Short Communications and Preliminary Notes

NUCLEOTIDE COMPONENTS OF THE MAMMARY GLAND

by

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RUTTER AND HANSEN[1] have demonstrated the presence of UDPG** in rat mammary gland by enzymic techniques, while an enzymic comparison of the UDPG content of mammary gland with that of rat and chicken liver showed little difference in the UDPG content of a tissue actively concerned with the formation of galactose and tissues which were not.

In the present communication, the authors have made a preliminary study of the nucleotide components of guinea pig mammary gland by ion exchange and paper chromatography, and have succeeded in isolating greater amounts of UDPG from mammary gland than that obtained by identical means from the liver of the corresponding animal. A preliminary account is also given of some of the nucleotide components of the mammary gland.

The excised mammary glands from a 14 day lactating guinea pig were washed well with ice-cold water to remove as much milk as possible, and subjected to an ice-cold perchloric acid extraction. The extracts were neutralised to pH 7 with KOH, $KClO_4$ removed by centrifugation, the supernatant adjusted to pH 9 with NH_4OH and applied to a Dowex 1 Cl' column. The column was washed well with water and successively eluted with 0.005 *N* HCl, and 0.01 *N* HCl containing 0.01, 0.03, 0.05 and 0.1 *N* NaCl respectively. Each fraction was collected in bulk, activated charcoal added to adsorb the nucleotides, and the adsorbed nucleotides re-eluted from the charcoal with 50% ethanol containing a trace of ammonia. Careful checks were carried out to ensure that complete adsorption and re-elution of the nucleotides was obtained.

The ethanol extracts were subsequently evaporated to a small volume in a current of cold air and aliquots analysed by paper chromatography in the neutral ethanol-ammonium acetate solvent of PALADINI AND LELOIR[2]. The nucleotide components of each fraction were identified and found to be distributed as follows.

0.005 N HCl eluate: AMP and UMP were found in this fraction, and identified by chromatographic comparison[2] with authentic samples and by chromatography in *iso*propanol-HCl (WYATT[3]) after hydrolysis in 1 *N* HCl for 1 hour.

0.01 N HCl/0.01 N NaCl eluate: Separation of the nucleotide components of this fraction by paper chromatography[2] indicated the presence of ADP. This component was further identified enzymically by the method of BERG AND JOKLIK[4], and by hydrolysis to the free base with chromatography in *iso*propanol-HCl[3]. Two phosphorus containing components were also obtained which, on hydrolysis with *N* HCl and subsequent chromatography[3] were shown to be cytosine and guanine compounds with typical absorption maxima at 275 mμ and 250 mμ respectively. In the neutral ethanol-ammonium acetate solvent[2], these compounds were chromatographically identical with authentic CMP and GMP.

0.01 N HCl/0.03 N NaCl: UDPG was found in this fraction and was identified enzymically by pyrophosphorylation of the paper eluate in accordance with the reaction of MUNCH-PETERSEN, KALCKAR, CUTOLO AND SMITH[5]. By this spectrophotometric assay, the eluate was shown to contain 70% UDPG. It is considered probable that the remaining 30% unaccounted for by enzyme assay may be UDPGal which is chromatographically identical with UDPG in this solvent.

0.01 N HCl/0.05 N NaCl: A nucleotide component was obtained in this solvent which, on hydrolysis with *N* HCl and subsequent chromatography[3], was shown to be a guanine compound with a typical absorption maximum at 250 mμ. Insufficient material was present to conduct a complete analysis, but the chromatographic location of this compound in the neutral ethanol-ammonium

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** The following abbreviations are used throughout: UDPG = uridine diphosphate glucose, UDPGal = uridine diphosphate galactose, UMP = uridine 5′-monophosphate, AMP = adenosine monophosphate, ADP = adenosine diphosphate, ATP = adenosine triphosphate, CMP = cytidine monophosphate, GMP = guanosine monophosphate.

acetate solvent[2] combined with the point of elution from the Dowex Cl′ column suggests that it may be guanine diphosphate.

0.01 N HCl/0.1 N NaCl: The sole component of this fraction was ATP which was determined by base analysis and by enzymic assay[6].

Several other unidentified components were located in 0.01 *N* HCl/0.03 *N* NaCl and 0.01 *N* HCl/0.05 *N* NaCl which will be the subject of a future detailed communication.

While the amount of UDPG isolated from mammary gland is of the order of 15 μ moles per 100 g, the amount isolated from the liver of the guinea pig under identical conditions was rarely in excess of 5 μ moles per 100 g. This higher concentration of UDPG in the mammary gland suggests that it may be functional in lactose synthesis where it probably acts as coenzyme of the glucose–galactose transformation[7].

The fact that the UDPG isolated from mammary gland does not pyrophosphorylate to the theoretical amount of UDPG present (based on U.V. absorption) also suggests that UDPGal may be present in the mammary gland.

These observations will be the subject of further study.

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PHOSPHOPROTEIN PHOSPHATASE FROM RAT SPLEEN

by

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We have presented evidence recently for the presence of a specific phosphoprotein phosphatase in ox spleen[1]. Though we were able to separate the enzyme from phosphomonoesterases to a certain extent, the final preparation was slightly active against glycerophosphate. A purified preparation of phosphoprotein phosphatase from rat spleen has been found in the present investigation to be completely free from phosphomonoesterase activity. The action of the enzyme has been tested on several other substrates, including β-casein which has recently been found by PERLMANN[2,3] to be resistant to the action of phosphomonoesterases.

The preparation of the enzyme from rat spleen was effected according to the method previously described by us[1]. The final acetone fractionation step was however omitted. The specific activities of such preparations were of the order of 150 units and represented a 40-fold purification of the enzyme.

For the estimation of enzyme activity 0.2 ml of the purified preparation was mixed with 1 ml of substrate solution, 0.4 ml of 0.01 *M* thioglycollic acid (activator), 1 ml of pH 5.8 acetate buffer and water to a final volume of 4 ml. After incubation at 37° C for 3 hours, the reaction was stopped by the addition of 2 ml of 20% trichloracetic acid and the inorganic phosphorus estimated in the filtrate. The results obtained with the several substrates are summarised in Table I.

It will be evident that while all the phosphoproteins tested are dephosphorylated by this enzyme, no liberation of phosphorus takes place with glycerophosphate. Further, no activity could be demonstrated with the latter substrate by either a change of pH or by the addition of Mg^{+2}. Dephosphorylation of the phosphoproteins was very low in the absence of the activator. In this respect the enzyme resembles the phosphoprotein phosphatase from ox spleen[1].

It is of interest to note that the enzyme attacks all the three casein fractions employed with equal vigour. The pH-activity curves for the three substrates are also quite similar, maximum activity being obtained in each case at pH 5.8. Further, the MICHAELIS constants for their hydrolysis are the same (average value, 0.55 m*M* of protein P) proving thereby that they have the same affinity

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