Vol. 107, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1982 Pages 209-216

DIFFERENCE IN ANDROGEN-DEPENDENT CHANGE OF NON-HISTONE PROTEINS BETWEEN DORSOLATERAL AND VENTRAL PROSTATES OF RATS

Yuhsi Matuo, Nozomu Nishi, Tetsuro Negi and Fumio Wada

Department of Endocrinology, Kagawa Medical School,
1750, Miki-cho, Kita-gun, Kagawa 761-07, Japan

Received May 6, 1982

SUMMARY: A novel species of non-histone protein having a molecular weight of approximately 20,000 (20K), abundantly localized in the dorsolateral prostate, was found to be decreased in the content by castration and to be restored by replacement of androgen, in addition to non-histone proteins of molecular weights $\geq$ 34,000. The content of 20K/DNA was more rapidly decreased by castration, but more slowly restored by replacement of androgen, with the dorsolateral prostate than the ventral prostate. Of other nuclear proteins, non-histone proteins of molecular weights $\geq$90,000 in the dorsolateral prostate were more susceptible to the decrease by castration, whereas those of all kinds of histones were hardly dependent on the androgen level.

Several findings have been reported on non-histone proteins whose contents depend on the androgen level with the ventral prostate of rats (1,2). However, little is known about the androgen-dependent change of nuclear proteins with the dorsolateral prostate of rats and its difference from that with the ventral prostate. The present report deals with studies on the androgen-dependent response of non-histone proteins with the dorsolateral prostate of rats, and on the difference of the response between the two prostates, using SDS-polyacrylamide slab gel electrophoresis.

MATERIALS AND METHODS

Male Sprague-Dawley rats (14-15 weeks,450-500 g) were fed ad lib. with water and Oriental MF solid diet (Oriental Yeast Co.,Osaka) in a plastic cage. Castration was performed under ether anesthesia via the scrotal route. Testosterone-treated rats received a daily dose of testosterone propionate (2 mg) in 0.2 ml of sesame oil into subcutaneous region. Control rats received 0.2 ml sesame oil. Rats were killed by cervical dislocation and prostate tissues were immediately dissected out. Nuclear fractions were prepared from the prostates by the method described previously (3) with some modifications. The prostates were minced and homogenized in 10 vol.(v/w) of 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.5), 3 mM $CaCl_2$ and 1 mM phenylmethylsulfonyl fluoride (PMSF) with Polytron for 30 sec and then with a Teflon-glass homogenizer by 20 up-down strokes, followed by filtration through two sheets of cheesecloth. The resulting homogenate was centrifuged at 1,500 x g for 10 min. The resulting

0006-291X/82/130209-08$01.00/0

Table I. Effect of castration and replacement of androgen on tissue weight, protein content and DNA content with dorsolateral and ventral prostates.

Group of rats[a)]	Relative tissue weight (%)		Relative content in homogenate (%)			
			Protein		DNA	
	DLP[b)]	VP[c)]	DLP	VP	DLP	VP
Normal	100[d)]	100[e)]	100[f)]	100[g)]	100[h)]	100[i)]
C-2	86	86	90	82	114	111
C-5	61	36	82	69	174	122
C-8	44	21	78	64	202	155
C-8/T-3	80	41	83	72	91	109
C-8/T-11	150	100	94	80	103	67

a) Normal values indicated were essentially the same as those with control and sham-operated rats. One group was composed of 4 rats with normal, C-2, C-5 and C-8/T-11, and 8 rats with C-8 and C-8/T-3. Results were obtained from 10 and 3 different experiments with normal and others, respectively. C-2, C-5 and C-8; castrated rats for 2, 5 and 8 days, respectively: C-8/T-3 and C-8/T-11; C-8 rats received a daily injection of testosterone propionate for 3 and 11 days, respectively. b) Dorsolateral prostate. c) Ventral prostate. d) 66 ± 11 mg/100 g body weight. e) 114 ± 17 mg/100 g body weight. f) 86.6 mg/g wet weight of tissue. g) 112.2 mg/g wet weight of tissue. h) 0.960 mg/g wet weight of tissue. i) 1.87 mg/g wet weight of tissue.

precipitate was suspended in 20 vol.(v/w) of 2.1 M sucrose containing 10 mM Tris-HCl (pH 7.5), 3 mM $CaCl_2$ and 1 mM PMSF with Polytron for 1 min, followed by centrifugation at 50,000 x g for 1 h in a swing bucket. The precipitate on the bottom was centrifugally washed three times with 5 vol.(v/w) of 0.15 M NaCl containing 1 mM PMSF. The resulting precipitate was used as nuclei. PMSF was freshly dissolved in dimethylsulfoxide at 200 mM.

