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Biochemical studies on the origin of the ATPase of the avian myeloblastosis virus

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Abstract. The adenosine triphosphatase activity of the avian myeloblastosis virus obtained from the blood of the virus-infected chicken was compared with that of the host cell myeloblasts. The specific activity of the viral enzyme is unusually higher than that of the myeloblasts. A significant difference in inhibitor sensitivity was observed with quercetin. When the virus was grown in chicken embryonic fibroblasts in culture, the resulting virus showed very little adenosine triphosphatase activity, comparable to that of the fibroblasts and similar sensitivity to inhibitors. Antibody raised against the purified enzyme of avian myeloblastosis virus inhibits the enzyme activity of the myeloblasts while the activity of the fibroblasts enzyme as well as that of fibroblast-grown virus remains unaffected.

Keywords. ATPase; avian myeloblastosis virus.

Introduction

Mommaerts *et al.* (1952) were the first to demonstrate an adenosine triphosphatase (ATPase) activity in the plasma of birds with induced myeloblastic leukaemia. Later on, Beard (1963) observed that the enzyme activity was associated with the virus present in the plasma. De-The' *et al.* (1964b) localized the enzyme by histochemical techniques, on the viral envelope and on the cell membrane of the myeloblasts where the virus matures. They also observed that in kidney tumours induced by the same virus, neither the cell membrane nor the virus showed any ATPase activity (De The' *et al.,* 1963a). The consensus is that the enzyme is not virus-specific but is incorporated into the virus from the host cell, myeloblasts, during cytoplasmic budding (Haddad *et al.,* 1960). Recently, Banerjee and Racker (1977) solubilized and partially purified an ATPase from virus-infected myeloblasts. The enzyme shows similarity to the cell-surface ATPase of the myeloblasts with respect to its molecular, catalytic and immunological properties (Banerjee, 1978a, 1979a). In order to know the origin of the enzyme, the virus was allowed to grow both in chicken myeloblasts and in chicken embryonic fibroblasts in culture. The ATPase activities of the host cells and the resulting virus as well as their relative

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sensitivity to some classical inhibitors were studied. The results of these studies are presented in the present communication.

Materials and methods

Mersalyl was a product of K and K Laboratories, Plain view, New York, USA, N, N' dicyclohexyl-carbodiimide (DCC) was obtained from Schwarz Biochemicals, Orangeburg, New York, USA. Sodium azide, ATP and ouabain were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Quercetin was procured from Nutritional Biochemicals Corporation, Cleveland, Ohio, USA. Mitochondrial inhibitor of ATPase from bovine heart mitochondria was prepared as described (Kanner *et al.,* 1976).

Myeloblasts from chicken, infected with the avian myeloblastosis virus (AMV) was kindly provided by Dr J. W. Beard and G. E. Houts authorized by the office of Resources and Logistics of the Virus Oncology Program. After infection, the myeloblasts were collected by centrifugation of the blood of the leukaemic chicken at low speed and washed free of virus, if any. The virus was purified from the plasma as described earlier (Banerjee and Racker, 1977). Chicken embryonic cells were cultured as described by Vogt and Ishizaki (1965) and infected with the AMV and grown according to the procedure of Bissel *et al.* (1972). About 150 ml of the culture medium was centrifuged at 50,000 *g* for 15 min in a 30 Ti rotor in a Beckman ultracentrifuge and the supernatant fraction was further centrifuged at 100,000 *g* for 1 h in the same rotor. The pellet was suspended in 3 ml of 10 mM Tris-HC1 buffer, pH 7.2 and centrifuged at 105,000 g for 30 min. The virus pellet was resuspended in 10 mM Tris-HCl buffer pH 7.2 and used for enzyme assay. The ATPase of AMV and myeloblasts were solubilized and purified as reported earlier (Banerjee and Racker, 1977; Banerjee, 1979a).

