CHROM. 6241

A microgel electrophoretic analysis of the proteins in single rat thyroid follicles

III. Incorporation of [3H]leucine into the large iodoproteins of the colloid

Gel electrophoresis of colloid samples from single rat thyroid follicles has demonstrated that the normal colloid always contains both 19S thyroglobulin and more slowly migrating proteins[1,2]. The latter might at least partly consist of 27S protein since it was demonstrated that this protein migrates more slowly in the gel than 19S thyroglobulin[3], and in a similar manner to the most slowly migrating band. However, densitometric recordings of the latter were often observed to contain shoulders indicating the presence of more than one molecular entity in the band. Since both molecular size and conformation as well as net charge density of the protein influence the migration it seems probable that a molecule with a lower molecular weight, but with a less dense structure than 19S thyroglobulin, could migrate more slowly in the gel matrix. The most slowly migrating band might therefore contain a low iodinated newly synthesized and consequently less dense[4], prethyroglobulin molecule. To investigate this the incorporation of a labelled amino acid into the large protein fractions in the colloid was studied.

Materials and methods

Male Sprague-Dawley rats (Anticimex, Sweden) weighing 200–250 g were used. The animals were fed on standard pellets (Astra-Ewos, Södertälje, Sweden) containing 10–15 mg iodine/kg pellet and tap water *ad lib*. The iodine content of the pellets was determined by activation analysis at AB Atom-energi, Studsvik, Sweden. 500 μCi [3H]leucine (L-[4,5-3H]leucine > 15,000 mCi/mmole, Radiochemical Centre, Amersham, Great Britain) was given to each animal by intravenous injection in a tail vein. For short term experiments (less than 1 h) the animals were anaesthetized and prepared for sampling of the colloid before the injection was given.

Sampling of colloid was performed *in vivo* during pentobarbital anaesthesia by micropuncture of single follicles as previously described in detail[3]. Colloid sampling was performed over a period of 30–45 min before the animal was killed. The sample of colloid was collected under liquid paraffin and transferred directly to a microgel electrophoresis capillary.

Separation of the proteins was performed by microgel electrophoresis in poly-acrylamide gels. Two types of gels were used: (i) 10 % polyacrylamide gels prepared as described earlier[2] and (ii) gels consisting of a 5–25 % linear polyacrylamide concentration gradient[5]. All the gels were cast in 5-μl glass capillaries (Microcaps, Drummond Sci, Co., Broomall, Pa., I.S.A.) and had a pH of 6.8. No spacer gels were used. The samples part of the electrophoresis capillary was filled with 0.01 M phosphate buffer containing 0.15 M NaCl (PBS) and 20% sucrose (w/w). Tris-glycine, pH 8.3, was used as electrode buffer. The electrophoreses were run with a square wave (75 V, 500 Hz and 25 % duty cycle) layered on an off-set voltage of 25 V (IB Electronic, Gothenburg, Sweden). The electrophoretic run was stopped when the tracking dye (Bromphenol Blue) had migrated 7 mm (10 % gels) or 5.6 mm (con-

centration gradient gels). The gels were pushed out from the electrophoresis capillaries, fixed in 80 % ethanol and stained with 0.5 % Amido Black in 7.5 % acetic acid. After destaining in 7.5 % acetic acid the gels were stored in acetic acid containing a small amount of Amido Black[3].

Densitometric recording of the electrophoretic separation patterns was performed directly from the gels with a Schnell photometer II (Jena, D.D.R.) equipped with a modified Vitatron UR 400 recorder[3]. Simultaneous with the recording of the optical density, the areas of the protein peaks were integrated and a staining unit (S. U.), directly correlated to the amount of protein, was calculated for each protein peak in the separation pattern[3,6].

The radioactivity of the proteins in the bands of the microgel was determined according to LARSSON[7]. The stained protein bands were cut out from the gels under a stereomicroscope ($\times$ 32) and were combusted together with Zn in glass capillaries. The radioactive free gas obtained was led into a Geiger-Müller tube which allowed measurement of tritium activities with an efficiency of 93 % and a background in the counting system of 2.5 c.p.m. with a S.D. of $\pm$ 0.03 c.p.m. A minimum of 1,000 counts per sample was counted.

