Oxidoreductases - dehydrogenases

E.C.1.3.99.1. Succinic Dehydrogenase

The aim:

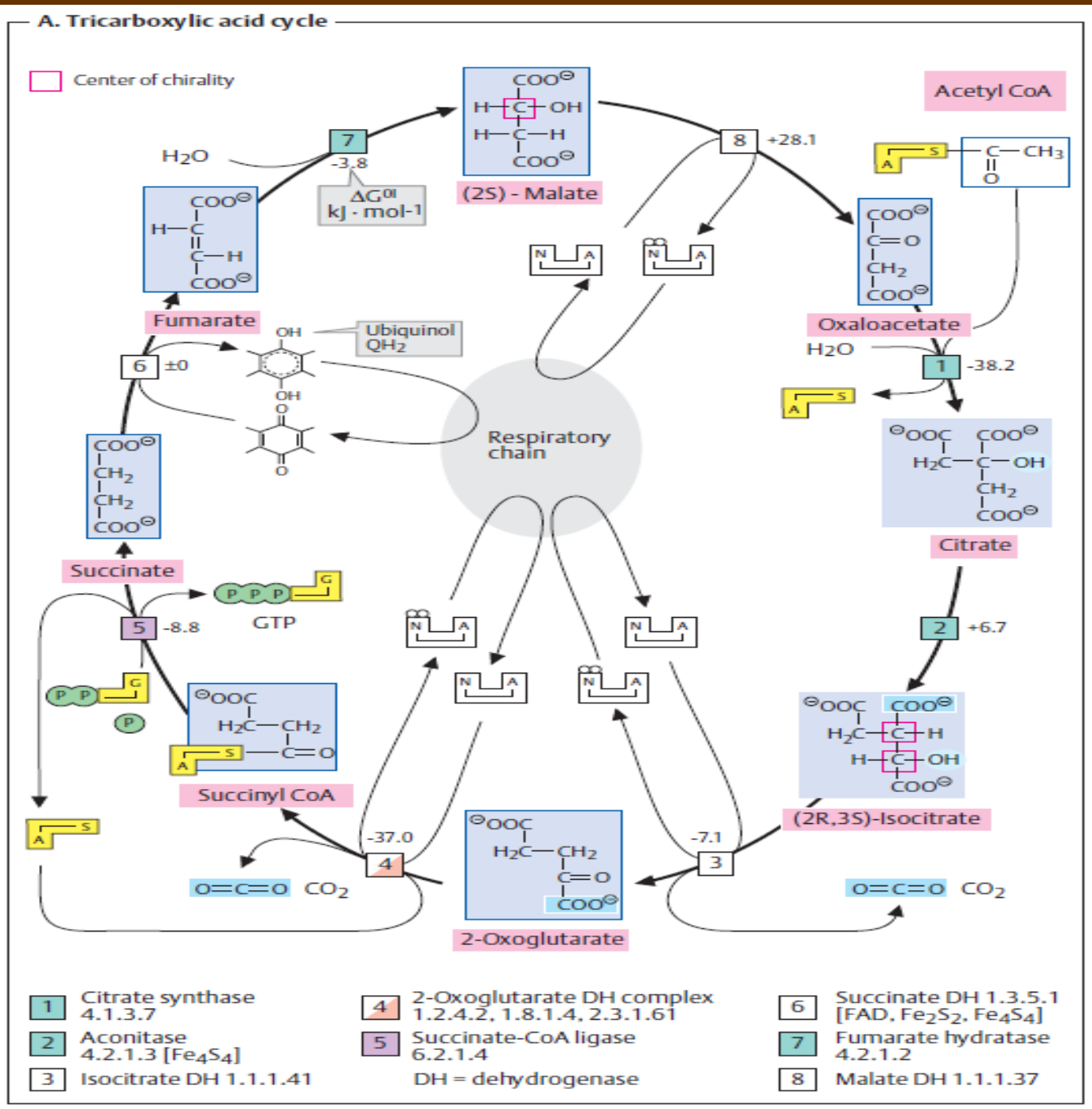
The isolation of enzyme from biological material and the estimation of activity of succinic dehydrogenase in different conditions of reaction (competitive and noncompetitive inhibitors)

The determination of activity:

- In order to demonstrate redox properties of enzyme in vitro selected stainings are used eg. dichlorophenolindophenol

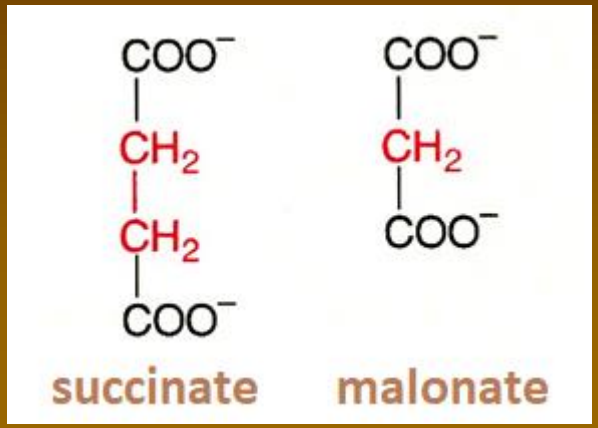
Properties of enzyme:

