cytoplasm*, 1st ed., pp. 49-55. London: Oxford University Press.
- Harris, H., Fisher, H. W., Rodgers, A., Spenser, T. & Watts, J. W. (1963). *Proc. R. Soc. B*, **157**, 177.
- Hilmoe, R. J. & Heppel, L. A. (1957). *J. Am. chem. Soc.* **79**, 4810.
- Kessler, B. & Chen, D. (1964). *Biochim. biophys. Acta*, **80**, 533.
- Murthy, M. R. V., Bharucha, A. D. & Raynaud-Jammet, C. (1969). *Can. J. Biochem.* **47**, 283.
- Siebert, G., Villalobos, J., jun., Ro, T. S., Steele, W. J., Lindenmayer, G., Adams, H. & Busch, H. (1966). *J. biol. Chem.* **241**, 71.
- Smellie, R. M. S. (1963). *Prog. nucleic Acid Res.* **1**, 27.
- Thang, M. N., Thang, D. C. & Grunberg-Manago, M. (1967). *Biochem. biophys. Res. Commun.* **28**, 374.
- Thang, M. N., Thang, D. C. & Grunberg-Manago, M. (1969). *Eur. J. Biochem.* **8**, 577.
- Yagi, K., Ozawa, T. & Konogi, H. (1959). *Nature, Lond.*, **184**, 1939.