TABLE I

RELATIVE ACTIVITY AND RELATIVE SPECIFIC ACTIVITY IN THE PROTEIN FRACTIONS REPRESENTING 19S THYROGLOBULIN (19S TG) AND MORE SLOWLY MIGRATING PROTEINS (> TG) IN SAMPLES OF COLLOID COLLECTED FROM SINGLE RAT THYROID FOLLICLES AT VARYING TIMES AFTER [^{3}H]LEUCINE ADMINISTRATION

Time (h)	Relative activity c.p.m. (%)		Relative specific activity c.p.m./S.U. (%)		N
	> TG	19S TG	> TG	19S TG	
0–1	79	21	94	6	2
1–2	63	37	84	16	6
2–3	33	67	55	45	4
7–8	6	94	12	88	2

Results

The incorporation of [^{3}H]leucine into 19S thyroglobulin and the more slowly migrating proteins was followed over a period of 8 h after the leucine administration. Samples of colloid collected during the first, second, third and eighth hour, respectively, were grouped together. At each time interval the radioactivity and amount of protein (staining unit, S. U.), were measured in each of the two protein bands in the gel. The specific radioactivities (c.p.m./S.U.) as well as the relative specific radioactivities in the two bands in each colloid sample were calculated. The means of the relative activity as well as the relative specific activity are given in Table I. From this it is evident that the measured activity and the relative specific activity of the most slowly migrating proteins were highest during the first hour and declined throughout the observation time. The relative specific radioactivity in the 19S thyroglobulin peak was low initially and increased progressively. After 7 to 8 h the relative specific

radioactivity was higher in the 19S thyroglobulin than in the more slowly migrating proteins.

Densitometric recordings of the protein separation patterns of the samples of colloid in 10 % polyacrylamide gels indicated that the most slowly migrating protein consisted of one, two or three poorly separated fractions. As a result of a better separation in concentration gradient gels the hetereogeneous composition was more clearly revealed (Fig. 1). The distribution of the specific radioactivies in the 19S thyroglobulin peak and in the fractions of the most slowly migrating peak was studied by measuring the activity of well separated fractions in two gradient gels. It was observed that after 18-min labelling, the highest relative specific activity was present in the larger of the two fractions, II in Fig. 1d, migrating immediately behind the 19S thyroglobulin band (Fig. 2a). In the other sample (Fig. 2b), collected about 7 h after the leucine administration, the highest specific activity was observed in the 19S band. In the more slowly migrating fractions all activity was localized in the band corresponding to fraction II in Fig. 1d.
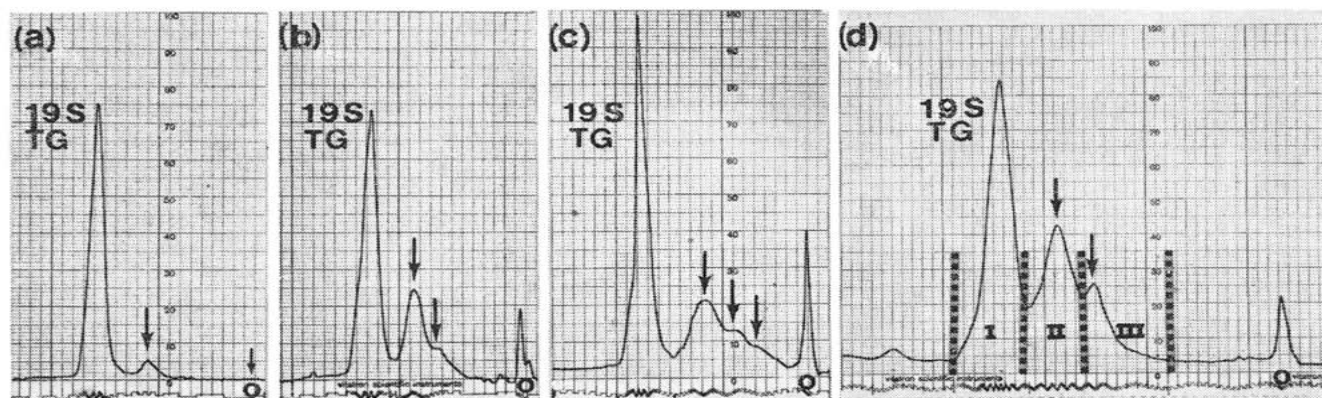