SDS-polyacrylamide electrophoresis was carried out at 25°C by the method of Laemmli (4), using a slab gel having a size of 176(height) x 150(width) x 2 (thickness)mm and concentrations of 14% acrylamide and 0.37% N,N'-methylenebis-acrylamide, unless otherwise noticed. Other conditions were the same as those described previously (3). Two-dimensional gel electrophoresis was carried out by the method described previously (5), using SDS-electrophoresis mentioned above as the second-dimension electrophoresis. The molecular weight markers used were phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,000) and α-lacto-albumin (14,400). Dried gel films were measured of absorbance at 560 nm by a densitometer (model CS-910,Shimadzu Seisakusho,Kyoto). A non-histone protein species separated in the gel is expressed as (apparent molecular weight of the species x 10^{-3})K. The contents of protein and DNA were determined by the methods of Lowry et al. (6) and Schneider (7), using bovine serum albumin and calf thymus DNA as standards, respectively.

RESULTS AND DISCUSSION

Comparison of androgen-dependent change in tissue weight, protein content and DNA content between dorsolateral and ventral prostates In order to verify the in vivo response of the prostates to castration and replacement of androgen, wet weight, protein content and DNA content were determined (Table I). The loss of the tissue weight by castration was more remarkable, whereas the

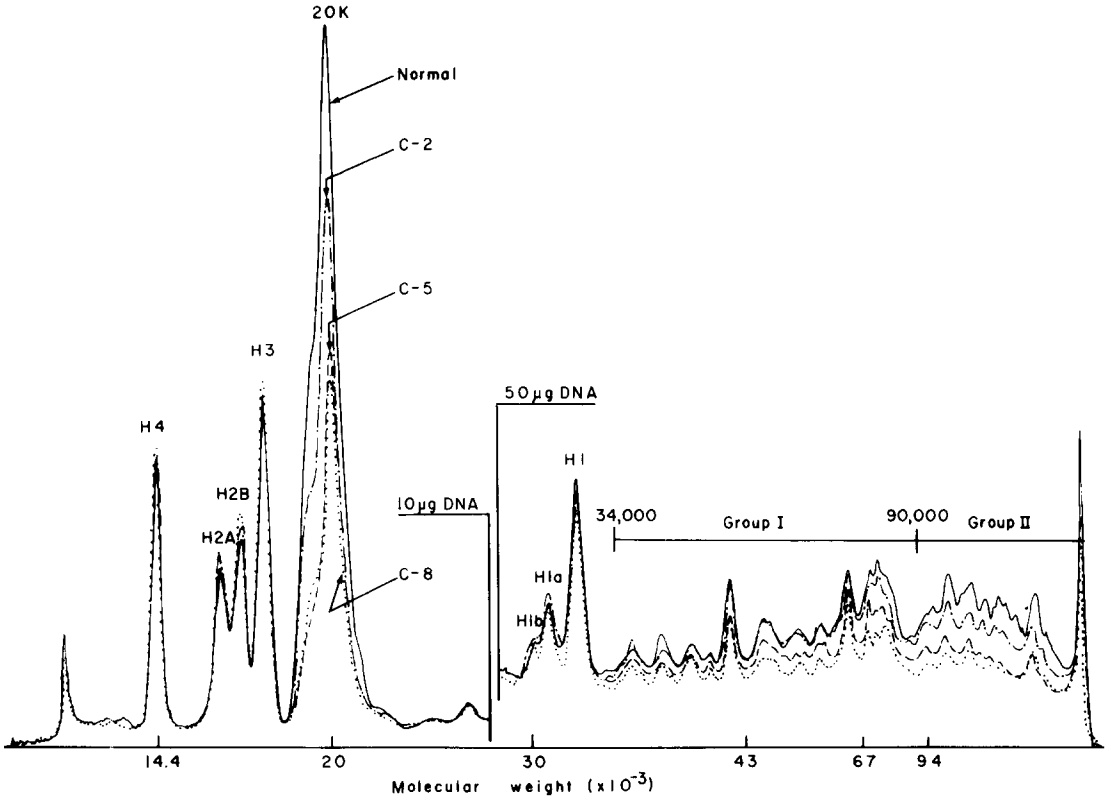

