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SMALL ANGLE X-RAY SCATTERING (SAXS) INVESTIGATIONS ON POTATO STARCH IN SUSPENSIONS

Abstract

Small angle X-ray scattering (SAXS) is one of the methods used for the analysis of the starch structure. In this manner, involving changes in electron density structure of starch becomes available. By variation of the media in which starch is suspended wider differentiation of starch structure is possible. Usually such studies are carried out in water. In this paper the SAXS method was successfully used for investigation of starch structure in such media as water, methanol, 1 M solution of HCl, 1 M solution of NaCl, and 0.1 M solution of I_2 in KI. Analysis of SAXS curves for those suspensions proved some differences in curve pattern. Especially interesting data were obtained from a comparison of SAXS curves for starch suspensions in 1 M HCl and 1 M NaCl. Effective scattering of starch suspension in 1 M HCl in the initial part of the curve, as compared to the 1 M NaCl case, indicates that the diffusion of Cl^- ions to various regions of the starch structures depends, among other things, on the pH of the solution. These results demonstrated that the SAXS method could be used for identification of the starch structures to which specific media diffuse and, therefore, for investigating the impact of various factors on this diffusion.

Introduction

Analysis of the structure of starch is a subject of great interest. Linearly bound glucose residues may form a spiral structure – the, so called, helix in the particle of amylose. Stability of that structure is preserved by hydrogen bindings.

In the structure of amylopectin, part of the branches glucose residues chains are distributed parallelly producing a three-dimensional species. Therefore, starch demonstrates the presence of crystalline regions and gives diffraction peaks [1-3]. Figure 1 shows, a simplified structure of starch. In the beam model of the amylopectin particle,

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one can distinguish dense parallel periodically arranged fragments considered as crystalline regions (Fig. 1a).
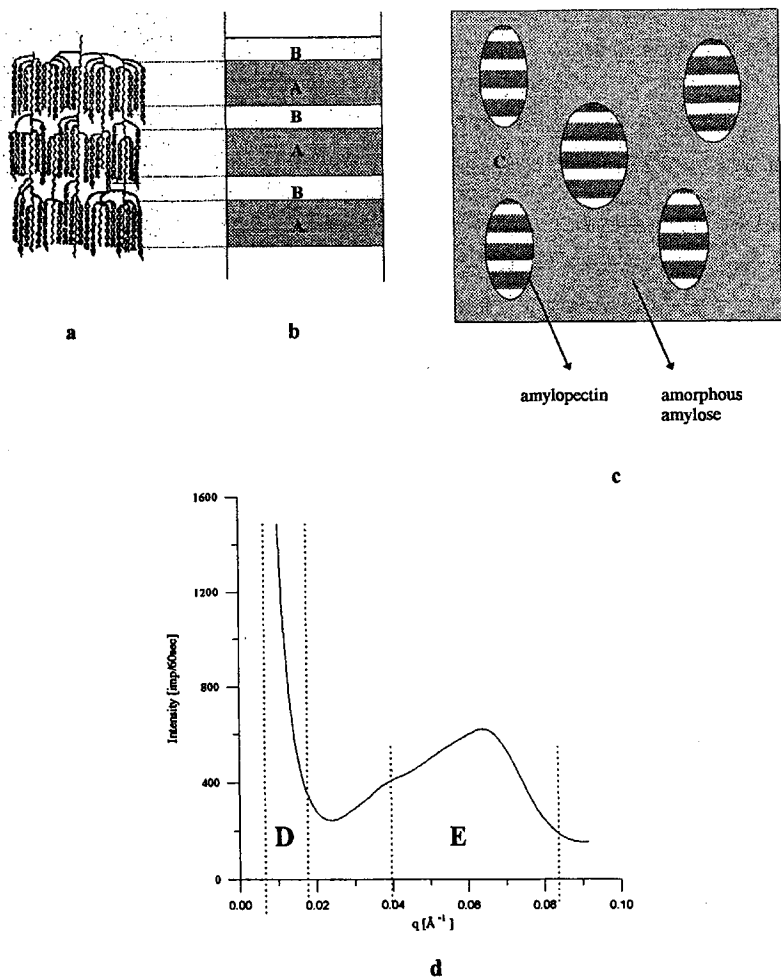

Fig. 1.   Scheme of starch structure (1a, 1b, 1c) and an example curve of SAX scattering of the potato starch suspension in water (1d).