For solubilization of the cell-surface ATPase from chicken embryonic fibroblasts, the cells were harvested from 40 tissue culture dishes and suspended in a suitable volume of 0.25 M sucrose, 0.5 mM EDTA, 1 mM ATP and 10 mM Tris-HCl buffer pH 8.0. The cells were centrifuged down at 5,000 *g* for 10 min in a Sorvall RC-2B centrifuge and resuspended in 5 ml of the above buffer. The cells were then stirred in the presence of 200 ml of ice-cold absolute ethanol for 30 min at 0°C to inactivate the mitochondrial or Na^+, K^+ -ATPase activities and finally centrifuged at 50,000 *g* for 20 min in a Sorvall centrifuge. The pellet was suspended in 10 ml of the above buffer except that the pH was adjusted to 10. Aliquots of 5 ml were sonicated in a bath type sonicator (T-80-80-I-RS) for 4 min, centrifuged at 160,000 *g* for 1 h and the supernatant fractionated by adding solid ammonium sulphate. The precipitate appearing at 0-60% saturation, was centrifuged at 40,000 *g* for 10 min and suspended in 4 ml of the above buffer at pH 8.0. This preparation was used as a soluble cell-surface ATPase from the fibroblasts.

ATPase activity was measured by determining the inorganic phosphate liberated from ATP according to the method of Taussky and Shorr (1953) and expressed as µmol of P_i formed per min per mg protein. The incubation mixture contained, in a final volume of 0.5 ml, 50 mM Tris HCl buffer pH 8.0,5 mM M_gCl_2 or $CaCl_2$, 5 mM ATP and a suitable amount of the enzyme preparation. Incubation was carried

out at 37°C for 10 min and the reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid. Phosphate was assayed in the supernatant after centrifugation. Protein was determined according to Lowry *et al.* (1951).

An antiserum was prepared by injecting into rabbits subcutaneously, 0.8 mg of purified AMV ATPase in Freund's adjuvant followed by a booster injection of 0.3 mg after 3 weeks. Blood was collected 1 week after the last injection. On an Ouchterlony gel diffusion plate (Ouchterlony, 1958), the purified enzyme showed one very strong precipitation line and a second thin line as shown earlier (Banerjee and Racker, 1977).

Results

The specific activity of AMV ATPase was about 30 μ mol/min/mg protein (table 1). This is the same specific activity reported for the most active preparation of Na^+, K^+-ATPase of plasma membrane (Jorgensen, 1975), or of the Ca^{2+}-ATPase of sarcoplasmic reticulum (MacLenan, 1970; Banerjee *et al.,* 1979) or of the ATPase isolated from the chloroplast (Lien and Racker, 1971). It is several times greater than myosin ATPase and about one-third the activity of the most active preparation of beef heart mitochondrial (F1) ATPase (Pullman *et al.,* 1960). The ATPase activity of the myeloblast, on the other hand, collected from the same virus-infected chicken was as low as 0.26 or even lower (about 1% of the activity of the viral

Table 1. Comparison of the catalytic activities of the ATPases of the avian myeloblastosis virus and host cell myeloblasts.

Addition	ATPase activity (μmol P_i/min/mg)			
	AMV	Myeloblasts	Purified enzyme of AMV	Purified enzyme of myeloblasts
None	33	0.26	59	1.1
Mersalyl, 1 mM	7.5		20	0.53
Azide, 2 mM	32.6	0.11	59	1.1
Mitochondrial inhibitor, 50 μg/ml	33	0.18	58	1.15
DCC, 125 μM	6	—[e]	18	0.66
Ouabain, 1mM	32	—	59	1.1
Trypsin[a]	9	—	24	0.7
ATP+Trypsin	30	—	78	1.2

A suitable amount of the virus or the cells or the purified enzyme was preincubated with the indicated concentration of the inhibitor at 37°C for 5 min in the presence of 0.2 ml of 0.25 M sucrose, 0.5 mM EDTA and 10 mM Tris-HCl pH 8.0 (SET buffer). The reaction was started by adding 0.5 ml of the solution containing 50 mM Tris HCl pH 8.0,5 mM each of ATP and Ca^{2+}

[a] In the case of trypsin, the ratio of protein to trypsin used was 2:1.

[b] The enzyme was protected from the trypsin effect by adding 20 mM ATP.