Fig. 1. Effect of castration on densitometric pattern of nuclear proteins with dorsolateral prostate. Each sample (10 or 50 µg DNA) prepared from rats shown in Table I was subjected to SDS-electrophoresis. In order to show the change of the content of 20K, the patterns with 10 µg DNA (left part) and those with 50 µg (right part) were rearranged on the same scale of an apparent molecular weight. The patterns with normal rats were essentially the same as those with control and sham-operated rats. With the sample from the same group of rats, the reproducibility of densitometric pattern was reasonably good. Other conditions were the same as those described in the footnote to Table I.

restoration of the tissue weight by replacement of androgen was slower, with the ventral prostate than the dorsolateral prostate. Protein content of the tissue was decreased by castration and restored to normal by replacement of androgen in the similar manners as the change of the tissue weight. On the other hand, the DNA content was increased by castration and the increase was more marked with the dorsolateral prostate than the ventral prostate.

Comparison of androgen-dependent change in electrophoretic pattern of histones between dorsolateral and ventral prostates Nuclei containing equal amount of DNA prepared from the prostates in the presence of PMSF were analyzed by SDS-electrophoresis. Yields of DNA in nuclei (40-60%) were hardly influenced by castration and replacement of androgen. The difference in the DNA yield did

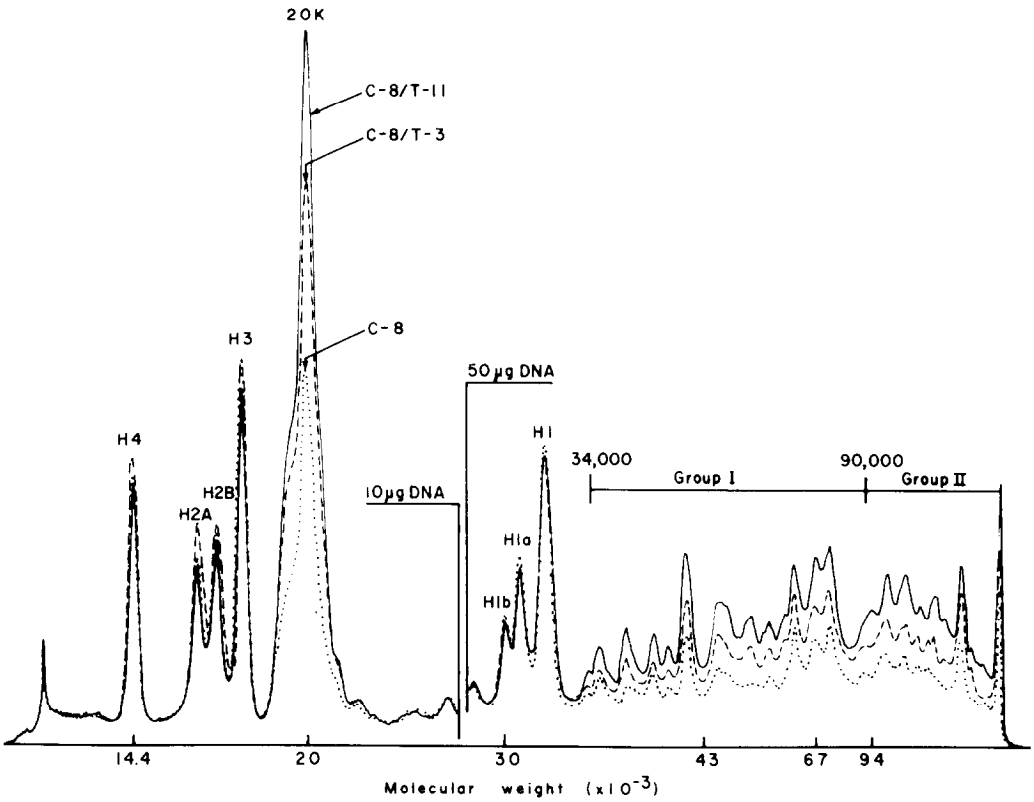

Fig. 2. Effect of replacement of androgen on densitometric pattern of nuclear proteins with dorsolateral prostate. The patterns with normal and C-8/T-11 rats were essentially similar, except that non-histone proteins in group I and II had a tendency to increase with C-8/T-11 rats. Experimental conditions were the same as those in the legend to Fig.1.