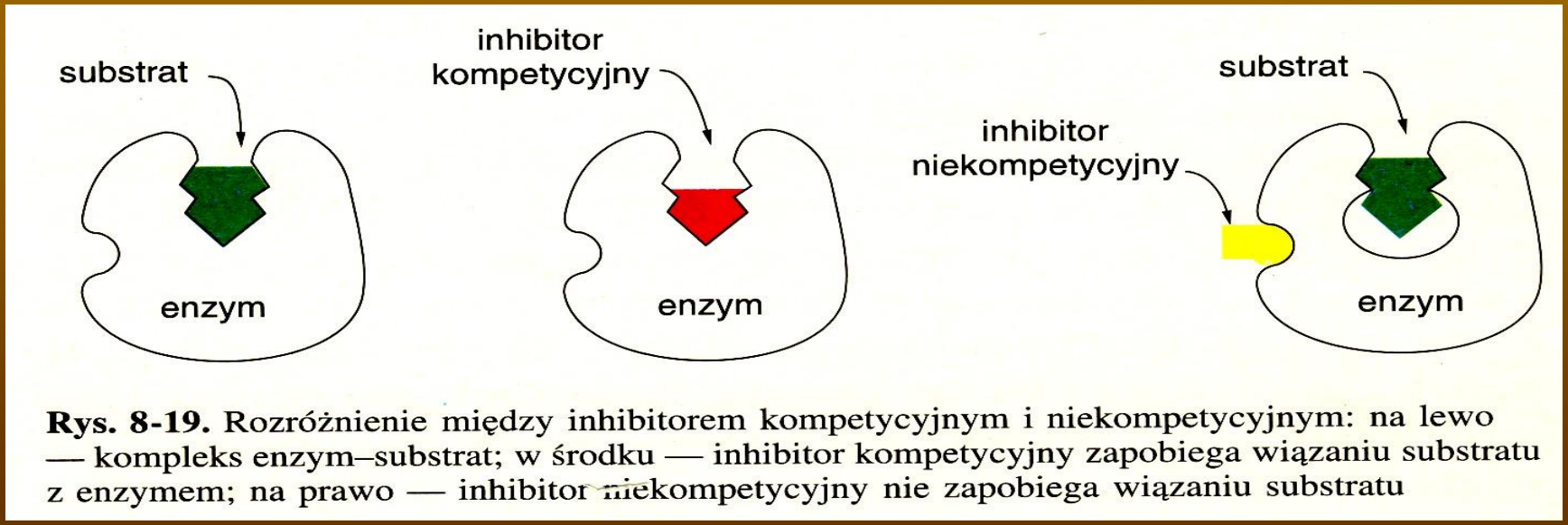
- Mitochondrial oxidoreductase
- Iron-sulfur protein, vitamin B2 – riboflavin is its prostetic group
- The enzyme is integral part of respiratory chain and binds Krebs cycle with the syntesis of ATP


A. Tricarboxylic acid cycle
Center of chirality
Acetyl CoA
(2S) - Malate
+28.1
-3.8
ΔG°' kJ · mol-1
H2O
Fumarate
Ubiquinol QH2
±0
Oxaloacetate
H2O
-38.2
Respiratory chain
Citrate
+6.7
Succinate
GTP
-8.8
Succinyl CoA
(2R,3S)-Isocitrate
-37.0
-7.1
CO2
2-Oxoglutarate
1 Citrate synthase 4.1.3.7
2 Aconitase 4.2.1.3 [Fe4S4]
3 Isocitrate DH 1.1.1.41
4 2-Oxoglutarate DH complex 1.2.4.2, 1.8.1.4, 2.3.1.61
5 Succinate-CoA ligase 6.2.1.4
DH = dehydrogenase
6 Succinate DH 1.3.5.1 [FAD, Fe2S2, Fe4S4]
7 Fumarate hydratase 4.2.1.2
8 Malate DH 1.1.1.37

Competitive inhibition:

Malate is the inhibitor of succinic dehydrogenase. Its structure is similar to substrate – succinate (the difference in one carbn atom) . Succinate can eliminate inhibitor from enzyme active center





Rys. 8-19. Rozróżnienie między inhibitorem kompetycyjnym i niekompetycyjnym: na lewo — kompleks enzym–substrat; w środku — inhibitor kompetycyjny zapobiega wiązaniu substratu z enzymem; na prawo — inhibitor niekompetycyjny nie zapobiega wiązaniu substratu

The action of cyanide on enzymes of respiratory chain :

Kalium cyanide like majority of cyanides is toxic for humans and animals. Its toxic action is related to the inhibition of respiration on cellular level via irreversible inhibition of cytochrome oxidase (cyanides bind to iron of cytochromes). Cytochrome oxidase is key enzyme of resporatory chain and secondly oxidative phosphorylation. In result regardless of oxygen transportation from lungs tissue hypoxia occurs.

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.

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						