[c] Not checked.

enzyme), as the preparation was contaminated with azide-sensitive as well as mitochondrial inhibitor-sensitive ATPase as shown in the table (viral ATPase is not sensitive to azide or the mitochondrial inhibitor). When the myeloblasts were treated with 98% ethanol, the mitochondrial or Na^+, K^+-ATPase activity, if any, was inactivated leaving only the myeloblastic cell-surface ATPase having specific activity of around 0.1 (data not shown) which is at least three hundred times less active than the viral ATPase. When the active ethanol precipitate was solubilized by sonication at alkaline pH and partially purified, the resulting enzyme preparation showed the same type of sensitivity to the inhibitors (as shown in table 1) when compared with that of the virus or the purified enzyme of the virus. The other molecular, catalytic and immunological properties were also found to be same as reported earlier (Banerjee, 1979). Thus, the most notable feature of the viral enzyme is the very high specific activity in comparison to the myeloblast enzyme of the host. The data suggests that the virus may pick up an ATPase from its host cell in a highly modified state with an increased catalytic ability as shown by the high specific activity, without altering its sensitivity to the inhibitors like mersalyl, DCC or trypsin.

An interesting observation was made regarding the sensitivity of the viral and myeloblastic ATPase to quercetin. Table 2 shows that viral ATPase was inhibited by quercetin when ATP hydrolysis was measured in the presence of either Mg^{2+} or Ca^{2+} The sensitivity of the enzyme to quercetin was considerably increased in the presence of Ca^{2+} using Mg-ATP as the substrate. In contrast, neither the myeloblastic ATPase nor the purified enzyme from the myeloblasts was inactivated by quercetin. When the viral enzyme was solubilized and purified, it lost the quercetin sensitivity and behaved like the myeloblastic ATPase.

Table 2. Effect of quercetin on the ATPase activity.

Enzyme	Addition	ATPase activity (μmol/P_i/min/mg)		
		Mg-ATP	Ca-ATP	Ca^{2+}+MgATP
AMV ATPase	None	23	30	24
	Quercetin[a]	14	20	5.5
Myeloblast ATPase	None	—[b]	—	0.26
	Quercetin	—	—	0.24
Purified myeloblast ATPase	None	0.9	1.1	0.9
	Quercetin	0.9	0.95	0.85
Purified AMV ATPase	None	60	80	64
	Quercetin	62	80	55

ATPase activity was assayed in the presence or the absence of quercetin after preincubation at 37°C for 5 rain as described in the legend to table 1. In the case of column 3, preincubation was carried out in presence of 5 mM Ca Cl_2. ATPase activity was then assayed either with 5 mM each of ATP and Ca or $MgCl_2$.

[a] The concentration of quercetin used was 20 μg/ml.
[b] Not checked.

To confirm the finding that the viral ATPase is a host-specific enzyme, the virus was allowed to infect and grow in chicken embryonic fibroblasts in culture. The fibroblast cells when assayed for ATPase activity were found to have very low activity and a part of it was contaminated with azide-sensitive ATPase (table 3).

Table 3. ATPase activity of the chicken embryonic fibroblasts.

Addition	ATPase activity (μmol P_i/min/mg)	
	Fibroblasts	Solubilized enzyme
None	0.048	0.1
Mersalyl, 1 mM	0.008	0.005
Azide, 2 mM	0.03	0.009
Quercetin, 10 μg/ml	0.04	0.009
DCC, 125 μM	0.014	0.006
Ouabain, 1 mM	0.05	—
Trypsin	0.022	—
ATP+trypsin	0.042	—

ATPase activity of the fibroblasts or the solubilized enzyme from it was assayed in the presence or absence of the inhibitors according to the procedure as described in the legend of table 1.

Like myeloblast cell-surface ATPase, the fibroblast surface ATPase was also inhibited in the presence of mersalyl or DCC. Inactivation by trypsin and protection with ATP was also the property seen in the case of the myeloblastic ATPase. Ouabain was, however, ineffective. When solubilized, the specific activity of the preparation dropped considerably, probably, due to inactivation of the azide-sensitive ATPase by ethanol and also due to partial inactivation of the surface enzyme or loss of some activating component, if any. The solubilized enzyme was also inhibited by mersalyl and DCC but not affected by azide or quercetin.

Table 4. ATPase activity of the virus grown in chicken embryonic fibroblasts.

