

# THE MECHANISM OF THE RETROGRADATION OF AMYLOSE

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The aqueous solution of amylose is a typical colloidal solution. The decidedly hydrophil amylose molecules in a dissolved state form irregular helices [1, 2, 3]. These irregularly deformed helices represent the state of the molecule poorest in energy. Complex-forming agents might cause these helices to orient into regular ones, in which case the energy required to raise the molecules to a higher energy level is supplied by the energy that becomes free during the formation of H-bonds.

The transition from the helical state (which should be considered from the energetic standpoint as the basic state) into the state of stretched chains also requires energy.

According to the results of X-ray investigations, the aged semi-crystalline amylose consists of stretched amylose chains linked to each other or partly even within the chain by H-bonds. During a long period, the existence of H-bonds has been a mere hypothesis but SAMEC proved their existence with the aid of infrared spectra [4]. Most probably the H-bonds furnish the entire energy required to stretch the chains.

There exists, however, an other stage of the process demanding energy, *viz.* the stopping of hydration of hydroxyls. This is, so to say, the reverse process of the dissolution where the hydration heat of the hydroxyls covers the work necessary to separate the amylose molecules from each other.

The fact that retrogradation automatically goes on and even can hardly be stopped shows that the amount of free energy of the system decreases. This can be the case only if the energy liberated during the formation of H-bonds exceeds the energy required for the stopping of hydroxyls and for the stretching of the helices. Thus :

$$- \Delta H_{\text{H-bond}} + \Delta H_{\text{dehydration}} + \Delta H_{\text{stretching of helices}} < 0$$

Here temperature has a very interesting role [5]. At low temperatures retrogradation goes on quickly, while at temperatures above 60° C it does not take place at all. This can be partly explained by the fact that at high

temperatures, due to the more energetic thermic movement of the dissolved molecules, the amylose molecules become kinetically freer, and the stretched chains partly wind up again. On the other hand, the H-bonds already formed split up again as retrogradation, it being an exotherm process, is hindered by increased temperature.

The micellar structure of the crystals of aged amylose explains why the more homogeneous one (as to its degree of polymerization) retrogrades faster. Amylose molecules of approximately identical size easily form a crystal lattice, while the heterodisperse fractions containing molecules of various sizes, do not.

There is a virtual contradiction between our statements that though a stretched chain represents a higher energy level as a randomly wound up helix, retrogradation involves, however, a loss of energy. This contradiction disappears, however, if we take into consideration that retrogradation does not merely mean the stretching of the helices but mainly the forming of H-bonds.

Considering the iodine reaction investigations [1, 3, 6] it can easily be imagined that a section of the chains keeps a helix-like structure, and a hydrogen bridge forms here within the helices, through the interaction of water molecules, between the hydroxyls located in the interior of the helices.

Reverting to the question of dehydration, we refer to the retrogradation experiment performed in a  $\text{MgSO}_4$  solution [5].

From a 13 %  $\text{MgSO}_4$  solution amylose precipitates within a few minutes but the decrease of iodine absorption though it takes place within a relatively short time, is not able to keep pace with the velocity of the precipitation.

The following happens: the elimination of hydrate water is performed by the electrolyte, hence amylose precipitates within a few minutes. This amylose has not, however, yet retrograded, therefore the process can be considered to be a simple salting out. The lack of hydrate water-hull promotes the forming of H-bonds, and their energy does not have to cover but the energy needed for stretching the helices. That this actually happens, is proved by the fact that in the 13%  $\text{MgSO}_4$  solution the retrogradation of amylose can be performed within two hours to the same extent as in distilled water within twenty days. (See Figs. 2 and 4 of our previous publication [5]).

In the very moment in which retrogradation begins, the system contains amylose molecules which are kinetically free. The forming of an associate consisting of 2–3 molecules occurs quickly. As the number of the members of the associate increases, however, the velocity of the diffusion decreases rapidly. A vast number of small centres form. These centres, however, merge with rather great difficulty to visible flocculata. Surface active agents inhibit retrogradation by stabilizing dispersion. They hinder the small centres from merging and prevent their crystallization. If, however, aged centres are introduced into a fresh molecular dispersed solution, the solution is, so to say, inoculated, retro-

gradation advances much faster [5]. The final value of retrogradation tends, of course, to the same level as the one of non-inoculated solutions.

During the retrogradation process a certain hydrolysis of the phosphate groups and the glucosidic bonds occurs, too.

The retrogradation of amylose consists of the following stages :

1. The randomly linked helices are stretched by an intake of energy.

2. The stretched chains and segments, resp., after losing their hydrate water-hulls, arrange themselves one after the other.

3. Between the hydroxyls spaced near to each other H-bonds form. If more of such bonds are formed between two molecules they can be separated from each other only by energetic means. The linking of a plurality of molecules produces strong opalization and finally causes flocculation visible to the naked eye.

The most favourable conditions for retrogradation are, therefore, the following :

1. neutral pH,
2. a homogeneous solution as to the degree of polymerization and a high average polymerization degree,
3. the absence of surface-active agents,
4. a high concentration of the solution,
5. a low temperature,
6. the presence of inoculation centres,
7. the presence of hydrate water eliminating materials (*e. g.* anorganic salts in high concentration),
8. the amylose molecule should not be ramified (wheat, corn or other starches).

Consequently, the retrogradation of amylose and starch solutions, resp., can be withheld by the following means :

1. solutions of low concentration should be used,
2. the pH should possibly be alkaline,
3. temperature should be high (60—70° C),
4. surface-active agents should be added to the system,
5. starch-containing amylose with ramified chains (potato starch) should be applied,
6. starch derivatives or partly decomposed starch should be used.

### Summary

The mechanism for the retrogradation of amylose consists of three stages :

- a) the chains stretch,
- b) they lose their hydrate water-hull, and arrange themselves after each other,
- c) H-bonds form between the hydroxyls of amylose and a crystalline structure is formed.

### Literature

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