not affect the electrophoretic pattern with either prostate. Of the nuclear proteins separated, histones were referred to as H1, H2A, H2B, H3 and H4 (3) (Figs. 1-4). Almost all of five kinds of histones, in addition to two species having electrophoretic mobilities between H1 and the marker protein of 30,000 daltons, were extracted from the nuclei with H_2SO_4 by the method of Fambrough and Bonner (8). The two species adjacent to H1 are tentatively designated H1a and H1b, the latter of which was also reported by Anderson et al. (9-11). The contents of H2A, H2B, H3 and H4/DNA were fundamentally similar between the prostates, whereas those of H1, H1a and H1b were slightly but significantly higher with the ventral prostate than the dorsolateral prostate. The different content of H1 histones was not caused by proteases in the nuclei (see below). With either prostate, the contents of all kinds of histones/DNA were hardly changed by castration and replacement of androgen (Figs. 1-3).

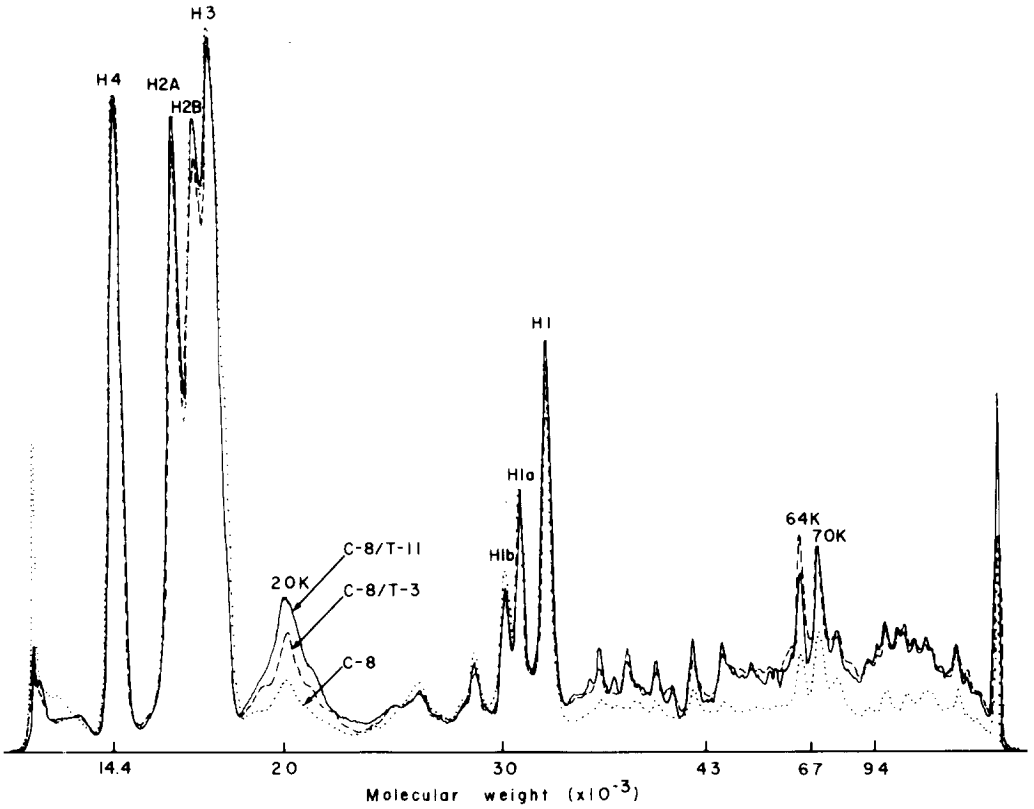

Fig. 3. Effect of castration and replacement of androgen on densitometric pattern of nuclear proteins with ventral prostate. Each sample containing 50 µg DNA was subjected to SDS-electrophoresis. Other conditions were the same as those in the legend to Fig. 1. The pattern with normal rats was essentially the same as that with C-8/T-11.

