Molecular Weight of Human Placental Lactogen Investigated by Gel Filtration

By P. ANDREWS

National Institute for Research in Dairying, Shinfield, Reading, Berks.

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Placental lactogen, a protein secreted by the human trophoblast, shows marked similarities to human growth hormone in immunological properties (Josimovich & MacLaren, 1962), in amino acid composition (Friesen, 1965) and sequence (Catt, Moffat & Niall, 1967a; Sherwood, 1967) and in the possession of prolactin-like activity (Josimovich & MacLaren, 1962; Forsyth & Folley, 1968). In contrast, molecular-weight determinations for the two hormones suggest that the growth hormone exists as monomer whereas the placental lactogen has a dimeric structure. For the growth hormone, molecular weights of 21 500 obtained in the ultracentrifuge (Li & Starman, 1964) and 20 500 by gel filtration (Andrews, 1966), both without the use of dissociating solvents, are close to the value required for a single-chain structure (Li, Liu & Dixon, 1966). On the other hand, sedimentation analysis of the placental lactogen gave molecular weights of 38 000 in a non-dissociating solvent and 18 500 in 5M-guanidine hydrochloride, suggesting that the hormone molecule consists of two polypeptide chains of equal weight held together by hydrogen bonds (Florini *et al.* 1966). The results of Catt, Moffat, Niall & Preston (1967b) also imply a dimeric structure, but a molecular weight of 30 000 estimated by the gel-filtration method (Friesen, 1965) suggests the possibility of a ready dissociation.

Investigation of the gel-filtration behaviour of human placental lactogen now indicates that it does, in fact, exist in dilute solution as a monomeric form similar to that of human growth hormone.

Materials and methods. Purified human placental lactogen was generously provided by Dr P. H. Bell, Lederle Laboratories, Pearl River, N.Y., U.S.A. (lot no. 4508C-75), Dr K. Catt, Department of Medicine, Monash University, Melbourne, Vic., Australia (lot no. 37), Dr H. Friesen, Royal Victoria Hospital, Montreal, Que., Canada, and Dr M. M. Grumbach, School of Medicine, University of California, San Francisco, Calif., U.S.A. All four preparations were assayed for prolactin-like activity by Dr Isabel A. Forsyth, with both the rabbit mammary intraductal test (Chadwick, 1963) and a local method of pigeon crop-sac assay (Rivera, Forsyth & Folley, 1967). Activities in the rabbit test were about 24 i.u./mg. in each case, and in the pigeon assay only 2–4 i.u./mg. (Forsyth & Folley, 1968).

Gel filtration was performed at 2–5° with a column (50 cm. × 2.3 cm. diam.) of Sephadex G-100 [Pharmacia (G.B.) Ltd., London W.13] equilibrated with 5 mM-tris-HCl buffer, pH 7.5, containing 0.1 M-KCl. The column was calibrated for estimating protein molecular weights with cytochrome *c*, myoglobin, chymotrypsinogen, myoglobin dimer, ovalbumin, bovine serum albumin and human γ -globulins as described by Andrews (1964, 1965). Hormone samples (0.5–2 mg.), dissolved in 2 ml. of the equilibration buffer, were run either alone or mixed with approximately the same weight of serum albumin or half the weight of myoglobin dimer, the added protein acting as an internal standard. Column effluents were collected in 3 ml. fractions. Myoglobin and its dimer in the effluent fractions were determined by extinction at 412 nm. and the other proteins at 230 nm.

Results. As the biological assays showed no significant differences between the four lactogen preparations, the assumption was that they were all of approximately equal purity and that the heterogeneity of some samples (Fig. 1) was due to the hormone existing in different states of aggregation. The material of the more homogeneous preparations and part of the material of the heterogeneous preparations had elution volumes corresponding to molecular weights in the range 18 600–20 400 in different experiments. No systematic variation of the values was detected in the small concentration range investigated. The elution diagrams for the heterogeneous preparations (Figs. 1c and 1d) show two other peaks, one, eluted at about 115 ml., corresponding to material with a molecular weight of 38 000–40 000 and the other, eluted at the column void volume, corresponding to more highly aggregated material.

