Biochimica et Biophysica Acta, 436 (1976) 863–868

BBA 77372

PROPERTIES OF A BICARBONATE-STIMULATED ATPase FROM RAT UTERUS

NOBUKO IRITANI* and WILLIAM W. WELLS**

Department of Biochemistry, Michigan State University, East Lansing, Mich. 48824 (U.S.A.)

(Received December 10th, 1975)

SUMMARY

Crude subcellular fractions from rat uterus contain a HCO_3^--stimulated Mg^{2+}-ATPase with properties analogous to those previously reported for the enzyme in gastric mucosa, pancreas, salivary gland and liver lysosome. Estradiol-17β treatment of ovariectomized rats resulted in an increase in uterine mitochondrial ($HCO_3^- + Mg^{2+}$)-ATPase and Mg^{2+}-ATPase activity. In an early response (105 min) to estradiol-17β treatment of ovariectomized rats, the lysosomal enzyme, β-*N*-acetylglucosaminidase increased in the nuclear and mitochondrial fractions and decreased in the microsomal and supernatant fractions.

INTRODUCTION

An increasing number of tissues, most of which have secretory functions, have been shown to possess relatively high levels of HCO_3^--stimulated ATPase [1–8]. A major portion of the activity is associated with the microsome-rich fractions. In other tissues, such as beef heart [9] and whole rat brain [10], HCO_3^--stimulated ATPase is closely linked to the mitochondria. Recently, we have demonstrated the presence of a HCO_3^--stimulated ATPase and carbonic anhydrase system in lysosome-rich fractions (tritosomes) prepared from livers of Triton WR-1339-treated rats [11].

During studies of the effects of hormones on the ATPases of rat uterus, crude homogenates were found to contain ATPase activity that was stimulated by HCO_3^-. The purpose of this report is to describe the properties of this activity in the uterus and estimate its levels in subcellular fractions from normal and ovariectomized rats and ovariectomized rats treated with estradiol-17β.

MATERIALS AND METHODS

Female rats of the Holtzman strain (Madison, Wisc.), initially weighing 250 g, were housed in groups of 3–4 and fed a commercial rat chow and water, ad libitum. In

* Present address: Tezukayami Gakuin College, Osaka, Japan.
** To whom inquiries should be addressed.

other experiments, female rats of the same strain and weight were ovariectomized and permitted to recover for 1 month before receiving estradiol administration. For hormone replacement, a 2 % (v/v) Tween-80 aqueous solution containing 0.2 μg (experiment I) or 1.0 μg (experiment II) estradiol-17β (Sigma)/0.1 ml per 100 g body weight was administered intraperitoneally in the morning and again after 1.5 h. 15 min after the second injection, the animals were killed, uteri rapidly removed, and chilled on ice in preparation for homogenization. Ovariectomized controls for each experiment consisted of groups of animals injected with proportionate amounts of the Tween-80 solution. Intact controls were animals of similar age and weight.

Enzyme preparation

Uteri from 3–4 animals were trimmed of connective tissue, placed in 20 vols. of 0.25 M sucrose containing 1 mM EDTA, (pH 7.0) 0–4 °C, and homogenized for 3–5 min with a Tekmar Model SDT tissue disruptor (Cincinnati, Ohio). The homogenate was passed through two layers of cheesecloth to remove fragments and excess lipid and connective tissue. The filtrate was centrifuged in a SS-34 rotor (Sorvall) at 3000 rev./min for 10 min. The pellet (nuclear fraction) was separated from the upper phase which was further centrifuged in the same rotor at 17 000 rev./min for 10 min. This pellet, designated the mitochondrial fraction, was separated from the supernatant phase, which was further centrifuged in a Beckman Model L-2 ultracentrifuge at 40 000 rev./min for 45 min. The resulting pellet and supernatant material were designated microsome and soluble fractions, respectively. The recoveries of the ATPases in the fractionation procedure were typically greater than 90 %.

