INCORPORATION OF ^{131}I-LABELED ANDROGEN-RECEPTOR INTO NUCLEI OF RAT PROSTATES

Kaoru NOZU* and Bun-ichi TAMAOKI
National Institute of Radiological Sciences
Anagawa-4-chome, Chiba-shi 280, Japan

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SUMMARY The ^{131}I-labeled receptor of adult rat prostate showed the same binding attitude toward 5α-dihydrotestosterone as the non-labeled receptor. The ^{131}I-labeled receptor was efficiently incorporated into the chromatin and its non-histon protein and DNA in the form of the complex with 5α-dihydrotestosterone, in contrast to the radioactive receptor alone or ^{131}I-labeled serum protein bound to the androgen.

INTRODUCTION

Fates of the steroid hormone-receptors in the cells of target organs have been examined by tracing the tritium-labeled steroids bound to the receptor proteins (1,2,3). In the present paper, the androgen-receptor was labeled with ^{131}I, in order to trace the receptor itself in the cell of rat prostate . After the ^{131}I-labeled receptor was examined of its ability of binding to 5α-dihydrotestosterone (hereafter, abbreviated as dihydrotestosterone), incorporation of the radioactively labeled receptor into nuclei and their components was examined.

MATERIALS AND METHODS

Radioisotopes [1,2-^{3}H]-dihydrotestosterone (44 Ci/mmol) and Na^{131}I (Protein iodination grade) were obtained from New England Nuclear Corp., Boston, Mass.

Tissue preparation Ventral prostates of adult rats (10 weeks of age, the Wistar strain) were homogenized in an ice-cold 0.25 M sucrose solution by a Polytron homogenizer (Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 800 x g for 20 min. The crude nuclear fraction which was obtained as the precipitate was further purified by a discontinuous sucrose density centrifugation as previously reported (4). Then, the supernatant fluid at 800 x g was centrifuged at 105,000 x g for 60 min, the cytosol fraction was

* Visiting scientist from Research Lab. of Kaken-yaku Kako Co. Otsu-shi, Japan

obtained as the supernatant fluid. The serum was prepared from blood of the same rats.

Fractionation and confirmation of androgen-receptor Cytosol protein was fractionated by a Sephadex G-100 column chromatography into the two macromolecular fractions namely, receptor A (corresponding to 9S) and receptor B (corresponding to 5S) (5) (Nozu and Tamaoki, unpublished). Receptor protein in the form of the complex with the tritiated dihydrotestosterone was detected by a continuous sucrose density gradient centrifugation (sucrose concentration, 5-20 % at 36,000 rpm for 16 hr) (5).

Iodination of protein with ^{131}I The cytosol receptor A and B, and serum protein were labeled with the mixture of $Na^{131}I$ and chloramine T by the same method as applied to iodination of protein hormones for radioimmunoassay (6).

Fractionation of nuclear components After incubation with ^{131}I-labeled receptor , the nuclei was precipitated by centrifugation at 10,000 x g for 5 min, and washed sequentially with 0.25 M sucrose solution, Triton X-100 solution (1 %), 0.15 M NaCl solution, then 1.5 mM NaCl-0.15 mM sodium citrate solution and 0.35 M NaCl solution. Then , chromatin fraction was obtained as the precipitate. The chromatin was dehistonized by treatment with 2.0 M NaCl-5.0 M urea solution containing 1.0 mM $MgCl_2$ and 10 mM phosphate buffer (pH 6.0). From the dehistonized chromatin, DNA fraction was prepared by removing non-histon protein with 3.0 M NaCl-7.5 M urea solution containing 1.5 mM $NaHSO_4$ 1.5 mM EDTA and 15 mM Tris buffer (pH 8.5), according to the method previously reported by O'Malley and his coworkers (7,8).

RESULTS AND DISCUSSION

Attitude of the ^{131}I-labeled receptor toward dihydrotestosterone After incubation of the ^{131}I-labeled receptor A with the tritiated dihydrotestosterone for 10 min at 20° C, formation of the receptor-steroid complex was examined by a continuous sucrose density gradient centrifugation. Peak in the distribution of the tritium due to the dihydrotestosterone bound to the receptor was found coincident with the peak of ^{131}I distribution due to the radioactive receptor (Fig. 1a). This result suggests that the ^{131}I-labeled receptor of the prostate showed very similar or identical attitude toward dihydrotestosterone to the one of the non-labeled receptor as already reported (9,10).

Coexistence of ^{3}H-dihydrotestosterone and ^{131}I-labeled receptor after incubation into the nuclei of the prostate After incubation of the tritiated dihydrotestosterone-^{131}I-labeled receptor A complex with the prostatic nuclei at 20° C

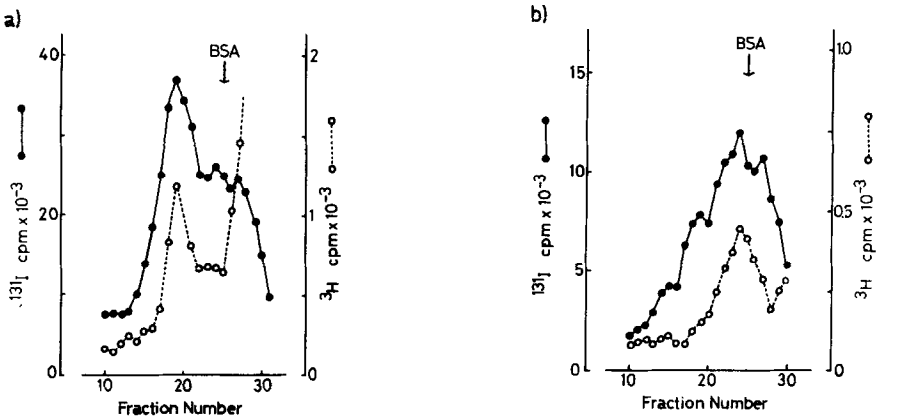

Fig. 1a. Sedimentation profile of the complex of ^{131}I-labeled receptor A with ^{3}H-dihydrotestosterone

