Investigations on Malt Amylase

IV. An Enzymic Method for the Determination of Viscosity-Molecular Weight Constants

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Viscosimetrical methods have frequently been used for the assay of the molecular weight of polymeric homologous substances giving solutions of high viscosity. An expression for the connection between the viscosity and the molecular weight is given by the modified Arrhenius-Staudinger formula [1,2]

$$\ln \eta_r = K_m c_{gm} M \tag{1}$$

where

η_r = the relative viscosity,
K_m = the viscosity-molecular weight constant,
c_{gm} = the concentration in primary moles per litre, and
M = the molecular weight.

This formula, however, is not valid for all polymeric homologous substances [3,4]. Reviews on this subject have recently been published by Ewart[5] and by Kinell[6].

The substances for which the modified Arrhenius-Staudinger formula is valid, are characterized by one constant, K_m. If such a substance can be submitted to enzymatic depolymerization under such conditions that all linkages are broken with equal ease, and if the break down can be followed both viscosimetrically and by end group determinations, the viscosity-molecular weight constant can easily be determined, if some approximations are made. This will be shown in the case of starch and malt α-amylase in this article. Starch is certainly not one substance but two, amylose and amylo-

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pectin, occuring in given proportions. Thus the values obtained from this mixture will depend on the K_m values for each of them and the proportions. This has, however, no influence on the principles of the method, and as starch has also been used in earlier measurements, the results from these measurements can be compared with the present results.

The number of moles of reducing sugars liberated per litre of a starch solution in unit time when submitted to hydrolysis by malt α-amylase can be determined both viscosimetrically and iodometrically. The number determined viscosimetrically is [7, 8]

$$2K_m M_o c_{gm}^2 \cdot \frac{d \ln \frac{1}{\eta_r}}{dt} \tag{2}$$

where

$$M_o = \text{the primary molecular weight, and}$$
$$t = \text{the time}$$

This expression includes two approximations, both founded on the presumption that the number of linkages broken is small in comparison with the number of linkages at complete polymerization (see equations (5) and (7) in the article quoted[7]).

The enzyme activity determined viscosimetrically, expressed in μA unites, is [7, 9, 10]

$$\mu A = c_s^2 \cdot \frac{d \frac{1}{\ln \eta_r}}{dt} \cdot 10^6 \tag{3}$$

A conversion factor for the amylase activities expressed in μA units and in mg maltose/min $\cdot$ ml is given in a recent paper by the present author [11]: the amylase unite 1 μA liberates reducing sugars corresponding to 0.018 mg maltose/min. If we want to calculate the number of moles reducing sugar liberated in unit time in one liter of starch solution on addition of the enzyme amount 1 μA, we must bear in mind that the activity of the enzyme in the reaction mixture is 0.001 μA and that the time is here counted in seconds. Hence, as the molecular weight of maltose is 342 and $c_s = c_{gm} \cdot 162 \cdot 10^{-3}$,

$$\frac{0.018 \cdot 10^{-3}}{342 \cdot 60} = 2K_m \cdot 162 \cdot \frac{10^{-3}}{162^2}$$

and $K_m = 0.71 \cdot 10^{-4}$.

This constant was calculated previously by Staudinger and Eilers [12] from measurements made by Biltz [13] and they found values between $0.9 \cdot 10^{-4}$ and $2.7 \cdot 10^{-4}$. Staudinger and Husemann [14] have also calculated this constant from experiments where starch was dissolved in formamide. They found the value to be $0.63 \cdot 10^{-4}$ with an uncertainty of 10 %.

SUMMARY

If the number of linkages broken pro unit time in enzymic depolymerization processes, where all linkages are broken with equal ease, can be calculated from end group determinations, and if the modified Arrhenius-Staudinger formula is valid for the substrate, the viscosity-molecular weight constant K_m of the substrate can be calculated from a conversion factor for the enzymatic activity. In this way the constant was calculated for starch, giving $K_m = 0.7 \cdot 10^{-4}$.

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