Carbohydrate Research, 93 (1981) 304–307
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Gelatinisation temperature of starch, as influenced by high pressure

JOHAN M. THEVELEIN*, JOZEF A. VAN ASSCHE,
Laboratorium voor Plantenbiochemie, Katholieke Universiteit te Leuven, Vaartstraat 24, B-3000 Leuven (Belgium)

KAREL HEREMANS,
Laboratorium voor Chemische en Biologische Dynamica, Katholieke Universiteit te Leuven, Celestijnenlaan 200 D, B-3030 Heverlee (Belgium)

AND SICCO Y. GERLSMA
Laboratorium voor Algemene en Technische Biologie, Technische Hogeschool Delft, Julianalaan 67, Delft 8 (The Netherlands)

(Received December 8th, 1980; accepted for publication, January 9th, 1981)

The effect of high pressure on thermal transitions has been studied for proteins[1], DNA and RNA[1], and phospholipid bilayers[2]. However, there have been few studies of carbohydrate systems. Suzuki *et al.* examined the effect of high pressure on the sol–gel phase diagram of an *O*-methylcellulose–water system[3], and the effect of high pressure on the gelatinisation of curdlan, a bacterial (1→3)-β-D-glucan, was recently investigated[4].

We have used high-pressure experiments to determine the molecular nature of the triggering system involved in heat activation of fungal spores and found that a protein thermal transition was most probably involved[5]. Similar experiments on the heat activation of the trehalase enzyme in the spores of *Phycomyces blakesleeanus* led us to consider a possible involvement of a carbohydrate thermal transition in the activation process[6]. Because of the obvious lack of data for such systems, we initiated studies of the effect of high pressure on the gelatinisation of starch granules.

On heating, starch granules undergo a transition from the birefringent, crystalline state to a non-birefringent, highly swollen state at a particular temperature known as the gelatinisation temperature. When the heating is performed at several temperatures, a sigmoidal transition curve is obtained, as shown in Fig. 1. The midpoint temperature lies between 60° and 61°. Application of high pressure results in an upward shift of the gelatinisation temperature with about 3–5°/1000 atm. However, pressure higher than 1500 atm does not further enhance the gelatinisation temperature

---

*To whom correspondence should be sent.

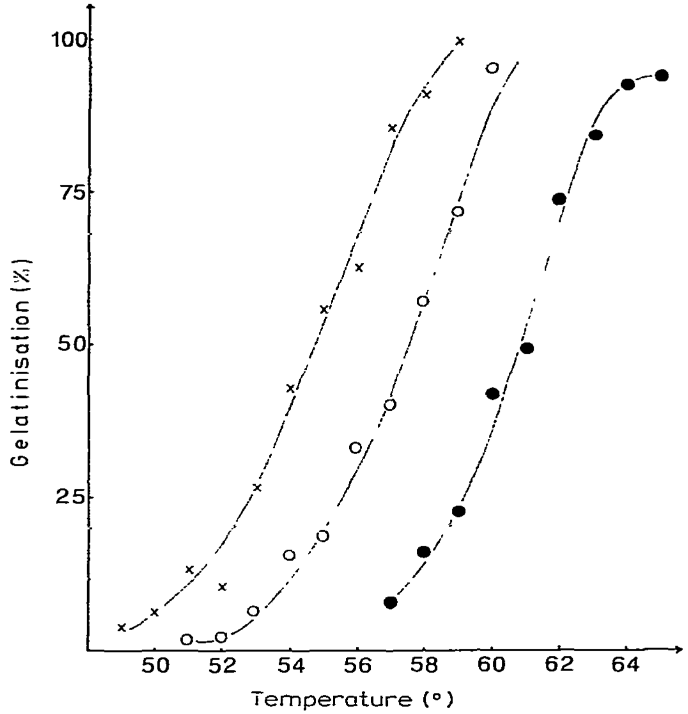

Fig. 1. Gelatinisation of starch granules as influenced by 1-propanol and high pressure: control (—●—), 2M 1-propanol (—×—), 2M 1-propanol + 1000 atm (—○—).

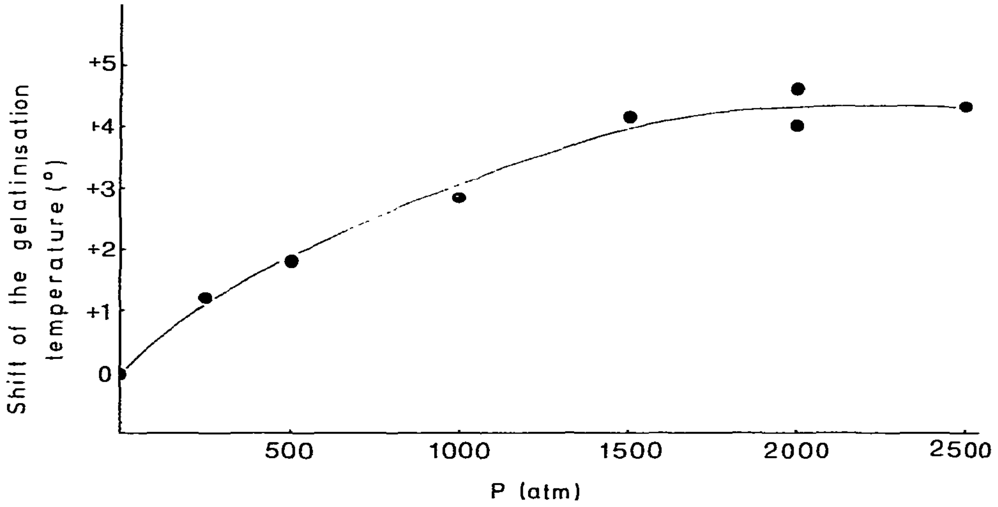

Fig. 2. Upward shift of the gelatinisation temperature of starch granules under the influence of high pressure (gelatinisation temperature = temperature at which 50% of the granules have lost their birefringence).

