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Evidence for the presence of oligophosphoglyceroyl-ATP in rat kidney

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The inability to account for large systematic variations in total purine nucleotide content of perfused rat hearts led to the demonstration that the soluble adenine nucleotides are in rapid equilibrium with a highly phosphorylated hetero-oligomeric derivative whose structure appears to be 3-phospho[glyceroyl-γ-triphospho-5′-adenosine-3′-3-phospho]$_4$glyceroyl-γ-triphospho-5′-adenosine [Hutchinson, Morris & Mowbray (1986) Biochem. J. **234**, 623–627]. Analogous techniques to those used with hearts for specifically labelling tissue purine nucleotides followed by extraction and purification of nucleotides from the trichloroacetic acid-precipitable fraction show the existence of a corresponding rapid equilibrium between ATP and an oligomeric tetraphosphoadenosine derivative in perfused kidneys.

INTRODUCTION

Large systematic variations in the contents of the soluble purine nucleotides in Langendorff perfused rat hearts extracted at time points 10–20 min apart could not be explained as interconversion with other known purine derivatives or macromolecules [1–3]. We suggested, therefore, that some unknown derivative(s) must exist capable of rapid exchange with the soluble nucleotides. Selective radiolabelling of heart purines showed that 10–15% of tissue radioactivity was, at very short times, incorporated into an acid/alcohol-precipitable form [4]. Whereas about 10% of this insoluble label is present in rapidly-turning-over RNA (P. C. Heyworth, W. L. Hutchinson & J. Mowbray, unpublished work), the bulk is present in a novel compound which appears to be a short phosphate-linked co-polymer of adenosine and glyceric acid for which we have proposed a structure [5] and the name of oligophosphoglyceroyl-ATP (PG-ATP). Here we present experiments with perfused rat kidneys, analogous to those conducted with rat heart, which suggest that PG-ATP is also an actively metabolized intermediate in kidney.

EXPERIMENTAL

Materials

Nembutal was purchased from Sigma, Poole, Dorset, U.K., sodium heparin from Weddel Pharmaceuticals, London E.C.1, U.K., bovine serum albumin fraction V from Miles Laboratories, Slough, Berks., U.K., mannitol from BDH, Poole, Dorset, U.K., and 8-[^{14}C]adenosine (58 Ci/mol) from Amersham International, Amersham, Bucks., U.K. All other materials were as previously described [5,6].

Kidney perfusion

Male Wistar rats (350–400 g body wt.) were used. The animals had free access to food and water before the experiment and were anaesthetized with an intraperitoneal injection of Nembutal (6 mg/100 g body wt.). To facilitate cannulation of the ureter, an osmotic diuresis was produced by the intravenous injection of 1 ml of 10% (w/v) mannitol. Immediately before cannulation of the renal artery, 200 units of heparin were injected intravenously. The right renal artery was cannulated via the superior mesenteric artery without interruption of flow and the kidney (1–1.1 g of wet tissue) perfused as an isolated preparation using a pulsatile flow apparatus as described by Ross [6]. The perfusate was Krebs–Henseleit saline containing 67 g of bovine serum albumin/litre, gassed with O_2/CO_2 (19:1). The medium was supplemented with 5 mM-glucose, and 0.01% streptomycin sulphate was added to inhibit bacterial growth. Kidneys were recirculation-perfused with this medium in the presence of 0.3 μM-8-[^{14}C]adenosine for 10 min at a flow rate of 23–35 ml/min per g wet weight and pressure equivalent to 12.8–13.3 kPa (96–100 mmHg) distal to the cannula. The kidneys were then washout-perfused with adenosine-free medium for 5 min, after which recirculation was allowed to proceed for 10, 20 or 30 min. Kidneys were freeze-clamped at liquid-N_2 temperature at the end of these periods. The frozen tissue was ground up, denatured in 10% (w/v) trichloroacetic acid/25% (v/v) methanol and treated to separation, phenol extraction and radioactivity estimation exactly as previously described for perfused hearts [1,4].

RESULTS AND DISCUSSION

Kidney perfusions

Urine flow (51 ± 9 μl/min), glomerular filtration rate (creatine clearance 0.60 ± 0.15 ml/min) and oxygen consumption (5.4 ± 1.1 μmol/min) were all monitored during perfusion and the preparations were all stable with respect to these indices and consistent with values for the model [7]. As with hearts [8,9], radioactivity was rapidly incorporated into total kidney nucleotides ($\sim 4 \times 10^5$ d.p.m./g of wet tissue) by the short labelling

Abbreviation used: PG-ATP, oligophosphoglyceroyl-ATP.