Comparison of androgen-dependent change in electrophoretic pattern of non-histone proteins between dorsolateral and ventral prostates Non-histone proteins were densitometrically separated into approximately 37 bands with either prostate (Figs. 1-4). Of these, the species of high relative content in the individual nuclei were 20K for the dorsolateral prostate, and 20K, 64K and 70K for the ventral prostate (Figs. 1 & 3). The most prominent difference between the prostates was that the content of 20K in the dorsolateral prostate was at least 20 times as high as that in the ventral prostate. The 20K in the nuclei of either prostate was effectively extracted with 0.35 M NaCl from the nuclei previously washed with 1% Triton X-100, but hardly with H_2SO_4. The contents of non-histone proteins were appreciably influenced by castration and replacement of androgen (Figs. 1-3). The decrease of 20K content with C-2 and C-5 rats was more remarkable with the dorsolateral prostate than the ventral

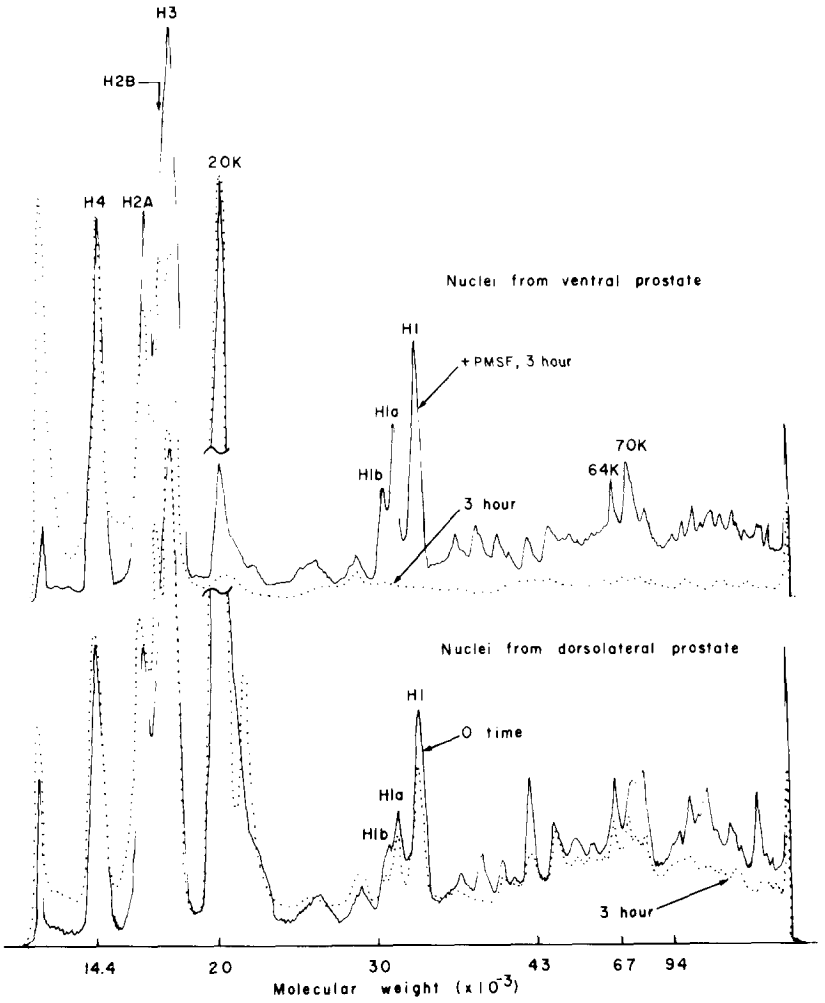

Fig. 4. Change of electrophoretic patterns of nuclear proteins from dorsolateral and ventral prostates by endogenous proteases. Nuclei containing 112.5 µg DNA prepared in the absence of PMSF were suspended in 0.1 ml of 10 mM Tris-HCl (pH 7.5), and incubated at 37°C in the presence (+PMSF) or absence of 1 mM PMSF. A part of the suspension (40 µg DNA) was subjected to SDS-electrophoresis by the method described in the text. Other conditions were the same as those in the legend to Fig. 1, except that 12.5% acrylamide and 0.33% N,N'-methylenebisacrylamide were used.

prostate, indicating that the response of 20 K to castration was faster with the dorsolateral prostate than the ventral prostate (Table II). On the other hand, the response of 20K to replacement of androgen was slower with the dorsolateral prostate than the ventral prostate. The content of 20K appeared to be inducible by the excess dose of androgen with the ventral prostate, but not with the dorsolateral prostate. Of other non-histone proteins than 20K with the dorsolateral prostate, non-histone proteins having molecular weights ≥

Table II. Effect of castration and replacement of androgen on the content of 20K with dorsolateral and ventral prostates of rats.

