

# The microheterogeneity of rat TBG

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Isoelectric focusing (IEF) of native sera from immature or adult rats and of purified or partially purified rat serum thyroid hormone-binding proteins, demonstrates that rat TBG is a microheterogeneous protein. Autoradiography and radioactivity scans of the IEF plates show that it consists of at least four main isoforms, with bands at pH 4.35, 4.45, 4.5 and 4.55. This pattern is remarkably similar to that reported for human TBG. This is the first demonstration of the polymorphism of this recently discovered major binding protein of the rat.

Thyroxine-binding globulin; Microheterogeneity; Thyroid hormone-binding protein; (Rat)

## 1. INTRODUCTION

We demonstrated recently the presence of TBG in the serum of euthyroid healthy rats, and showed the dramatic transient increase of the protein during post-natal development. The analogies so far uncovered between rat and human TBG include the post-albumin electrophoretic migration, the carrying of the highest affinity serum-binding sites for T3 and T4 ( $K_d$  of nanomolar order) and the specific inhibition by diphenylhydantoin [1,2].

Using isoelectric focusing (IEF), autoradiography and radioactivity scanning techniques we now demonstrate the polymorphism of rat TBG. We find four main TBG isoforms, with isoelectric points (pI) very near to those reported for human TBG [3–5]. These results constitute the first report of the microheterogeneity of rat TBG.

## 2. MATERIALS AND METHODS

[<sup>125</sup>I]T4 and [<sup>125</sup>I]T3 (spec. act. > 1.2 Ci/μg) were from

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*Abbreviation:* TBG, thyroxine-binding globulin

Amersham Int. England. Pools of 8 day and adult sera were from Charles River, France (Sprague-Dawley, outbred CD strain). TBPA was purified from adult rat serum as described [6].

A protein preparation enriched in TBG was obtained by submitting 8 day sera, prelabelled with [<sup>125</sup>I]T3, to preparative cylindrical electrophoresis on polyacrylamide gels (1.2 × 8.5 cm), in the conditions established previously for our analytical electrophoretic studies [1]. Successive 2 mm slices were counted to localize the binding of T3. All the gels showed, as expected, a single labelled zone, with post-albumin migration. The 3–4 slices of this zone were extracted with saline, and concentrated in a Micro-Prodicon concentrator (Bio-Molecular Dynamics, Beaverton, OR, USA).

IEF was carried out on LKB Ampholine PAG plates pH 4–6.5 (Bromma, Sweden), using the LKB Multiphor chamber and power supply. The sera or protein solutions were incubated 1 h at room temperature with the radioiodinated hormone (5 μl/100000 cpm). Protein concentrations were 30 mg/ml in 8-day sera and 65 mg/ml in adult sera, 9 mg/ml in the TBG and 25.5 mg/ml in the TBPA solutions. Samples were applied at the cathodic site of plates, by filter paper (5 × 10 mm, Whatman Inc., Clifton, NY). Runs were carried out, without prefocusing, at maximum 25 W, 10°C, for 3 h. The pH gradient was assessed using the isoelectrofocusing calibration kit (Pharmacia AB, Uppsala, Sweden). The gels were dried at 60°C under vacuum for 4 h.

Autoradiography of the focused labelled protein bands was performed by exposing the dried gels to Kodak X-Omat S films for 15 min–2 h, at –80°C, with X-Omatic rapid screens.

Distribution of radioactivity to the labelled protein bands was evaluated on the dried gels using a TLC Multi Tracemaster 10 (Berthold Analyt. Instruments, Nashua, NH, USA), and by densitometric scan of the autoradiographies with a dual

wavelength TLC scanner CS930 (Shimadzu Corp., Kyoto, Japan).