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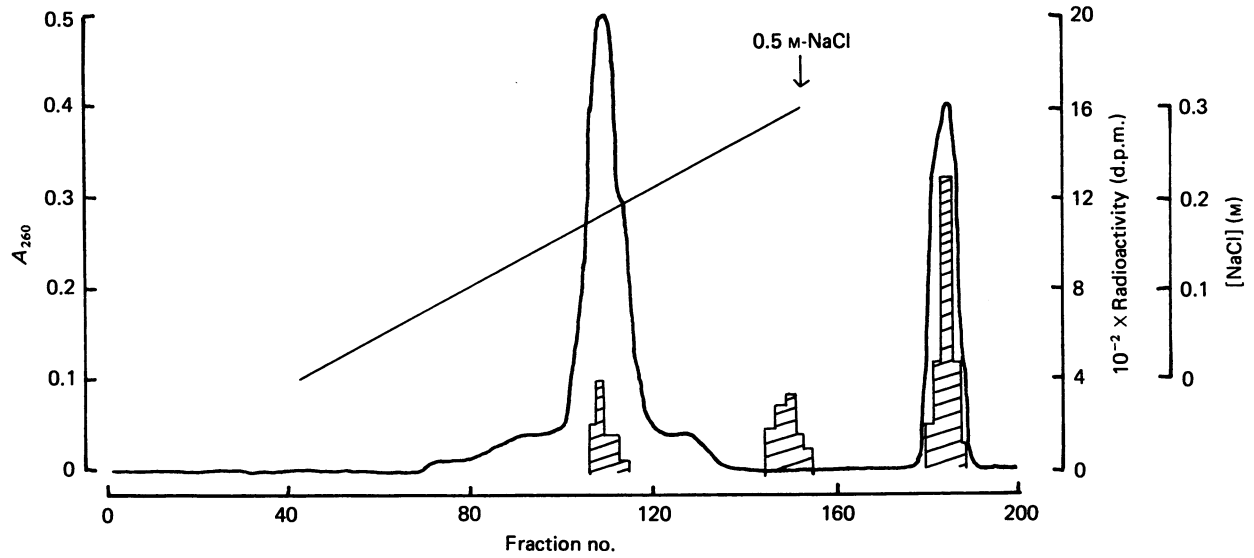

Fig. 1. Anion-exchange chromatography of the acid/alcohol-precipitable nucleotide fraction from kidney

Alcohol-precipitated material from the phenol extracts from the acid/alcohol-insoluble fractions of three kidneys was dissolved in less than 0.5 ml of 1 mM-EDTA/7 M-urea/10 mM-sodium acetate buffer, pH 4.5, applied to an 18 cm × 1 cm column of DEAE-cellulose and washed on with 50 ml buffer. The column was developed with a linear gradient of 0–0.3 M-NaCl in the same buffer at 25 ml/h, and 0.9 ml fractions were collected. At 0.3 M-NaCl the salt gradient was replaced by 0.5 M-NaCl and a further 40 fractions were collected. Radioactivity (▨) was assayed in 50 μl samples.

regime using [^{14}C]adenosine followed by washout of the unphosphorylated nucleoside. Around 7% of the incorporated activity was recovered in the acid/alcohol precipitate. This proportion was not appreciably altered by extending the recirculation period after adenosine washout (see under 'Kidney perfusion' in the Experimental section) from 10 to 20 or 30 min. This agrees very well with the findings reported for heart [4, 8] and implies that, in kidney too, rapid specific-activity equilibrium is established between the acid-insoluble material and the soluble nucleotides.

Phenol extraction

The labelled acid-insoluble material was subjected to the buffered phenol/chloroform/isopentanol extraction procedure used to separate heart nucleotide-rich material from precipitated protein [4]. Initial solubilization, even with sonication [4], proved more difficult than with hearts and only about 40–50% of the radioactivity was readily recovered in the aqueous fraction. This aqueous fraction was shown by later specific-activity measurements on the DEAE-cellulose-purified material (see below) to correspond to amounts of purine base ranging from 0.24 to 0.36 μmol/g of tissue, though the true quantity of this nucleotide derivative in kidney may be up to twice this amount because of inefficient extraction.

DEAE-cellulose chromatography

A sample of the crude aqueous phenol fraction from pooled preparations was applied to a DEAE-cellulose column (20.7 cm × 1.8 cm) in 10 mM-sodium acetate/7 M-urea, pH 4.5, containing 1mM-EDTA. The column was run at 24 ml/h and, at the appropriate time, a 0–0.3 M-NaCl gradient was applied in the buffer. Finally a 0.5 M-NaCl wash was applied to remove any remaining material. Fig. 1 presents the results from such a chromatogram. Two minor radiolabelled peaks were eluted before 0.3 M-NaCl: one coincident with the main u.v.-absorbing fractions and a second eluted in the position near to that expected for PG-ATP under these conditions. More than 65% of the label was obtained in the 0.5 M-salt fraction. This implies that the material is somewhat more polar than the principal PG-ATP species purified from hearts [5], though like the less-abundant, perhaps higher-M_r, form also found in hearts (P. C. Heyworth, W. L. Hutchinson & J. Mowbray, unpublished work). The peak fractions of this major form were pooled and desalted on a Sephadex G-15 column. The desalted material was in turn pooled and concentrated by freeze-drying down to 0.9 ml. The specific radioactivity of this purified fraction in the example shown was 29.9×10^3 d.p.m./μmol. This compares with specific radioactivities in precursor ATP within the range $(28.3–31.1) \times 10^3$ d.p.m./μmol and agrees with the specific-radioactivity equilibration implied by the lack of effect of prolonging the perfusion time discussed above.

Measurement of the phosphate/purine ratio of samples of this purified fraction gave a value of 3.96:1, just as we have reported finding for PG-ATP [4,5]. Although the chromatographic behaviour of this kidney material and of its breakdown products coincide with those of heart PG-ATP [4,5; P. C. Heyworth, W. L. Hutchinson & J. Mowbray, unpublished work], no attempt has yet been made to purify sufficient of it to show that it contains ribose, glycerate and unmodified adenine in addition to phosphate.

CONCLUSION

Kidney very rapidly incorporates [^{14}C]purine precursor into an acid/alcohol-precipitable species, which may be present in quantities ~ 0.7 μmol/g of tissue, reaches rapid specific-radioactivity equilibrium with ATP, has a

phosphate/purine ratio of 4 and is able to be purified by exactly the same procedures as those used for PG-ATP, the novel oligomeric glyceroyladenosine tetraphosphate derivative found in rat heart.

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