Addition	ATPase activity (μmol/P_i/min/mg)
None	0.06
Mersalyl, 1 mM	0
Azide, 2 mM	0.057
Quercetin, 20 μg/ml	0.052
DCC, 125 μM	0
Ouabain, 1 mM	0.06
Trypsin*	0
ATP+trypsin*	0.10

ATPase activity of the virus grown in chicken embryonic fibroblasts was assayed in the presence or absence of the inhibitor according to the procedure described in the legend of table 1.

When the virus was grown in the fibroblasts, and the ATPase activity of the resulting virus was assayed, it demonstrated very low activity comparable to that of the fibroblasts as shown in table 4. Sensitivity of the enzyme to the inhibitors remained almost the same as that of the host cells except that the enzyme became more sensitive to mersalyl, DCC and trypsin. The important difference that could be noticed is the specific activity of the ATPase when the virus was grown in fibroblasts. Unlike the viral ATPase grown in myeloblasts, the enzyme of the virus grown in fibroblasts was not modulated to show very high specific activity and was not inhibited in the presence of quercetin.

The qualitative similarity of the ATPase activity of AMY as well as of its host cells as regards the sensitivity towards inhibitors, led us to investigate the immunological properties of the enzymes. The antiserum raised against the purified viral ATPase inhibited the ATPase activity not only of the virus or of the purified viral enzyme but also of the myeloblast and the enzyme purified from it, as shown in table 5. However, the ATPase activity of the chicken embryonic fibroblast and of the solubilized enzyme obtained from it as well as that of the virus grown in fibroblasts were not inhibited by the antiserum when treated under identical conditions. Incidentally, it should be mentioned that the antiserum crossreacted with the soluble enzyme from myeloblasts but not with that of the fibroblasts in an Ouchterlony gel diffusion test.

Table 5. Effect of antiserum on the ATPase activity.

	ATPase activity (μmol/P_i/min/mg)				
	AMV	Purified enzyme from		Solubilized enzyme from fibroblasts	Virus enzyme grown in fibroblasts
		AMV	Myeloblast		
Normal serum	26	80	1.1	0.015	0.062
Antiserum	13	40	0.55	0.015	0.06

A suitable volume of the virus, or the cell or the purified enzyme was preincubated with 20 μl of normal serum or antiserum at 37°C for 75 min followed by the assay of ATPase activity as described in the legend of table 1.

Discussion

Histochemical evidence shows that the avian myeloblastosis virus AT Pase is not a virus-specific enzyme but originates from the host cells (Haddad *et al.,* 1960). In leukaemic thymuses, the enzyme is localized at the cell membrane where the virus matures (De The' *et al.,* 1963b). When the virus is grown in chondrocytes devoid of any ATPase, the resulting virus does not show any ATPase activity but contains cartilage fibrils which are the component of the cell membrane of the chondrocytes (De The' *et al.,* 1962). Thus it appears that the cell membrane is involved in the formation of the viral envelope. Similar host cell enzymes have

also been demonstrated in herpes virus (Epstein and Hott, 1963) and influenza virus (Ada and Lind, 1961). De-The (1964a) has presented evidence based on electron microscopic studies that the ATPase of the AMV originates from the cell membrane of the myeloblasts.

The studies presented in this paper support the original finding of De-The' *et al.,* on the distribution of the virus and myeloblast phosphatase activity (De-The' *et al.,* 1964b). We have indicated three important aspects of the host-virus relationship in connection with the viral association of the enzyme. Firstly, the virus when grown in chicken fibroblasts having very poor ATPase activity also exhibits the same enzyme activity indicating that the virus enzyme may be of host-origin. Secondly, the ATPase activity can be modulated in the virus depending on the host cells in which the virus is maturating. In other words, the virus shows some specificity in modulating its ATPase to a highly active state as observed in the case of myeloblasts but not in case of fibroblasts. The allotopic sensitivity (Racker, 1967) of the viral ATPase to quercetin which is lost upon solubilization of the enzyme may indicate that some component either present in the virus or contributed by the myeloblasts to the viral envelope during cytoplasmic budding, may be responsible for the induction of a remarkably high ATPAse activity in the virus, a phenomenon not observed in the case of fibroblasts. Thirdly, the cell-surface ATPase activity of the fibroblasts shows almost similar inhibitor sensitivity to that of the myeloblasts. Insensitivity of the enzymes of both myeloblasts and fibroblasts to the inhibitors of the mitochondrial ATPase or the Na^+, K^+-ATPase as well as their extreme sensitivity to a mercurial like mersalyl are properties often observed in the case of the cell surface ATPase known as 'ecto-ATPase' (Banerjee, 1978a, b; 1979a, b; 1981; De Pierre and Karnovsky, 1974).