### 3. RESULTS

#### 3.1. IEF of the thyroid hormone-binding proteins of rat serum

The autoradiogram presented in fig.1 allows comparison of the focused [ $^{125}$ I]T4 labelled proteins of 8-day sera, male adult sera, an enriched TBG preparation, and a purified TBPA preparation. It also shows the autoradiogram of [ $^{125}$ I]T3 labelled 8-day sera. The concomitant migration of free radioiodinated T4 and T3 is also shown. It may be seen that, in both immature and adult sera, the binding of T4 is shared between an apparently homogeneous TBPA, presenting one isoform with *pI* 5.1 and a heterogeneous TBG, presenting at least four major isoforms, with *pI*s of 4.35, 4.45, 4.50 and 4.55, respectively. As could be expected from our previous findings [1,2] the bulk of bound T4 is on TBG in the pups and on TBPA in the adults. For the enriched TBG preparation, which served as marker of the TBG zone of the native sera, a slight enrichment in the most acidic isoform is observed, which might result from an artefactual selection during the preparative process. The studies with [ $^{125}$ I]T3 evidence binding of the ligand to the same main four TBG isoforms, and apparent absence of binding to TBPA. In this case, a weak smearing of radioactivity along the gel and a slight labelling of a number of non-identified, more alkaline than TBG bands, may also be observed. IEF of [ $^{125}$ I]T3-labelled adult serum (not shown) led to even more intense smearing, possibly due to the fragility of the T3-TBG complex and to the scarcity of high-affinity T3-binding sites in adult serum, demonstrated formerly [2].

#### 3.2. Distribution of bound T4 and bound T3 to the different TBG isoforms

The focused T4- and T3-binding proteins were evaluated by scanning the dried gels with a radioactivity distribution analyzer (fig.2). The areas integrated by the analyzer gave with T4, ~65% binding to TBG vs 35% binding to TBPA in the pups and ~24% binding to TBG vs 76% to TBPA in the adults. The distribution of bound T3 in the pups was ~82% to TBG vs 18% to TBPA and/or nonspecific binders; in adults, the impor-

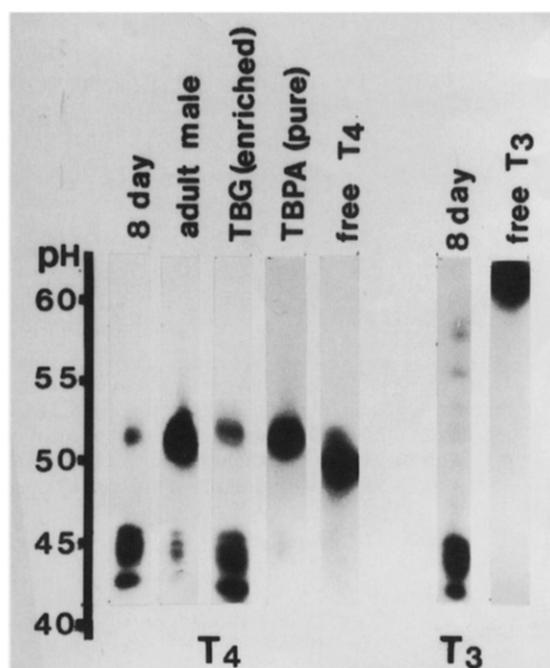


Fig.1. Autoradiogram of [ $^{125}$ I]T4 or [ $^{125}$ I]T3 labelled rat serum proteins following isoelectrofocusing. Studies with 8-day and adult male serum, partially purified TBG and purified TBPA.

tant smearing of radioactivity did not allow reliable quantitation. The integration of the T4-labelled TBG isoforms in the 8-day and in the adult sera showed a comparatively similar distribution of the radioactive ligand to the four main peaks with however a slight preferential binding to the most acidic isoform in the pups (~38% of the binding to TBG I, *pI* 4.35) vs a slight preferential binding to the more alkaline isoforms in the adults (~27% to TBG IV, *pI* 4.55). As to the percent distribution of T3 in the 8-day serum, it was 20, 22, 30 and 28 to TBGs I, II, III and IV, respectively, pointing to a shift of T3 binding towards the more alkaline forms, when compared to the binding of T4. It should be pointed out that the height of the peaks on the diagram does not necessarily correlate with the area of the corresponding band, which may have a more or less large base. These results have been confirmed by densitometric scan of the autoradiographies.

Repeated scanning of IEF gels obtained in identical conditions showed good reproducibility. Inter- and intra-assay variability was less than 5%.