(Fig. 2). Pressures higher than 2500 atm could not be tested with the available apparatus. From the data of Fujii *et al.*[4], we calculated for the gelatinisation temperature of curdlan a pressure-induced shift of 1.2 and 3.5°/1000 atm between the gel-formation temperatures 57.4°, 59.0°, and 61.0°, 64.5°, respectively. (The gelatinisation temperature is the temperature where an equal modulus of elasticity is obtained for gels formed at different pressures.) From the data of Suzuki *et al.*[3], a pressure-induced shift between 1.5 and 3°/1000 atm was calculated, depending on the percentage of *O*-methylcellulose in the *O*-methylcellulose–water system.

Apparent activation volumes ($\Delta V^{\neq}$) were calculated from the following equation[2]: $d(\ln G)/dP = -\Delta V^{\neq}/RT$, where G is the percentage of gelatinised granules after a 4-min heating time and R is 82.06 $cm^3.atm/K.mol$. Rate constants and not equilibrium constants were used for all calculations, since our experiments are essentially kinetic in nature: longer heating times result in higher gelatinisation percentages. Values of 31.7 cm^3/mol at 60°, 21.9 cm^3/mol at 61°, and 17.6 cm^3/mol at 62° were obtained in the pressure range between 1 and 500 atm. For the apparent activation enthalpy ($\Delta H^{\neq}$), a value of 312 kJ/mol was found with the following equation: $\Delta H^{\neq} = -R[d(\ln G)/d(1/T)] - RT$, where R is 8.314 J/K.mol and T is 333.8 K.

Heating of the starch granules in 2M 1-propanol lowers the gelatinisation temperature by ~6°, as observed previously by Gerlsma[7]. Application of high pressure simultaneously with the addition of 1-propanol antagonises the 1-propanol effect to the same extent as it raises the normal gelatinisation temperature in water (Fig. 1).

The structure of the starch granules is supposed to be stabilised by hydrogen bonds. An increase in the hydrogen-bond rupturing capacity of the medium (enhanced, for instance, by the addition of alcohols) lowers the gelatinisation temperature[7]. It is known that high pressure favours the formation of hydrogen bonds[1]. Therefore, the retarding effect of high pressure on the gelatinisation of carbohydrate systems has been supposed to be the result of the stabilisation of hydrogen bonds which maintain the carbohydrate granule in its original state. Such an explanation was given by Fujii *et al.*[4] for the retarding effect of high pressure on the gelatinisation of curdlan. Studies carried out by Atake and Angell[8] on the glass transition temperature in molecular liquids and plastic crystals confirm the smaller pressure-dependence of the transition temperature for hydrogen-bonded systems in comparison with non-hydrogen-bonded systems. In addition, Fujii *et al.*[4] supposed that "a decrease of free water as solvent" caused by the compression could also be responsible for the stabilising effect of high pressure. The importance of water availability in starch gelatinisation has been shown by Collison and Chilton[9] and by Wootton and Bamunuarachchi[10].

EXPERIMENTAL

A 0.4% suspension of potato-starch granules (Scholten, The Netherlands) was heated for 4 min in a metal cylinder (volume, 0.5 ml). Pressure was applied at room temperature with a water pump. The rise in temperature caused by the application of pressure is far too small to induce any gelatinisation of the starch granules. The high-pressure column with the starch granules was heated for 4 min in a water bath at several temperatures and thereafter cooled for 30 s in an ice bath before the pressure was released. Starch gelatinisation is an essentially irreversible process during the first hours after the heating of the granules. Hence, germination percentages do not change for at least several hours after heating, which allows measurements to be made at leisure. Since longer heating times result in higher gelatinisation percentages, measurements were kinetic in nature. Therefore, rate constants and not equilibrium constants were used for all calculations. Gelatinisation percentages were determined under a polarising microscope by counting a total of 200–300 granules (gelatinisation percentage = percentage of starch granules that have lost their birefringence).

ACKNOWLEDGMENT

The Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek is thanked for a fellowship (J.M.T.).

REFERENCES

1 K. Suzuki and Y. Taniguchi, in M. A. Sleigh and A. G. MacDonald (Eds.), *The Effects of Pressure on Organisms*, Cambridge University Press, Great Britain, 1972, pp. 103–124.
2 F. Ceuterick, J. Peeters, K. Heremans, H. De Smedt, and H. Olbrechts, *Eur. J. Biochem.*, 87 (1978) 401–407.
3 K. Suzuki, Y. Taniguchi, and T. Enomoto, *Bull. Chem. Soc. Jpn.*, 45 (1972) 336–338.
4 S. Fujii, K. Miyagawa, and T. Watanabe, *Carbohydr. Res.*, 84 (1980) 265–272.
5 J. M. Thevelein, J. A. Van Assche, A. R. Carlier, and K. Heremans, *J. Bacteriol.*, 139 (1979) 478–485.
6 J. M. Thevelein, J. A. Van Assche, and K. Heremans, *Eur. J. Biochem.*, 111 (1980) 171–175.
7 S. Y. Gerlsma, *Staerke*, 22 (1970) 3–9.
8 T. Atake and C. A. Angell, *J. Phys. Chem.*, 83 (1979) 3218–3223.
9 R. Collison and W. G. Chilton, *J. Food. Technol.*, 9 (1974) 309–315.
10 M. Wootton and A. Bamunuarachchi, *Staerke*, 32 (1980) 126–129.