

# THE MECHANISM OF STARCH-IODINE REACTION

## I. Critical investigation of actual viewpoints

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One of the most characteristic features of starch known for long is its assuming blue colour on contacting with iodine.

During the evolution of starch chemistry, the fact emphasized already by MAQUENNE and ROUX [1] in 1905 that starch consists of two components, has fully been proved. One of the components is amylopectin which constitutes, according to the species of starch, about 70—80% of the starch granule (some waxy starches might consist of up to 100% of amylopectin, while amylopectin constitutes just 20—30% in some pea-starches). Amylopectin is of highly branched structure and gives a violet-red or brown-red colouring with iodine, depending on its type. Blue-colouring with iodine occurs only in the component called amylose which consists of glucose with bonds  $\alpha$ -1,4 and is unramified.

Some authors attribute the blue colour reaction to a phenomenon of adsorption in which colloidal aggregates are involved. Some others have referred to the formation of complexes, again others have tried to prove the existence of a determined compound. The latter theory was widely acknowledged at the time when starch was still believed to consist of a determined molecule.

Some years ago, iodine was generally assumed to be bound by amylose only, while amylopectin was altogether excluded from the reaction. Recently, some investigators produced pure amylopectin and having determined the quantity of iodine that might be bound, it was found to be 0—0,7% [2, 3]. It cannot be stated for certain whether the taking up of iodine is due to incomplete fractionation and to the failure to remove the traces of amylose or due to the longer chains of some amylopectin molecules. Consequently, we should, in fact, speak of starch-iodine reaction, unless we investigate pure amylose specimens.

The idea of the helical amylose model was first suggested by HANES. According to him, one period contains six glucose units, and a cycle of less than six units doesn't give any colour reaction. FREUDENBERG et al., by constructing the helix model, have proved that it may hold the iodine molecule. In their

view the conditions are exactly identical with those occurring in the case of non-polar solutions of iodine [4].

Evidences of the helical structure are the following :

a) *The Schardinger-dextrins.* Amylase of bacterial origine (*Bac. macerans*) hydrolyses starch into the so-called Schardinger-dextrins, *i. e.* results in cyclomylases of 6,7 and 8 members. This might be explained by the fact that every sixth glucose member of the chain-shaped molecule approaches the other in space, thus forming coils consisting of six glucose units.

b) *The so-called "V" X-ray diffraction pattern.* KATZ [5] has dealt with the roentgenographic investigation of starches. He called type "V" the crystalline starch precipitated from its solution by alcohol and found the roentgenogram of iodine—starch to be very similar to it. RUNDLE [7] determined the sizes of the elementar cells of type "V" and concluded that amylose is of a helical structure.

Ss. W. NEDSWEZKI [6] was the first to prove in 1934 by viscosimetric measurements that the iodine reaction takes place in two steps. He claims the first step to be a typical chemical reaction which is followed by the adsorption. The evidences of the chemical reaction are the following :

a) According to the potentiometric titration curve, the iodine-binding occurs in the first step isopotentially at a potential characteristic for the special type of starch. In the second stage the potential, too, increases.

b) The Rundle complementation test [7]. RUNDLE added iodine to amylose solution, removed with a precipitating agent the complex formed and, having investigated the amylose residue, found that it didn't contain iodine at all, while in the precipitated complex amylose was iodine-saturated.

In RUNDLE's opinion the amylose chains distorted to helical shape form dipols within the helices, and produce in the direction of axis of the helix resultant dipol moments, which are increasing with increasing length of the helix. The iodine molecules that have penetrated into the helix and settled in the direction of the axis of the helix acquire induced dipol moment, the value of which depends on the number of iodine molecules established in the neighbourhood in both directions. The summarized effect of the external and internal dipols produces the contraction of helices when complexes are formed.

In FRENKEL's opinion [6] this dipol theory is of little probability. According to him, HILBERTS' [8] theory is more acceptable. HILBERT suggests that in the moment of complexing, the amylose forms hydrogen bonds with the complexing agent and the molecular forces are transiting into the state of internal saturation. The presence of the hydrogen bond is corroborated by the fact that the molecular spectrum changes so substantially that it obviously shows the rearrangement of the electron shell.