Analytical procedures

ATPase activity (EC 3.6.1.3) was determined as previously described [11]. The total ATPase activity was measured in 1 ml in the presence of various amounts of protein, 20 mM Tris · HCl buffer (pH 7.5), 5 mM $MgCl_2$, 3 mM disodium ATP (Sigma Chemical Co.), 0.2 mM ouabain (Calbiochem) and 20 mM $NaHCO_3$. The Mg^{2+}-ATPase activity was determined under identical conditions except that 20 mM $NaHCO_3$ was omitted. The difference between the total ATPase and the Mg^{2+}-ATPase activity is referred to as the HCO_3^--stimulated ATPase activity. The reaction was carried out for 20 min at 37 °C. ATPase activity was assayed by measuring the rate of inorganic phosphate liberated from ATP as previously reported [11]. Nucleotide and anion reactivity were compared using the standard assay and providing substitute nucleotides (3 mM) for ATP or a series of oxyanions for HCO_3^- (10 mM). β-*N*-Acetylglucosaminidase (EC 3.2.1.30) activity was measured as reported previously [12]. Carbonic anhydrase (EC 4.2.1.1) was estimated by the procedure of Cutolo [13] as reported by Davis [14] and Iritani and Wells [11]. Assays were linear with respect to protein concentration and incubation period employed. Protein was measured by the procedure of Lowry et al. [15] using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Kinetics of uterine HCO_3^--stimulated ATPase

K_m and V for ATP and HCO_3^- were obtained from Lineweaver-Burk plots by least-square lines employing a computer program which determines the value of n, equivalent to n_H of the Hill equation as previously described [16]. The correlation coefficient expressing the degree of fit to a straight line for all calculations reported herein was > 0.93.

Both (Mg^{2+}+HCO_3^-)-ATPase and Mg^{2+}-ATPase were present in the mitochondria, microsomal, nuclear and soluble (post 100 000 × g supernatant) fractions of adult rat uterus. Kinetic parameters of these activities for the mitochondrial and microsomal fractions are presented in Table I. For the Mg^{2+}-ATPase, the apparent K_m of ATP for mitochondria and microsomes were 0.18 and 0.29 mM, respectively. Apparent Michaelis constants for the HCO_3^--stimulated activity of each fraction for ATP were 0.48 mM (mitochondria) and 0.28 mM (microsome), and for HCO_3^- were 3.29 mM (mitochondria) and 3.34 mM (microsome). Reciprocal plots were nonlinear for both substrates, both activities and both subcellular fractions (Table I, n_H = 1.43–2.07).

TABLE I

KINETIC PARAMETERS OF THE HCO_3^--STIMULATED AND Mg^{2+}-ATPase OF RAT UTERUS

The fractions were obtained from rat uteri and kinetic parameters were determined as described in the text. Each value is the average of duplicate analyses of pooled uteri from 3–4 rats.

Fraction	Enzyme	Substrate	K_m (mM)	V (μmol/h per mg protein)	n_H	Correlation coefficient
Mitochondria	Mg^{2+}-ATPase	ATP	0.18	5.30	1.43	0.99
Mitochondria	(HCO_3^-+Mg^{2+})-ATPase	ATP	0.48	1.85	2.07	0.98
Mitochondria	(HCO_3^-+Mg^{2+})-ATPase	HCO_3^-	3.29	1.74	1.44	0.99
Microsome	Mg^{2+}-ATPase	ATP	0.29	12.20	1.65	0.99
Microsome	(HCO_3^-+Mg^{2+})-ATPase	ATP	0.28	4.05	1.68	0.93
Microsome	(HCO_3^-+Mg^{2+})-ATPase	HCO_3^-	3.34	4.60	1.49	0.99

Carbonic anhydrase and Ca^{2+}-ATPase

The hydrolysis of ATP by a mitochondrial and microsomal fraction requiring Ca^{2+} was observed (2.3 and 6.1 μmol/h per mg protein, respectively) and this activity was stimulated by HCO_3^- (20 mM) (0.7 and 0.9 μmol/h per mg protein, respectively). Carbonic anhydrase activity was 0.52 and 0.43 units/mg protein for mitochondrial and microsomal fractions, respectively.

Nucleotide specificity of HCO_3^--stimulated ATPase and effect of various anions

GTP and UTP were equivalent to ATP as substrate for the phosphohydrolase activities from both microsomes and mitochondria. ADP and AMP were hydrolyzed 55–65 % and 4–14 % as rapidly as ATP. Anions affected the rat uterine HCO_3^--

stimulated and Mg^{2+}-requiring ATPase from microsomes and mitochondria in analogy with those observed for rat liver lysosomes [11]. Both Mg^{2+}-ATPase and that stimulated by HCO_3^- were inhibited significantly (37–73 % of control) by 10 mM OCN^- and $HA_sO_4^{2-}$ and the Mg^{2+}-ATPase was stimulated 44–48 % by SeO_3^{2-}.

