Showing the amount of FFA liberated after incubation of post-heparin guinea-pig plasma with intralipid, control and diabetic rat serum

| | Concentrations of rat serum 0.2 ml | | 1.0 ml | |
|---|---|---|---|---|
| | Control | Experimental | Control | Experimental |
| Mean values ± SEM (mM MFFA liberated) | 151.1 ± 19.5 | 251.9 ± 13.0 | 298.9 ± 25.6 | 431.7 ± 26.7 |
| t-values | | 5.0 | | 3.5 |
| Number of observations | 5 | 5 | 6 | 8 |

Values shown were corrected from the circulating FFA before incubation at 37 °C.

activated only by apolipoprotein C-2 whose origin is extrahepatic[1]. It was therefore thought worthwhile to study the activators of LPL in the serum of alloxan diabetic rats.

The assay was conducted, based on the observation of Whayne and Felts[5]. They showed that guinea-pig post-heparin plasma did not hydrolyze intralipid unless it was activated by the addition of human or rat serum. The methods used in the experiment were conducted according to Chu et al.[6]. Serum of control and alloxan diabetic rats were used to activate the post-heparin plasma of guinea-pigs for the assay.

The response of the system is shown in the figure, which shows that the amount of FFA liberated during the digestion occurs actively till 2.0 ml of rat serum. It was therefore decided to use 2 concentrations to observe the difference between the normal and alloxan diabetic serum.

It can be seen from the table that at 0.2 ml and 1.0 ml of serum concentrations of control and diabetic rats that there has been a significant increase of FFA liberated when diabetic serum was used. This would probably mean that the presence of larger quantities of the activators of LPL in the diabetic serum activated the post-heparin treated guinea-pig plasma to a greater extent than the controls.

The mean values of blood sugar and FFA of the control and diabetic rats were respectively 120 and 444 mg %; 70.4 and 254.6 mM/100 ml of serum. The corrected elevation of plasma activators of LPL in the alloxan diabetic rat serum when compared to controls were 251.9 and 431.7 in 2 concentrations of 0.2 and 1.0 ml of serum respectively.

From these observations one would feel that lipolysis of the circulating triglycerides were occurring very rapidly in the insulin deficient diabetes. On the contrary, inspite of the elevated plasma activators the LPL activity is absent owing to lack of insulin[2]. It seems that insulin could influence LPL activity but could not influence the proteins which help to activate the process. These apoproteins belong to CII which usually predominate in the VLDL[6].

The bond between atherosclerosis and diabetes is still not very clear, since VLDLs play an important role in the atherosclerotic process[7], and apolipoprotein C has been demonstrated in the atherosclerotic lesion of both hyperlipidaemic and normolipidaemic individuals[8]. The presence of significantly high level of plasma activators in insulin deficient diabetes could possibly be the clue to the link between the 2 diseases.

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## Viral adenosine triphosphatase

R.K. Banerjee[1,2]

*Departments of Biochemistry and Molecular and Cell Biology, Cornell University, Ithaca (New York 14853, USA), 13 February 1978*

*Summary.* The catalytic and immunological properties of an adenosine triphosphatase from different types of virus have been studied. The avian myeloblastosis virus has been found to be specialized in holding this enzyme in a highly active state as compared to other virus with respect to their host cell enzyme. Catalytically myeloblastosis virus and Rous virus ATPase behave alike, while that of the Reo virus is significantly different.

An adenosine triphosphatase (ATPase) activity, associated with avian myeloblastosis virus (AMV)[3], has been purified and characterized[4]. This enzyme is incorporated into the virus during cytoplasmic budding from its host cell myeloblast[5]. The partially purified myeloblastic ATPase did exactly match the properties of the virus enzyme[6]. ATPase activity has also been detected in the Rous virus and Reo virus. The Reo virus enzyme has been shown to differ strikingly from that of the AMV or Rous virus. The results have been incorporated in the present communication.