Fig. 1. Densitometric recordings of the first part of three 10 % acrylamide gels (a–c) containing a 19S band (19S TG) and a more slowly migrating band. Note the shoulders on the most slowly migrating band. Separation on continuous polyacrylamide concentration gradients (d) gives better resolution of the large protein components. o = Origin.
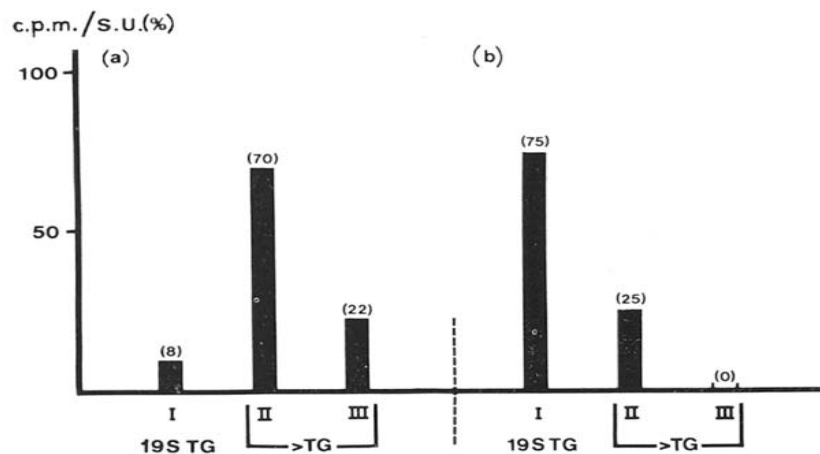

Fig. 2. The relative specific radioactivity calculated as c.p.m./S.U. in the three fractions (I–III) indicated in Fig. 1d, 18 min (a) and about 7 h (b) after the administration of [³H]leucine.

Discussion

After administration of [³H]leucine both 19S thyroglobulin and a complex of more slowly migrating proteins were found to be labelled. During the first 3 h the specific radioactivity was higher in the latter complex than in 19S thyroglobulin but at 7 h this relationship was inversed.

It has previously been demonstrated that 27S protein migrated more slowly than 19S thyroglobulin and in a similar manner to the most slowly migrating band[3]. The 27S molecule is probably formed by association of 19S thyroglobulin units and consequently aggregated after the synthesis of 19S thyroglobulin[8]. Therefore, the fact that the highest relative specific activity at short times after administration of [³H]leucine was found in the slower migrating band can probably not be attributed to the 27S molecule but to precursors in the 19S thyroglobulin synthesis.

Previous observations in this laboratory indicated that the more slowly migrating protein complex is less iodinated than 19S thyroglobulin[9]. Since the degree of iodination influences the density of the thyroglobulin molecule and a low iodinated molecule has a lower density than a normally iodinated 19S thyroglobulin molecule[4], it seems reasonable to assume that the former will be retarded in the gel. Therefore, the rapidly [³H]leucine-labelled protein fraction migrating immediately behind the 19S thyroglobulin most probably represents a newly synthesized and low iodinated prethyroglobulin molecule.

The significance of the shoulders of the main slowly migrating peak is uncertain. Control experiments showed that a purified 27S protein fraction migrates similarly to the most slowly migrating band (fraction III) in gradient gels. However, at a short time after [³H]leucine administration considerable incorporation of activity was observed in fraction III (Fig. 2a) and the specific activity was more than twice that of 19S TG. This indicates that fraction III does not represent 27S protein alone but that it also contains a newly synthesized prethyroglobulin.

Department of Anatomy, University of Göteborg (Sweden)   S. SMEDS
                                                                                U. BJÖRKMAN

1 S. SMEDS, *Biochem. Biophys. Res. Commun.*, 38 (1970) 1168.
2 S. SMEDS, *Endocrinology*, in press.
3 S. SMEDS, *J. Chromatogr.*, 44 (1969) 148.
4 A. B. SCHNEIDER AND H. EDELHOCH, *J. Biol. Chem.*, 246 (1971) 6592.
5 S. SMEDS AND U. BJÖRKMAN, *J. Chromatogr.*, 71 (1972) 499.
6 S. SMEDS, *Endocrinology*, in press.
7 S. LARSSON, *Anal. Biochem.*, in press.
8 G. SALVATORE, G. VECCHIO, M. SALVATORE, H. J. CAHNMANN AND J. ROBBINS, *J. Biol. Chem.*, 240 (1965) 2935.
9 S. SMEDS, unpublished observation.