Fig. 1b. Coexistence of tritium and radioiodine in the nuclear receptor, after incubation of ^{3}H-dihydrotestosterone-^{131}I-labeled receptor complex with the prostate nuclei

for 20 min, the nuclear fraction was sedimented and washed repeatedly with 0.25 M sucrose solution. From the nuclei, the receptor was extracted with 0.5 M KCl solution as previously reported (11), and analyzed of distribution of the two radionuclides by the same sucrose density gradient centrifugation as applied to the previous experiment. The ^{131}I-labeled nuclear receptor was sedimented at 5 S in agreement with the previously reported value (11,12) of the intact receptor extracted similarly from the nuclei. Furthermore, it was noted that the peak found in the distribution of the tritium coincided with the peak of the radioiodine, suggesting that receptor A was incorporated into the nuclei in the form of the complex with dihydrotestosterone (Fig. 1b).

Temperature dependency of incorporation of the ^{131}I-labeled receptor A into the nuclei The ^{131}I-labeled receptor bound to dihydrotestosterone was incubated with the nuclei at the temperature from 0 to 50° C. The maximal rate of incorporation of ^{131}I into the chromatin fraction was observed at 35° C, whereas accumulation of ^{131}I in total nuclei increased almost linearly with the raised temperature.

Incorporation of the ^{131}I-labeled receptor into the nuclear components with or without formation of the complex with dihydrotestosterone After the cytosol fraction was separated into the receptor A and B by a Sephadex column chromato-

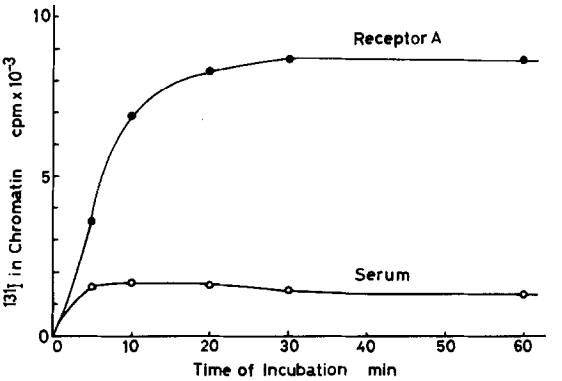

Fig.2. Time course of incorporation of the ^{131}I-labeled receptor A and serum protein into the chromatin of the prostate nuclei

graphy, each receptor was labeled with ^{131}I. An aliquot of each radioactive receptor was incubated with dihydrotestosterone to form the steroid-receptor complex, while the other aliquot was reserved as the control. The receptor alone and the steroid-receptor complex were incubated with the prostate nuclei under the identical condition. After the incubation, the nuclear fraction which was sedimented at 10,000 x g was treated by several procedures mentioned in Methods, to fractionate it into the nuclear components. As shown in Table I, the complex of ^{131}I-labeled receptor A with dihydrotestosterone was most efficiently incorporated into the chromatin, its non-histon protein and DNA fractions among the four fractions examined. It is interesting that the present result was principally compatible with the previous findings (1,13) that the tritium labeled steroid-receptor complex was associated with the non-histon protein of the chromatin. On the other hand, receptor B was not active in incorporating into the nuclear components. Relatively higher incorporation of the iodinated receptor A alone suggests that a part of receptor A was present in the form of the complex with the endogenous androgen.

Time course of incorporation of the ^{131}I-labeled receptor into the chromatin of the prostate nuclei After formation of complex of the ^{131}I of the androgen receptor A with dihydrotestosterone, the complex was further incubated with the prostate nuclei for 0 to 60 min, while the ^{131}I-labeled serum protein bound to the androgen served as the control. As shown in Fig. 2, rates of incorporation of radioiodine

TABLE I. Incorporation of Receptor A and B into Nuclear Components in vitro with or without Formation of the Complex with Dihydrotestosterone

Nuclear component*	Receptor A		Receptor B	
	With DHT**	Without DHT	With DHT	Without DHT
Chromatin	26,800***	17,000	3,300	3,800
Dehistonized chromatin	24,900	14,500	3,300	3,500
Non-histon protein	14,700	10,000	1,500	1,400
DNA	10,200	4,500	1,800	2,100

* Procedures of fractionating the nuclei into the nuclear components were described in the text.
** Dihydrotestosterone
*** Radioactivity of ^{131}I in cpm.

from the receptor and serum protein increased with time of incubation up to 10-20 min and then reached plateaus. Moreover, incorporation of the ^{131}I of the androgen receptor A complex into the chromatin fraction was found markedly higher than the one of the ^{131}I-labeled serum protein bound to the androgen.

These results obtained by the present experimentation suggest that the nuclear membrane retains the capability of differentiating the receptor A from receptor B or serum protein, and also the receptor A-dihydrotestosterone complex from the receptor A alone. As it was established that the receptor A itself in the form of the complex with the androgen was associated with the chromatin, particularly with non-histon protein and DNA, it is postulated that the association of the steroid-receptor complex initiates transcription of new cords from the DNA for new protein synthesis.

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