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Oxidoreductases - dehydrogenases

succinate dehydrogenase EC 1.3.5.1

Exercise 1

The aim of the experiment is to obtain the extract of succinate dehydrogenase (EC 1.3.5.1, succinate-coenzyme Q reductase) from the bovine heart muscles and to determine the activity of enzyme.

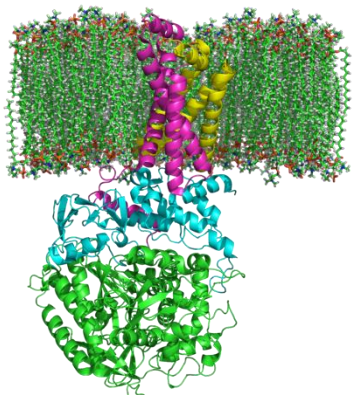

Fig 1.*The structure of succinate dehydrogenase in a phospholipid membrane.*

Protocol

The 5g of bovine myocardium tissue purified from fat, put in a beaker and fill with 50 cm^3 of distilled water, and stir for 10 minutes at room temperature.

After this time squeeze the pulp through gauze and then mix with **50 cm^3 of** distilled water. This step must be repeated until the muscle pulp is completely discolored.

This washed pulp grind in a mortar with silica (0.5 tablespoons) for 5 minutes, add 10 cm^3 of phosphate buffer, pH 7.2.

Obtained homogenate centrifuge for 30 minutes with 2000 x g. Keep the supernatant for the determination of succinate dehydrogenase activity.

Prepare six test tubes containing compounds of the incubation mixture according to the table 1.

Incubate all test tubes at room temperature. Measure and note the discoloration time of each test tube. Explain the results.

Tab 1. The list of compounds of the incubation mixture

The components in cm^3	**Number of test tube**					
	1	**2**	**3**	**4**	**5**	**6**
Phosphate buffer, pH 7.2	2	2	2	2	2	2
Sodium succinate, 0.01 mol/dm^3	x	1	1	1	1	1
Dichlorophenolindophenol,0.011%	1	1	1	1	1	1
Potassium cyanide, 0.02 mol/dm^3	0.2	0.2	0.2	0.2	x	x
Sodium malonate,0.01 mol/dm^3	x	x	x	x	x	1
Distilled water	5	4	3.8	4.2	4.2	3
Enzymatic extract	0.2	0.2	0.4	-	0.2	0.2
Time for discoloration, minutes						