Acid-soluble nucleotides of lactating mammary gland

The occurrence of UDPG* among the nucleotide components of rat[1], and guinea pig[2] mammary gland has led to the suggestion, that by acting as coenzyme in a glucose-galactose transformation of the type described by CAPUTTO *et al.*[3], it may be instrumental in effecting lactose synthesis[2]. As a preliminary to the investigation of this possibility existing in ruminants, a study has been made of the acid-soluble nucleotides present in lactating cow and goat mammary gland.

For the separation and identification of the nucleotides, use has been made of the method of HURLBERT *et al.*[4], whereby perchloric acid extracts of tissue are subjected to extended gradient chromatography on columns of anion exchange resin. The perchloric extracts were obtained in one case from 100 g mammary tissue of a 200 day lactating cow, and in the other from 50 g tissue of a 14 day lactating goat. Columns (18 × 1.6 cm) of Dowex 1 (formate) anion exchange resin (X-10, 200–400 mesh) were used for the separation of the nucleotides, gradient elution being carried out with formic acid increasing in concentration from zero to 4 *M*, followed by 4 *M* formic acid containing ammonium formate increasing in concentration from zero to 0.8 *M*. Fractions of approximately 10 ml were collected at 10 minute intervals and their optical density at 260 mμ and 275 mμ measured.

The distribution of the extracted nucleotides from the goat mammary tissue thus obtained is shown in Fig. 1; the nucleotides from the cow mammary tissue showed a similar pattern, except that fractions G and J were absent. The further separation and identification of the nucleotides is outlined below.

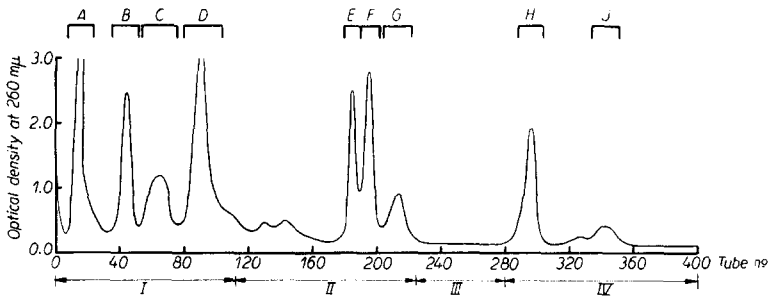

Fig. 1. Chromatography of the acid-soluble nucleotide fraction from 50 g of goat mammary gland. The optical density at 260 mμ is plotted for each 10 ml of eluate. For A the values too high to be plotted are 18, 37, 18 and 5, and for D are 3.5 and 3.5. (I) H_2O, 4 *M* formic acid; (II) 4 *M* formic acid + 0.2 *M* ammonium formate; (III) 4 *M* formic acid + 0.4 *M* ammonium formate; (IV) 4 *M* formic acid + 0.8 *M* ammonium formate.

The fractions bracketted together in Fig. 1 and the corresponding fractions of the cow mammary extract, after freeze-drying and resolution in water were separately rechromatographed on smaller columns (10 × 1 cm) of the same resin. Gradient elution was carried out with solutions of ammonium formate increasing in concentration from zero to 2 *M*, followed by 2 *M* ammonium formate containing formic acid increasing in concentration from zero to 0.75 *M*. The eluate was collected in 5 ml portions and the optical density at 260 mμ and 275 mμ measured. After rechromatography the constituents of fractions A to F and H were found to be qualitatively identical for the goat and the cow tissue. The identification of each constituent was based primarily on its chromatographic behaviour, ultra-violet spectrum at 260 and 275 mμ in acid and alkali[4,5], phosphorus content[6], and ribose content[4]. For confirmation, a direct comparison was made where possible with an authentic sample by paper chromatography in neutral ethanol/ammonium acetate[7]. The fractions were found to be constituted as follows:

Fraction A: On rechromatography the fraction was resolved into 3 main parts identified by the methods outlined above, as DPN, CMP, and AMP. The identification of DPN was confirmed by its spectrum in cyanide solution[8], and that of CMP and AMP by comparison with the behaviour of authentic specimens of CMP and AMP on paper chromatograms. In addition, a fourth substance

* The following abbreviations are used: UDPG = uridine diphosphate glucose, DPN = diphosphopyridine nucleotide, CMP = cytidylic acid, AMP = adenylic acid, GMP = guanylic acid, TPN = triphosphopyridine nucleotide, UMP = uridylic acid, CDP = cytidine diphosphate, ADP = adenosine diphosphate, UDP = uridine diphosphate, UDPAG = uridine diphosphate acetylglucosamine, UTP = uridine triphosphate.

present in traces only, and containing no phosphorus nor apparent ribose, was identified from its spectrum and chromatographic comparison with an authentic sample as orotic acid.

Fraction B: This consisted almost entirely of GMP, but also contained a small amount of TPN further characterised by its light absorbing properties in the presence of cyanide[8].

Fraction C: This contained largely UMP with a smaller amount of CDP. The identity of the UMP was checked by comparison with authentic UMP but no analogous comparison of the CDP was possible.

Fraction D: ADP and AMP were the only constituents of this fraction, the latter being presumably produced by breakdown of ADP. Direct paper chromatographic comparison with an authentic sample was made only with AMP.

Fraction E: Four substances were obtained on rechromatography of this fraction. Two were identified as UMP and UDP in the usual way, and by direct comparison with authentic specimens. The solutions containing the remaining two substances were adsorbed on washed, neutral charcoal and eluted with 50% alcohol containing *N*/100 ammonia. After concentration *in vacuo* the solutions were hydrolyzed with *N*/100 hydrochloric acid for 10 minutes at 100°. Each solution contained a reducing sugar[9]. That from the larger fraction, when chromatographed on paper[10], behaved in the same way as a specimen of N-acetyl-D-glucosamine, and gave a positive acetyl glucosamine reaction with *p*-dimethylamino-benzaldehyde[11]. The sugar component from the smaller fraction was identical with that obtained from fraction F below, and consisted of glucose and galactose[10].

The main sugar-containing component of fraction E on account of its ultra-violet light absorbing properties, phosphorus content, acetylglucosamine content and chromatographic behaviour was identified as UDPAG[12]. The minor constituent was similarly identified as UDPG[2], present as a contaminant from fraction F. The UDP and UMP isolated in this fraction can be supposed to arise by breakdown of UDPG and UDPAG.

Fraction F: On rechromatography, four substances were again obtained. In a manner identical to that described for fraction E, UMP, UDP, UDPG, and UDPAG were again identified. On this occasion however, UDPG was the main constituent.

Fraction G: This fraction occurring only in the goat tissue extract was not identified. It appears to contain adenosine and phosphate, and is eluted from the column at the same point as the Ad-X obtained by HURLBERT *et al.*[4] from rat liver extracts.

Fraction H: ATP was identified only in the fraction obtained from the goat tissue. The small amount originally present in the cow tissue extract had presumably broken down during the preparation for rechromatography, but traces of ADP and AMP were found in both extracts.

Fraction J: This fraction from goat tissue on rechromatography gave an extremely small sample which corresponded in its light absorbing properties and point of elution from the column to those of an authentic sample of UTP.

While a direct qualitative comparison shows little significant difference between the nucleotides from the two sources, quantitatively wide variations can be observed. Such a comparison is however probably of limited value since the tissue extracts were obtained from animals at different stages in lactation. It is nevertheless interesting to note that the concentrations of uridine-diphospho-compounds in the tissues were high. UDPAG, which has not hitherto been identified as a mammary gland constituent, was present in the goat and cow tissue to the extent of 25 μmoles and 12 μmoles per 100 g, and UDPG to the extent of 40 μmoles and 15 μmoles per 100 g respectively.

The possible significance of these high concentrations of UDPG in relation to lactose synthesis in ruminants is at present being investigated.

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