Ovariectomy and estradiol treatment

The effects of ovariectomy and estradiol-17β on uterine subcellular Mg^{2+}-ATPase and ($HCO_3^- + Mg^{2+}$)-ATPase activity are shown in Table II. Treatment with 0.2 μg estradiol-17β following ovariectomy resulted in an increase in total HCO_3^--stimulated ATPase levels primarily resulting from changes in mitochondrial activity, but with the higher dose (1.0 μg), an increase was also observed in the microsomal fraction. Total Mg^{2+}-ATPase activities of the subcellular fractions were not appreciably changed following ovariectomy and estradiol-17β treatment, but steroid treatment caused an increase in mitochondrial and nuclear activities and a decrease in the Mg^{2+}-ATPase activity of microsomal and supernatant fractions.

TABLE II

EFFECT OF OVARIECTOMY AND ESTRADIOL-17β ON THE SUBCELLULAR DISTRIBUTION OF HCO_3^--STIMULATED AND Mg^{2+}-ATPase IN RAT UTERUS

Female rats were approximately 320 g and 3 months old. Ovariectomy was performed 1 month prior to administration of 0.2 μg (Expt. I) estradiol-17β in 2 % Tween-80/100 g body weight, or 1.0 μg (Expt. II) estradiol-17β/100 g body weight, or 2 % Tween-80/100 g body weight. Controls were untreated female rats of the same age and body weight. Animals received 2 injections 1.5 h apart and were killed 15 min after the second injection. Each value represents the average of duplicate analyses of pooled uteri from 3–4 rats. See the Materials and Methods section for subcellular fractionation procedures.

Group + treatment	Mitochondrial	Microsomal	Nuclear	Supernatant	Total
HCO_3^--ATPase (I.U./g fresh tissue)					
Intact Control	0.37	0.23	0.20	0.27	1.07
Expt. I					
Ovariectomy	0.40	0.18	0.17	0.10	0.85
Ovariectomy + estradiol-17β	0.58	0.18	0.21	0.03	1.00
Expt. II					
Ovariectomy	0.29	0.09	0.10	0	0.48
Ovariectomy + estradiol-17β	0.36	0.24	0.08	0	0.68
Mg^{2+}-ATPase (I.U./g fresh tissue)					
Intact Control	1.58	0.94	0.99	2.08	5.59
Extp. I					
Ovariectomy	1.34	1.17	0.68	1.55	4.74
Ovariectomy + estradiol-17β	1.89	0.63	0.83	1.17	4.52
Expt. II					
Ovariectomy	1.28	1.07	0.70	1.96	5.01
Ovariectomy + estradiol-17β	1.49	0.77	0.85	1.30	4.41

Distribution of β-N-acetylglucosaminidase

The total protein content of uteri from ovariectomized rats was reduced when compared with normal controls (Table III, 40.45 and 43.37 versus 50.52 mg/g fresh tissue for experiments I and II, respectively). The protein content of the microsomes and supernatant fractions decreased in both experiments and also in the mitochondrial fraction in experiment II, while that of the nuclear fractions increased in response to both levels of estradiol-17β employed. Since we were interested in whether HCO_3^--ATPase distribution after estrogen treatment of uteri from ovariectomized rats might correlate with the relocation of lysosomes, we compared the activity of the lysosomal marker enzyme, β-*N*-acetylglucosaminidase in each fraction (Table III). Ovariectomy caused a reduction of total β-*N*-acetylglucosaminidase activity compared with normal controls chiefly in the microsomal and supernatant fractions. In response to estrogen treatment over a period of 105 min, there was a relocation of this enzyme activity from microsomal and supernatant to the nuclear fractions in both experiments. Exactly the same redistribution pattern was observed for a second lysosomal marker enzyme, acid phosphatase (data not shown). These data for lysosomal enzyme distribution in uteri from ovariectomized rats 15 min after the second injection of estradiol-17β are in good agreement with the studies of Szego and her colleagues [17]. In their studies, uterine lysosomes have been implicated in the events following hormone uptake leading to enzyme induction and new protein synthesis.

Liver lysosomes contain a HCO_3^--stimulated ATPase that may participate in an H^+ gradient across the lysosomal membrane [11]. The enzyme is not unique to

TABLE III

EFFECT OF OVARIECTOMY AND ESTRADIOL-17β ON THE SUBCELLULAR DISTRIBUTION OF PROTEIN AND β-*N*-ACETYLGLUCOSAMINIDASE IN RAT UTERUS

See Table II for description of pertinent experimental conditions.