*Materials and methods.* AMV and AMV-infected myeloblast were kindly provided by Dr J.W. Beard and G.E. Houts, authorized by the office of Resources and Logistics of the Virus Oncology Program. Rous sarcoma virus was cultured on chicken embryonic cells in our laboratory by standard procedure[7]. Reo virus was generously supplied by Dr A. Shatkin of Roche Institute of Molecular Biology, Nutley, New Jersey. The ATPase activity of the Rous virus, AMV-infected myeloblast and chicken embryonic fibroblast was assayed by colorimetric estimation of Pi[8] as

described in our earlier paper[4]. The enzyme of the AMV-infected myeloblast was solubilized and purified according to the procedure described for AMV-ATPase[4] with slight modification[6]. The cells (350 mg) free of any contaminating virus and suspended in 5 ml of 0.25 M sucrose, 0.5 mM EDTA, 1 mM ATP and 10 mM Tris-Cl (pH 7.2) were treated with 80 vol. of ice cold absolute ethanol. Rest of the procedure was as described before[4].

*Results.* The ATPase activity of AMV, Rous virus or Reo virus differed from one another in several aspects. Both AMV and Rous virus ATPase preferred $Ca^{++}$ to $Mg^{++}$ for optimum activity for the hydrolysis of ATP, whereas the Reo virus ATPase had the absolute requirement for $Mg^{++}$. Table 1 shows that AMV-ATPase had a 100 times higher rate than that of Reo virus, and about a 1000 times higher than that of the Rous virus under their standard assay condition. Another significant difference was noted as regards their sensitivity to trypsin and chymotrypsin. The ATPase activity of both AMV and Rous virus was significantly inactivated in presence of trypsin, while the activity could be protected against trypsin action in presence of ATP. Reo virus ATPase, on the other hand, was slightly stimulated in the presence of trypsin. The stimulation was more than 100% when incubated with chymotrypsin, while AMV-ATPase was inhibited 50% under identical condition. Unlike AMV-ATPase incubation of Reo virus with chymotrypsin at a ratio of 1:1 at 40 °C for 1.5 h was associated with 50% solubilization of the enzyme activity, which could be recovered at 240,000 × g supernatant with about 2fold increase of specific activity. There are of course some similarities as regards their sensitivity to some inhibitors of ATPase. All these virus ATPases were highly sensitive to dicyclohexylcarbodiimide and mercurials like mersalyl but insensitive to azide or ouabain. The same type of sensitivity to these inhibitors was noted in case of ATPase of AMV-infected myeloblast, or chicken embryonic fibroblast, or if AMV was grown on the latter.

One interesting observation was made when ATPase activity of AMV was compared to that of its host cell. The AMV-ATPase showed 100fold higher specific activity over its host cell enzyme (30 compared to 0.3 of the myeloblast). The ATPase activity of the Rous virus grown on chicken fibroblast, on the other hand, had almost the same specific activity as that of the fibroblast itself. In this connection, one interesting effect of quercetin was observed on this AMV-ATPase. Table 2 shows that quercetin strongly inhibited the membrane-bound ATPase of AMV while it had no effect on the purified enzyme. Quercetin had no significant effect on the AMV-infected myeloblast ATPase or the enzyme purified from it. Again, AMV enzyme was inhibited by higher concentration of $Ca^{++}$ above 5 mM. $Ca^{++}$ at a concentration of 10 mM inhibited the AMV enzyme 33% while it had no effect at all on the purified enzyme. These allotopic properties indicate that the AMV membrane may hold the enzyme in such a conformation that it induces a very high catalytic activity on this enzyme. This phenomenon is absent in case of Rous virus.

There is marked difference in the antigenic property of the ATPase from different sources. Table 3 shows that the antibody prepared against the purified ATPase from AMV inhibited the ATPase activity of the AMV itself as well as the purified enzyme from AMV and AMV-infected myeloblast. It did not inhibit the ATPase activity either of the chicken fibroblast or that of the AMV grown on fibroblast. Reo virus ATPase was also insensitive to this antibody.