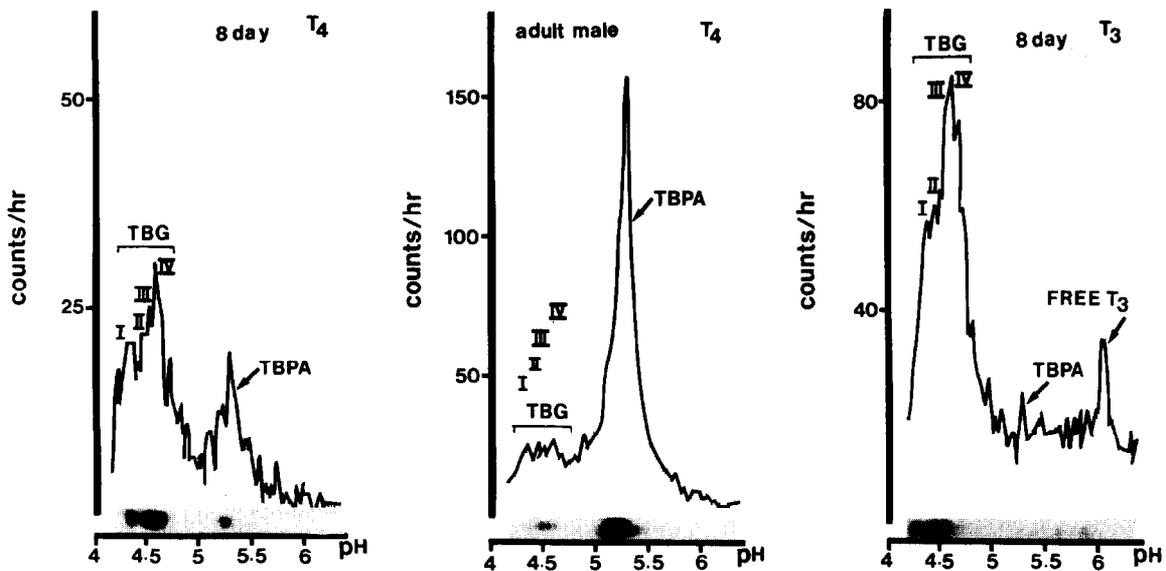


Fig.2. Scan of radioactivity of the gels following IEF of [ $^{125}$ I]T<sub>4</sub> labelled 8-day sera and adult sera and of [ $^{125}$ I]T<sub>3</sub> labelled 8-day sera.

#### 4. DISCUSSION

We demonstrate that rat TBG is a microheterogeneous protein, consisting of four main acidic isoforms, with isoelectric points between pH 4.35 and 4.55. The four isoforms are present in both immature and adult sera and they all bind T<sub>4</sub> as well as T<sub>3</sub>. The distribution of bound T<sub>4</sub> to the iso-TBGs appears in favor of the most acidic isoform in the 8-day sera and in favor of the most alkaline isoform in the adult sera.

These results evidence a striking similitude between the microheterogeneity of the rat and that of the human TBG. Indeed for the latter virtually the same four main isoforms have been reported, with *pI* values between 4.25 and 4.55 [3], 4.2 and 4.6 [4] or 4.3 and 4.55 [5]. Thus to the previously demonstrated similarities between the proteins of the two species, i.e. comparable electrophoretic and equilibrium binding characteristics [1,2], the present data add novel indications of functional as well as structural relationships. Whether the polymorphism of rat TBG is due, like that of human TBG [3-5,7] to differences in sialic acid content and variations in amino acid composition remains to be verified. The field is also open for a detailed investigation of the shifts among the rat

isoforms in various physiologic or pathologic conditions.

The relative proportions of TBG and TBPA labelling, in pups and adults, measured in our IEF experiments, are in fair agreement with the relative binding activities of the two proteins we had formerly evidenced using electrophoretic and equilibrium binding techniques, i.e. ~3:1 in favor of TBG in the pups vs 3:1 in favor of TBPA in the adults. As TBPA is present in similar concentrations in the 8-day and in the adult rat serum [1], our present studies provide additional evidence that the ability of TBPA to carry the thyroid hormones is dramatically governed by the coexistent TBG levels.

The physiologic importance in man of the serum concentrations of unbound thyroid hormones is at present firmly established, even to the routine measurement of the parameter for the clinical evaluation of the thyroid function. Still the role of the serum-binding proteins in controlling this essential index is not understood [8,9]. In studies of this problem, the rat has been widely used as a model, but the setting of experiments [10,11] and the extrapolation of results to man have come up against the supposed non-existence (for a review, see [12]) of rat TBG. The finding in the normal rat

of a protein, close to the human TBG, both functionally and structurally, should allow substantial progress in elucidating the mechanisms of the normal or impaired delivery of the circulating T4 and T3 to the target tissues.

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