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References

Ada, G. L. and Lind, P. E. (1961) *Nature,* **190,** 1169.
Banerjee, R. K. and Racker, E. (1977) *J. Biol. Chem.,* **252,** 6700.
Banerjee, R. K. (1978a) *Indian J. Biochem. Biophys.,* **15,** 135.
Banerjee, R. K. (1978b) *Experientia,* **34,** 1430.
Banerjee, R. K. (1979a) *Eur. J. Biochem.,* **97,** 59.
Banerjee, R. K. (1979b) *Indian J. Biochem. Biophys.,* **16,** 334.
Banerjee, R. K. (1981) *Mol. Cell Biochem.,* (in press).
Banerjee, R., Epstein, M., Kandrach, M., Zimniak, P. and Racker, E. (1979) *Mem. Biochem.,* **2,** 283,
Beard, J. W. (1963) *Adv. Cancer Res.,* **7,** 1.
Bissell, M. J., Hatie, C, and Rubin, H. (1972) *J. Natl. Cancer Inst.,* **49,** 555.
Brunette, D. M. and Till, J. E. (1971) *J. Membrane Biol.,* **5,** 215.
DePierre, J. W. and Karnovsky, M. L. (1974) *J. Biol. Chem.,* **249,** 7111.
De-The', G., Heine, U., Ishiguro, H., Sommer, J. R., Beard, D. and Beard, J. W. (1962) *Fed. Proc.,* **21,** 919.
De-The', G., Ishiguro', H., Heine, U., Beard, D. and Beard, J. W. (1963a) *J. Natl. Cancer Inst.,* **30,**1267.
De-The', G., Heine, U., Sommer, J. R., Arvy, L., Beard, D. and Beard, J. W. (1963b) *J. Natl. Cancer Inst.,* **30,** 201.

De-The', G. (1964a) *Natl. Cancer Inst. Monogr.,* **17,** 651.
De-The', G., Becker, C and Beard, J. W. (1964b) *J. Natl. Cancer Inst.,* **32,** 201.
Epstein, M. A. and Holt, S. J. (1963) *Cell Biol.,* **19,** 337.
Haddad, M. N., Weinstein, D., Bonar, R. A., Beaudreau, G. S., Becker, C, Beard, D. and Beard, J. W. (1960) *J. Natl. Cancer Inst.,* **24,** 971.
Jorgensen, P. L., (1975) *Q. Rev. Biophys.,* **7,** 239.
Kanner, B. I., Serrano, R., Kandrach, M. A. and Racker, E. (1976) *Biochem. Biophys. Res. Commun.,* **69,** 1050.
Lien, S. and Racker, E. (1971) *Methods Enzymol.,* **A23,** 547.
Lowry, O. H., Rosebrough, N. J. Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.
MacLennan, D. H. (1970) *J. Biol. Chem.,* **245,** 4508.
Mommaerts, E. B.. Eckert, E. A., Beard, D., Sharp, D. G. and Beard, J. W. (1952) *Proc. Soc. Exp. Biol. Med.,* **79,** 450.
Ouchterlony, O. (1958) *Progress in allergy,* Ed. P. Kallos (Karger, Basel) **5,** 1.
Pullman, M. E., Penefsky, H. S., Datta, A. and Racker, E. (1960) *J. Biol. Chem.,* **235,** 3322.
Racker, E. (1967) *Fed. Proc.,* **26,** 1335.
Taussky, H. H. and Shorr, E. (1953) *J. Biol. Chem.,* **202,** 675.
Vogt, P. K. and Ishizaki, R. (1965) *Virology,* **26,** 664.