*Discussion.* The ATPase of the virus as described in this communication may be unique as it differs from the mitochondrial, bacterial or $Na^{+}$-$K^{+}$-ATPase. As regards the insensitivity to azide and ouabain, and sensitivity to mersalyl and trypsin, the AMV and Rous virus ATPases behave alike. Regarding chymotrypsin sensitivity and metal ion requirement, Reo virus ATPase looks different from

Table 1. Effect of some reagents on ATPase activity

| | Specific activity (μmoles/min/mg) | | |
|---|---|---|---|
| | AMV | Rous Virus | Reo Virus |
| Control | 33 | 0.035 | 0.33 |
| + Mersalyl 1 mM | 7.5 | 0.01 | 0.07 |
| + Azide 2 mM | 32.6 | 0.034 | 0.32 |
| + Ouabain 1 mM | 32 | 0.034 | 0.32 |
| + Dicyclohexylcarbodiimide 125 μM | 8 | 0.02 | 0.19 |
| + Trypsin (2:1) | 9 | 0.012 | 0.41 |
| + ATP 20 mM + Trypsin | 30 | 0.046 | 0.28 |
| + Chymotrypsin (2:1) | 16 | - | 0.67 |

AMV or Reo or Rous virus (2.5 μg, 60 μg and 136 μg respectively) was incubated with the indicated concentration of the reagent at 37 °C for 5 min in a final volume of 0.2 ml containing 0.25 M sucrose, 10 mM Tris Cl (pH 8), 0.5 mM EDTA followed by assay of Ca-ATPase activity with 50 mM Tris Cl pH 8.0 and 5 mM Ca-ATP. In the case of trypsin and chymotrypsin, incubation was at 37 and 40 °C respectively, using virus to proteolytic enzyme ratio of 2:1. Trypsin and chymotrypsin action was terminated by adding equal amount of soybean trypsin inhibitor and trasylol respectively before assay.

Table 2. Effect of quercetin on ATPase activity

| | Specific activity (μmoles/min/mg) | | | |
|---|---|---|---|---|
| | AMV | | AMV-infected myeloblast | |
| | Virus enzyme | Purified enzyme | Myeloblast enzyme | Purified enzyme |
| Control | 26.3 | 59 | 0.26 | 0.90 |
| + Quercetin 5 μg/ml | 5.3 | 59 | 0.24 | 0.95 |

The enzyme was incubated with an indicated amount of quercetin in a final volume of 0.2 ml of sucrose-Tris-EDTA solution as in table 1. ATPase activity was assayed using Mg-ATP as substrate. The enzyme was purified from AMV or myeloblast by ethanol extraction, sonication in alkaline pH, ammonium sulfate precipitation, Bio-gel A − 0.5 m chromatography and sucrose density gradient centrifugation.

Table 3. Effect of antiserum of virus ATPase on the ATPase activity

| | Specific activity (μmoles/min/mg) | |
|---|---|---|
| | Normal serum | Antiserum |
| AMV ATPase (virus-bound) | 30 | 16 |
| AMV ATPase (purified) | 60 | 30 |
| AMV-infected myeloblast ATPase (purified) | 1.1 | 0.6 |
| ATPase of AMV grown on chicken fibroblast | 0.067 | 0.07 |
| ATPase of chicken fibroblast | 0.05 | 0.05 |
| Reo virus ATPase | 0.35 | 0.36 |

In a final volume of 0.2 ml containing sucrose Tris EDTA as in table 1, the AMV (2.5 μg) or the purified enzyme (2.2 μg), 40 μg of the purified enzyme from AMV-infected myeloblast, 64 μg of AMV grown on chicken fibroblast, 136 μg of chicken fibroblast or 60 μg of Reo virus was incubated with 20 μl of normal or antiserum at 37 °C for 75 min followed by the assay of Ca-ATPase activity. In the case of Reo virus, Mg-ATPase activity was assayed. The titration curve (not shown) indicated not more than 50% inhibition of enzyme activity, even after using 100 μl of the antiserum. The AMV and the purified enzyme start saturation of inhibition from 20 μl and 10 μl respectively. For comparison, 20 μl was therefore used in this experiment.

