Exp.10 Assay of carbohydrate (part II)

Introduction：

D. Bial's test distinguishing pentoses from hexoses:

Pentoses undergo dehydration much more rapidly than hexoses. As a result, furfural is readily produced by heating pentoses in acid solution.

The furfural can reacts with orcinol in the presence of Fe^{2+} to yield a green-colored iron complex. The reaction as performed is a timed reaction, and hexoses do not interfere, although on prolonged heating a red precipitate may be produced from hexoses.

E. Seliwanoff's test for ketoses:

This test is based upon the fact that ketohexoses form furfural derivative more readily than aldohexoses. Since fructose is the only biologically important ketohexoses, this test is used to detect the presence of fructose in either the free or combined state.

The 4-hydroxy-methyl-furfural, which is formed when fructose is heated in a strongly acid solution, reacts, with resorcinol to form a red colored compound. Fructose in the combined state is converted to the monosaccharide by the hydrolytic action of the acid.

At very high concentrations, the aldohexoses such as glucose, etc. will give a positive test. Pentoses react in this test to give a blue to green product.

F. Iodine test for starch:

The iodine color obtained with polysaccharides, such as starch and glycogen, is believed to be due to a coordination complex between the helically coiled polysaccharide chains and the iodine centrally located within the helix.

The color depends on the length of the linear (α-1,4-linked) or unbranched chain available for complex formation. Thus, amylase, the linear starch component, gives a deep blue color; amylopectin, the branched chain component, gives a purple color, glycogen gives a reddish brown color, while dextrin, formed from the partial hydrolysis of starch, gives colors varying from brownish red to colorless depending on the size of the molecule.

The color is decreased as the temperature is increased and is intensified as temperature is lowered, and is destroyed by the presence of alcohol and by alkali.

Sugars which have the property of readily reducing alkaline solutions of the oxide of metals like copper, bismuth and mercury is called reducing sugars. Test for reducing power is as followed:

Reagents:

1. 1% solutions of carbohydrates:
   xylose, glucose, fructose, maltose, sucrose, starch and glycogen
2. 1% glycine
3. Bial's reagent
4. Seliwanoff's reagent

5. Iodine solution

Procedure：

D. Bial's test distinguishing pentoses from hexoses:

Add 0.5 ml of the sample into separate test tubes, then add 0.5 ml of Bial's reagent into each tube. Mix. Heat these tubes in boiling water bath for exactly 60 seconds. Blue-green color is obtained if the test is positive. All other colors obtained may be considered to be a negative test. Record your results.

E. Seliwanoff's test for ketoses:

Add 0.5 ml of 1% solutions of glucose, fructose, sucrose, and water into separate test tubes, then add 0.5 ml of Seliwanoff's reagent into each tube. Mix. Heat these tubes in boiling water bath for exactly 60 seconds. Record your results. Then continue heating, observing the color change at 30 seconds intervals for 3 minutes.

F. Iodine test for starch:

Add 0.5 ml of :(1)1% fructose, (2)1% glycogen, (3)1% starch, (4)1% starch into separate test tubes, then add one drop of iodine solution into each tube. What is the color? Do you see any color change?

Then heat (F-3) tube in boiling water bath for ~5minutes. Record the changing of the color?

Then add 95% alcohol in (F-4) tube until the blue color

disappears. Record the minimum amount of alcohol that is necessary to decolorize the starch solution.

Results：

Record your results.

(有無反應請以+/-表示；以及顏色變化有無沉澱，如下 A-(1)範例)

Sample \ Reagent		D	E
		Bial's Test	**Seliwanoff's Test**
(1)	**Xylose**		 **min**
(2)	**Glucose**		 **min**
(3)	**Fructose**		 **min**
(4)	**Sucrose**		 **min**
(5)	**Maltose**		 **min**
(6)	**Starch**	**×**	**×**
(7)	**Glycine**	**×**	**×**
(8)	**Unknown**		 **min**

Reagent / Sample		F
		Iodine Test
(1)	Fructose	
(2)	Glycogen	
(3)	Starch	Starch: Biol color:_____→_____
(4)	Starch	Starch: EtOH V=_____滴
(5)	Unknown	

Discussions：