Comenius University in Bratislava, Faculty of Medicine
Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

LABORATORY PROTOCOL GM-WS - 3rd seminar

Determination of Ca^{2+}-dependent ATP-ase activity in membranes of human erythrocytes

Name, group:	Date:

Principle:

Ca^{2+}-ATP-ase in presence of Mg^{2+} (ATP binds to ATP-ase only as a complex with Mg^{2+}) and Ca^{2+} (activator of Ca^{2+}-ATP-ase) hydrolyses ATP to ADP and inorganic phosphate:

$$ATP + H_2O \rightarrow ADP + H_3PO_4$$

Released phosphate is determined through phosphomolybdic acid spectrophotometrically.

Procedure:

In the first test tube containing mixture I we measure amount of phosphate that was produced by **activated ATP-ase (total activity) and non-enzymatic hydrolysis of ATP**. In the second test tube containing mixture II without Ca^{2+} ions we measure amount of phosphate that was produced by **non-activated ATP-ase (basal activity) and non-enzymatic hydrolysis of ATP.** In the third test tube we measure amount of phosphate that was produced **only by non-enzymatic hydrolysis of ATP**. After detecting and measuring amount of produced phosphate we calculate total and basal activity of Ca^{2+}-ATP-ase and by subtracting basal activity from total activity we get **Ca^{2+}-dependent activity of Ca^{2+}-ATP-ase**.

1st part - production of phosphate				
sample	1	2	3	ref. sample
mixture I (contains both Ca^{2+} and Mg^{2+} ions)	1.5 ml	---	---	---
mixture II (contains Mg^{2+} but no Ca^{2+} ions)	---	1.5 ml	---	---
mixture III (contains Ca^{2+} but no Mg^{2+} ions)	---	---	1.5 ml	---
suspension of erythrocyte membranes	0.5 ml	0.5 ml	0.5 ml	---
We let the samples stand at laboratory temperature for 15 minutes.				
20% TCA	1 ml	1 ml	1 ml	---
2nd part - spectrophotometrical detection of produced phosphate (new test tubes)				
from the test tubes from the 1st part we pipet	1.5 ml	1.5 ml	1.5 ml	---
water	---	---	---	1.5 ml
ammonium molybdate (in dispenser)	0.4 ml	0.4 ml	0.4 ml	0.4 ml
$SnCl_2$ (in dispenser)	0.3 ml	0.3 ml	0.3 ml	0.3 ml
After 10 minutes we measure absorbance against reference sample at 720 nm.				

Calculation:

Erythrocyte membrane suspension contains 200 mg of proteins in 100 ml of solution.

sample	1	2	3
absorbance			
nmol of phosphate from cal. curve (=using enzyme from 0.25 ml of suspension)			
nmol of phosphate/g of proteins			
nkat/g			

Reference values of activity of ATP-ase in erythrocytes: **cca. 80 nkat/g of proteins**

Conclusion:

Total Ca^{2+}-ATP-ase activity = ...nkat/g
(Activity in test tube 1 minus activity in test tube 3)

Ca^{2+}-dependent Ca^{2+}-ATP-ase activity = ...nkat/g
(Activity in test tube 1 minus activity in test tube 2)

Basal Ca^{2+}-ATP-ase activity = ...nkat/g
(Activity in test tube 2 minus activity in test tube 3)

Thanks to Ca^{2+} ions the activity of Ca^{2+}-ATP-ase increased by .....%.
(Basal activity of Ca^{2+}-ATP-ase is considered to be 100 %).

Literature for next week:
- Aerobic and anaerobic oxidation of glucose.
- Practical exercises in biochemistry (Asklepios, 1993) – Chapter 5 - Theoretical part
- Lippincott´s: Chapter 8 - Glycolysis, formulas: Fig. 8.12; 8.15; 8.16; 8.18.