AMV-ATPase. Ethanol extraction followed by sonication at an alkaline pH solubilizes the AMV-ATPase[4], while the same treatment completely inactivates the Reo virus enzyme. As the active site of the ATPase in AMV, AMV-infected myeloblast, Rous virus, Reo virus and chicken embryonic cells or fibroblasts is oriented outside to hydrolyse the ATP of the external medium, it may be termed as 'ecto-ATPase'[6,9]. The activity of the enzyme was therefore measured directly by suspending the cells or the virus in the incubating medium. It is worth mentioning that the specific activity of the purified enzyme from AMV is double as compared to the intact virus (tables 2 and 3). It looks as if most of the enzyme molecules in the purified preparation are inactivated. However, the high specific activity of the virus ATPase, the marked loss of activity after solubilization, which could not be restored even in presence of the viral lipids, and also the differential sensitivity of the enzyme towards quercetin (table 2) before and after solubilization, all point to the conclusion that AMV holds the enzyme in a conformation that induces a high ATPase activity of the enzyme. Thus the apparent inactivation may be due to change of conformation after solubilization. Antigenically Reo virus ATPase differs from AMV-ATPase as it originates from different host cells. The immunological cross-reaction between an ecto-ATPase from chicken oviduct and the AMV-ATPase, as reported previously, supports the host origin[4]. The reason why the antibody against the AMV-ATPase did not inhibit the ATPase activity of the chicken fibroblast, or AMV grown on the latter, may be due to masking of the antigenic site in the membrane. Conclusive evidence will emerge from cross-reaction studies after solubilization. It thus appears that Reo virus ATPase is completely different from AMV or Rous virus enzyme as regards their catalytic and antigenic properties. Although Rous virus ATPase behaves similarly to that of the AMV, regarding their sensitivity towards inhibitors, AMV-ATPase has been highly specialized in showing very high catalytic rate not observed in other viruses.

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## Adenylate cyclase and phosphodiesterase from brain tissue: Different stabilities during incubation of cerebral cortical slices

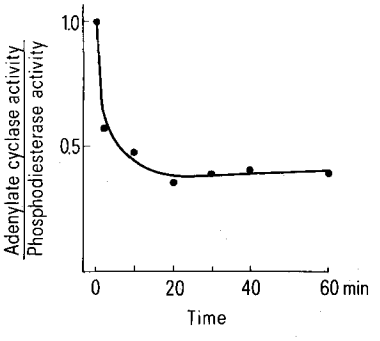
J. Schultz, Gertrud Kleefeld and A. du Moulin

*Pharmazeutisches Institut der Universität, Auf der Morgenstelle 8, D-7400 Tübingen (Federal Republic of Germany),* *6 March 1978*

*Summary.* Adenylate cyclase and phosphodiesterase were prepared from brain slices from guinea-pig. The specific activity of adenylate cyclase declined rapidly with increasing incubation time of tissue slices, while phosphodiesterase activity was almost uneffected by the incubation of brain slices.

Receptor-adenylate cyclase interactions in brain have been studied mainly with slices or homogenates of brain tissue[1]. Both preparations offer distinct advantages, and the resulting data should be viewed together when evaluating the mechanism of hormone elicited cyclic AMP formation. However, no investigations have been reported describing the effect of incubation of brain slices on the enzymic activities of adenylate cyclase (AC) and phosphodiesterase (PDE). In this report, we present data from experiments with cell-free AC and PDE prepared from cerebral cortical slices which had been incubated identical to the usual protocol carried out in studies with brain tissue slices.

*Methods.* Preparation and incubation of brain cortical slices from guinea-pigs was exactly as described earlier[2]. After various periods of incubation, slices were quickly transferred into a Dounce homogenizer and disrupted in ice-cold buffer containing 48 mM Tris-HCl, 12 mM $MgCl_2$ and 0.1 mM EGTA, pH 7.4. After addition of 5 ml 1 mM $KHCO_3$-solution, the homogenate was centrifuged (5000 × g for 15 min). The precipitate was washed once with $KHCO_3$-solution and the final pellet was dispersed in Tris/$MgCl_2$ buffer. Cyclic AMP formation was determined in a total volume of 200 µl containing 40 mM Tris-HCl, 10 mM $MgCl_2$, 0,1 mM EGTA, 10 µM $CaCl_2$ above EGTA, 1 mM 1-methyl 3-isobutylxanthine (IBMX, EGA-Chemie, Steinheim, BRD), 13,8 mM creatinphosphate, 0.7 mg creatinkinase and 120 µg enzyme protein, pH 7.4. After equilibration at 37 °C, ATP (2 mM) was added to start the reaction. During the incubation, no hydrolysis of newly formed cyclic AMP occurred. After 5 min, the reaction was stopped by addition of 50 mg dry $Al_2O_3$ and 1 ml 60 mM




Ratio of activities of AC and PDE prepared from cerebral cortical slices which had been incubated for various periods of time (abscissa) in oxygenated Krebs-Ringer-bicarbonate buffer at 37 °C before enzyme preparation.