According to the concept accepted so far, the structure of iodine amylose is represented by FREUDENBERG's theory of helix confirmed by the forming

of Schardinger-dextrins. FRENKEL [6] and some others claim that the helix results from the linear structure instantaneously, under the influence of some complexing agents. In this case, the formation of Schardinger-dextrins offers no evidence for the helical structure! Should amylase be a complexing material? This seems rather improbable, since its molecular size is far too big, as already tertiary alcohols cannot be held within the normal helix: they form a period of a larger number of members. Amylose would require an enormous ring, but why does it result then in dextrans of 6, 7 and 8 units? Consequently, either this cannot be accepted as evidence, or the theory of instantaneous helix-formation should be revised. In the presence of iodine, when amylose is undoubtedly of helical structure, the amylopectin, under the influence of amylase, decomposes, but iodine-amylose remains intact [6]. The fact that amylopectin decomposes in the presence of iodine, is an additional proof of the fact that iodine has no general inhibitory effect on the enzyme, though seems to have a local effect. This problem will be taken up later.

The interpretation of FRENKEL's experiment proving the instantaneous helix formation can be disputed, too. He submerges frozen starch solution into iodine solution [6]; in his opinion iodine diffuses into ice and fails to become blue exclusively because the helices are unable to form. In fact, the melting surface alone becomes blue during melting. We admit that iodine may diffuse, though we may as well assume that the helix existed previously and its active hydroxyls, which form the colouring complex with iodine, create in frozen state a steady hydrogen bond with the hydroxyls of water.

In the case of iodine-amylose, recent experiments decidedly support the helical structure, proved earlier by various authors, but the problem whether the helix forms instantaneously, or is originally present in the starch, remains to be decided yet. It is generally believed that helices form instantaneously. This supposition is supported by two circumstances:

a) Iodine is taken up as follows: first the saturation of one helix is completed, while the saturation of a second begins only afterwards. This fact was proved by RUNDLE's [7] experiments.

b) The aforesaid melting experiment of FRENKEL, the correct interpretation of which can still be discussed.

It will be pointed out later that even RUNDLE's experiment can be differently interpreted. It might yield an evidence contrary to what it was meant to.

The first problem to be solved is whether or not ready helices exist before the addition of the complexing agent.

Before answering this question, let us consider the other questions to be answered.

As pointed out previously, the theory of helical structure has been developed by HANES and FREUDENBERG. FREUDENBERG's idea consists, essentially, of two points:

- a) the amylose chain is of helical structure,
- b) iodine dissolves in the helices as in hydrocarbons (= physical solution occurs).

Recent experiments seemed to refute both statements. The helical structure seemed to be inconsistent with the fact that not only starch, polyvinyl-alcohol and cellulose swelled with  $ZnCl_2$ , but other agents, as well, such as certain colloidal suspensions of finely dispersed precipitates give colouration with iodine. Similar reaction might be observed with finely dispersed prae-seodymium-acetate and lanthanum-acetate, sodium carboxyhydrindene compounds and acetoin, too.

Neither of these agents yield colour reaction either in molecularly dispersed solution, or in well-developed crystalline form, but create in the precipitated state a rather "unclear" lattice. Iodine molecules may be assumed to penetrate into the slits of these particles and the changes of light-absorption of iodine to be in connection with such penetration [9].

For this reason, the theory of helical structure is not generally accepted. It must be added that the theory of physical solution seems to be rejected for good. It is known that iodine dissolves in hydrocarbonlike solvating agents with violet colour reaction. In the case of iodine—starch, the colour is blue, and neither molecular extinction nor the value of the absorption maximum are similar to those of hydrocarbonous solutions. According to MEYER, iodine inserts itself among the micella aggregates of amylose and amylopectin.

RUNDLE and BALDWIN [7] have established that amylose-iodine complex in solution causes dichroism. Absorption of light is much more intensive in the direction of the flow-lines than normally to them. This proves that the molecules are orienting themselves and, as the actual velocity, evidently, cannot orient the iodine molecules, they can be located in no other place than in the interior of the amylose helices. This phenomenon can't be observed in the case of waxy starch, amylopectin and glycogen, none of which contains amylose.

The helical theory can be confirmed to a certain degree by the fact that the previously mentioned colloidal precipitates colouring with iodine all contain hydroxyls, so that the hydroxyls might be assumed to interfere in the colouration. The very fact that air-dry amylose of type "V" is able to bind as much as 25% iodine from iodine vapours, obviously, points to the existence of some special mechanism.

The second question arising is the following: By what mechanism is the binding of iodine promoted?

Our experiments performed in order to clear up the questions raised will be published in subsequent communications.

### Summary

The paper gives a critical investigation of the actual viewpoints about the starch — iodine reaction, with an eye on the following problems :

1. Whether or not, ready helices exist before addition of complexing agent :
2. By what mechanism is the binding of iodine promoted.

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