Group+treatment	Mitochondrial	Microsomal	Nuclear	Supernatant	Total
Protein (mg/g fresh tissue)					
Intact Control	5.66	3.86	9.90	31.1	50.52
Extp. I					
Ovariectomy	5.78	3.67	7.90	23.1	40.45
Ovariectomy+estradiol-17β	5.79	2.10	9.45	21.6	38.94
Expt. II					
Ovariectomy	5.20	3.30	7.57	27.3	43.37
Ovariectomy+estradiol-17β	4.71	2.61	8.80	23.4	39.52
β-*N*-Acetylglucosaminidase (I.U./g fresh tissue)					
Intact Control	0.17	0.18	0.20	0.13	0.68
Expt. I					
Ovariectomy	0.17	0.12	0.18	0.07	0.54
Ovariectomy+estradiol-17β	0.19	0.08	0.24	0.04	0.55
Expt. II					
Ovariectomy	0.18	0.12	0.15	0.10	0.56
Ovariectomy+estradiol-17β	0.22	0.09	0.21	0.05	0.58

liver lysosomes but is distributed throughout the cell. A similar broad distribution of a HCO_3^--stimulated ATPase with similar kinetic properties was observed in the rat uterus. The HCO_3^--ATPase did not redistribute appreciably in uterine tissue in response to estradiol-17β, whereas the lysosomal enzyme, β-*N*-acetylglucosaminidase, increased in the nuclear fraction and decreased in the microsomal and soluble fractions in response to the hormone. However, the lysosomes associated with the crude mitochondrial fraction, as judged by β-*N*-acetylglucosaminidase activity in that fraction, actually increased, suggesting a complex redistribution of lysosomes with respect to response to estradiol-17β treatment. In addition, the reduced β-*N*-acetylglucosaminidase activity in the supernatant fraction 15 min after the second steroid injection might be interpreted as the stabilization of the lysosomal membranes following the hormone treatment. However, earlier time points would be needed to present a more detailed analysis of lysosomal fragility.

ACKNOWLEDGEMENT

We acknowledge support by Grant AM 10209 from the U.S. Public Health Service.

REFERENCES

1 Kasbekar, D. K. and Durbin, R. P. (1965) Biochim. Biophys. Acta 105, 472–482
2 Sachs, G., Mitch, W. E. and Hirschowitz, B. I. (1965) Proc. Soc. Exp. Biol. Med. 119, 1023–1027
3 Wiebelhaus, V. D., Sung, C. P., Helander, H. F., Shah, G., Blum, A. L. and Sachs, G. (1971) Biochim. Biophys. Acta 241, 49–56
4 Sachs, G., Shah, G., Stryck, A., Cline, G. and Hirschowitz, B. I. (1972) Biochim. Biophys. Acta 266, 625–638
5 Simon, B., Kinne, R. and Knauf, H. (1972) Pflügers Arch. 337, 177–184
6 Izutzu, K. T. and Siegel, I. A. (1972) Biochim. Biophys. Acta 284, 478–484
7 Simon, B., Kinne, R. and Sachs, G. (1972) Biochim. Biophys. Acta 282, 293–300
8 Simon, B. and Thomas, L. (1972) Biochim. Biophys. Acta 288, 434–442
9 Racker, E. (1962) Fed. Proc. 21, 54
10 Kimelberg, H. K. and Bourke, R. S. (1973) J. Neurochem. 20, 347–359
11 Iritani, N. and Wells, W. W. (1974) Arch. Biochem. Biophys. 164, 357–366
12 Blosser, J. C. and Wells, W. W. (1972) J. Neurochem. 19, 1539–1547
13 Cutolo, E. (1957) J. Biochem. 6, 270–274
14 Davis, R. P. (1963) in Methods of Biochemical Analysis (Glick, D., ed.), Vol. 11, pp. 315–317, Wiley-Interscience, New York
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
16 Ting-Beall, H. P., Clark, D. A., Suelter, C. H. and Wells, W. W. (1973) Biochim. Biophys. Acta 291, 229–236
17 Szego, C. M. (1974) in Recent Progress in Hormone Research, Vol. 30 (Greep, R. O., ed.), pp. 171–233, Academic Press, New York