Group of rats[a]	Relative content of 20K/DNA (%)[b]	
	Dorsolateral prostate	Ventral prostate
Control	100	100
Sham-operated	100	100
C-2	72	98
C-5	48	90
C-8	45	56
C-8/T-3	73	92
C-8/T-11	91	160

a) See the footnote to Table I. b) determined by planimeter, including adjacent minor components.

90,000 (shown as group II in Figs.1 & 2) were more susceptible to the decrease by castration than those in the group I ($34,000 \leq$ M.W. $< 90,000$), whereas these different responses to castration were not observed with the ventral prostate. Restoration of these non-histone proteins by replacement of androgen was uniformly slower with the dorsolateral prostate than the ventral prostate.

As far as we know (1-3), the 20K in the prostates is a kind of tissue-specific non-histone proteins, and a novel species having a dependency on the androgen level. By two-dimensional electrophoresis of nuclei from the dorsolateral prostate, isoelectric point of the 20K was estimated to be approximately 11.5.

Evidence that androgen-dependent change of non-histone proteins is not due to the hydrolysis by endogenous proteases during preparation When the nuclei of both prostates prepared in the absence of PMSF were incubated at 37°C and pH 7.5 for 3 h, and subjected to SDS-electrophoresis, the contents of H1 histones and non-histone proteins were more remarkably degraded with the ventral prostate than the dorsolateral prostate (Fig. 4). The content of 20K was markedly decreased with the ventral prostate by the 3 h incubation, but not with the dorsolateral prostate. At any time of incubation tested, the increase of 20K was hardly observed with either prostate, indicating that 20K was not a degradation product of other nuclear proteins. These degradation were effectively inhibited by addition of 1 mM PMSF to the incubation mixture

(Fig. 4, +PMSF, 3 hour). Hagiwara et al. (12, 13) found that alkaline protease capable of hydrolyzing non-histone proteins and H1 histone is present at the state bound with chromatins of various tissues of rats, in addition to neutral protease capable of hydrolyzing other histones. Both of alkaline and neutral protease activities were found to be hardly influenced by castration and replacement of androgen with either prostate (data not shown). These results indicate that the androgen-dependent change of non-histone proteins including 20K is not caused by the endogenous protease during the preparation of nuclei.

ACKNOWLEDGEMENTS

We are very thankful to Prof. Takekazu Horio (the Division of Enzymology Institute for Protein Research, Osaka University) for his fruitful discussions and constant help.

REFERENCES

1. Chung,L.W.K.,and Coffey,D.S.(1971) Biochim.Biophys.Acta 247,570-583.
2. Kadohama,N.,and Anderson,K.M.(1976) Exptl.Cell Res.99,135-145.
3. Miyazaki,K.,Hagiwara,H.,Nagao,Y.,Matuo,Y.,and Horio,T.(1978) J.Biochem. 84,135-143.
4. Laemmli,U.K.(1970) Nature 227,680-685.
5. Hirano,N.,Kohda,S.,Miyazaki,K.,Matuo,Y.,Yamashita,J.,and Horio,T.(1981) Gann 72,346-353.
6. Lowry,O.H.,Rosenbrough,N.J.,Farr,A.L.,and Randall,R.J.(1951) J.Biol.Chem. 193,265-275.
7. Schneider,W.C.(1957) Methods in Enzymology 3,pp.680-684,Academic Press, New York.
8. Fambrough,D.,and Bonner,J.(1966) Biochemistry 5,2563-2570.
9. Anderson,K.M.,Chance,H., and Kadohama,N.(1975) Exptl.Cell Res.94,176-190.
10. Shirley,M.A., and Anderson,K.M.(1977) Can.J.Biochem.55,9-18.
11. Kadohama,N.,and Anderson,K.M.(1977) Can.J.Biochem.55,513-520.
12. Hagiwara,H.,Miyazaki,K.,Matuo,Y.,Yamashita,J.,and Horio,T.(1980) Biochim. Biophys.Res.Commun.94,988-995.
13. Hagiwara,H.,Miyazaki,K.,Matuo,Y.,Yamashita,J.,and Horio,T.(1981) Biochim. Biophys.Acta 660,73-82.