Even in the absence of dissociating agents, human placental lactogen evidently exists as a monomer in dilute solution at physiological pH. The molecular weight is probably 19 000–20 000, very similar to that of human growth hormone monomer. A monomer-dimer relationship between the two main components of the heterogeneous lactogen preparations is indicated, but the separation of the two forms, and the absence of dimer from some preparations, precludes the possibility of a rapid equilibrium between them. More probably the dimer and higher aggregates are stable, and analogous to minor

Fig. 1. Gel filtration on Sephadex G-100 of four different preparations of purified human placental lactogen. The amounts used were: (a) 0.8 mg., (b) 1.4 mg., (c) and (d) 2.0 mg. The elution volumes of proteins used to calibrate the column for molecular weight estimation are indicated by arrows, and the abbreviations are: cc, cytochrome c (mol.wt. 12400); ch, chymotrypsinogen (mol.wt. 25000); γ g, human γ -globulins (apparent mol.wt. 205000; see Andrews, 1965); mb, myoglobin (mol.wt. 17800); mb₂, myoglobin dimer (mol.wt. 35600); sa, bovine serum albumin (mol.wt. 67000).

constituents found in, for example, some sheep prolactin preparations (Squire, Starman & Li, 1963). Reversible association of the placental lactogen monomer in more concentrated solutions, proceeding at least as far as dimer formation, presumably accounts for the values of about 40000 obtained for its molecular weight (Florini *et al.* 1966; Catt *et al.* 1967b). Observations on the molecular weight of human placental lactogen now closely resemble those on several mammalian growth hormones, which also associate reversibly but basically appear to have monomeric structures with molecular weights of about 20000 (Andrews, 1966; Ellis, Marler, Chen & Wilhelmi, 1966; Santomé, Dellacha & Paladini, 1968). The placental lactogen and human growth hormone together conform to the uniformity in molecular size exhibited by prolactins and growth hormones from other mammalian species.

Andrews, P. (1964). *Biochem. J.* **91**, 222.

Andrews, P. (1965). *Biochem. J.* **96**, 595.

Andrews, P. (1966). *Nature, Lond.*, **209**, 155.

Catt, K. J., Moffat, B. & Niall, H. D. (1967a). *Science*, **157**, 321.

Catt, K. J., Moffat, B., Niall, H. D. & Preston, B. N. (1967b). *Biochem. J.* **102**, 27 c.

Chadwick, A. (1963). *J. Endocrin.* **27**, 253.

Ellis, G. J., Marler, E., Chen, H. C. & Wilhelmi, A. E. (1966). *Fed. Proc.* **25**, 348.

Florini, J. R., Tonelli, G., Breuer, C. B., Coppola, J., Ringler, I. & Bell, P. H. (1966). *Endocrinology*, **79**, 692.

Forsyth, I. A. & Folley, S. J. (1968). *Proc. 2nd int. Seminar Reproductive Physiology and Sexual Endocrinology, Brussels* (in the Press).

Friesen, H. (1965). *Endocrinology*, **76**, 369.

Josimovich, J. B. & MacLaren, J. A. (1962). *Endocrinology*, **71**, 209.

Li, C. H., Liu, W. L. & Dixon, J. S. (1966). *J. Amer. chem. Soc.* **88**, 2050.

Li, C. H. & Starman, B. (1964). *Biochim. biophys. Acta*, **86**, 175.

Rivera, E. M., Forsyth, I. A. & Folley, S. J. (1967). *Proc. Soc. exp. Biol., N.Y.*, **124**, 859.

Santomé, J. A., Dellacha, J. M. & Paladini, A. C. (1968). In *Growth Hormone*, p. 29. Ed. by Pecile, A. & Müller, E. E. Amsterdam: Excerpta Medica Foundation.

Sherwood, L. M. (1967). *Proc. nat. Acad. Sci., Wash.*, **58**, 2307.

Squire, P. G., Starman, B. & Li, C. H. (1963). *J. biol. Chem.* **238**, 1389.