Also amorphous regions can be recognised, where few amylopectin chains are not ordered. Those regions are placed alternately, which derives from daily and nightly starch accretions. Thus the structure of amylopectin grain can be presented in a way shown in Fig. 1b. It is a lamellar, semi-crystalline structure. Figure 1c shows placement of amylopectin grains in relation to the amorphous amylose. The above scheme is greatly simplified, but sufficient in terms of possibilities of using the SAXS method for starch structure investigations [4-7]. Actually, crystalline and amorphous layers of

amylopectin are bedded in shape of mutually shifted growth rings, which gives a tetragonal superstructure.

Small angle X-ray scattering always occurs if in the investigated material there are areas (regions) that vary in terms of electron density from the environment. The greater the difference in electron density, the greater intensity of scattering. Also the size of those regions affects the shape of the scattering curve. The bigger the scattering objects, the smaller the angel of the scattering. The SAXS method can, therefore, be used for investigating objects (areas) of dimensions of 1–1000 nm.

Investigating the starch structure in terms of electron density distribution (within the limits of region size of 1–1000 nm, not on the atomic level) one may expect some differences between the amorphous part and the crystalline part of amylopectin (A-B) and between amylopectin and amylose (A-C, B-C). In dry starch, the differences in electron density between the mentioned regions are very small. Therefore, SAX scattering is so small that it is impossible to interpret it in terms of starch composition. The situation changes when electron density of one of the phases is changed using different methods. It can be obtained by preparing a water suspension of starch. It turns out that water diffuses to the areas (regions) of starch in a non-uniform way and therefore it differentiates its structure in terms of electron density. Figure 1d shows an SAXS curve for native potato starch suspended in water.

As it can be seen, the SAXS curve after its initial fast decrease, shows a peak at a value of the q vector equal to 0.063 Å^{-1}, which corresponds with the interplanar spacing d = 100 Å The initial section of the curve, marked in broken lines (D), is responsible for the presence of differences in electron density of great areas of amylopectin in relation to amorphous amylose (A, B-C). Presence of the peak (E) derives from the occurrence of alternate regions A and B in the structure of amylopectin and the d value approximately determines thickness of the layer A+B [4]. As mentioned above, such form of the SAXS curve was registered for water suspensions of starch, when water easily diffuses to regions B, and not to regions A, which increases the difference of electron density and causes the occurrence of the peak. Change in electron density of region C causes changes in the first part of the SAXS curve.

The points presented above lead to a conclusion that the SAXS method can be used to investigate the process of absorption of different media to different areas of the starch structure. Thus, the aim of this paper was to examine the SAX scattering of starch suspensions in various liquid media.

Material and methods

Because the aim of this paper was to prove that diffusion of different compounds to the structure of starch affects the small angle scattering, measurements of a number of suspensions of potato starch in water, HCl, NaCl, iodine, and methanol were taken

using the SAXS method. Table 1 presents the chemical composition of the investigated suspensions.

Samples were prepared directly prior to the measurements. Potato starch was mixed with an appropriated solvent and the suspension was placed in a cuvette (thickness 0.5 mm) with windows covered with plastic foil.

T a b l e 1

Chemical composition of the investigated suspensions.

Sample	Starch concentration	Solvent
S1	45%	water
S2	45%	CH_3OH
S3	45%	1 M HCl in water
S4	45%	1 M NaCl in water
S5	45%	0.1 M I_2 in 1M KI in water

SAXS measurements

Measurements were performed on a slit-collimated Kratky camera using a Cu anode tube as the radiation source. A proportional counter with a nickel filter and a pulse-height analyser were used to measure the scattered intensity. The scattered intensity measurements were carried out for each of the investigated samples as well as for the empty cuvette (background scattering). The background scattering curve was each time subtracted from the scattering curve for an investigated sample. Absorption coefficient was also measured for each sample. Subsequently, the SAXS scattering curve were recalculated considering the differences in adsorption coefficient.

The measurements were carried out in the range 2θ from 0.076 to 6.52° in 0.0076 to 0.038° intervals and counting time of 100 sec. Scattering curves are presented in the intensity versus q

$$(q = \frac{4\pi \sin\theta}{\lambda}, \text{where } \lambda \text{ - X-ray wavelength, 2θ - scattering angle}).$$

Geometry of SAXS camera and other conditions of the SAXS experiments allowed for the treatment of the scattering curves obtained as slit-smeared data for beam of infinite length.

Results and discussion

SAX scattering occurs when there are areas in the investigated sample which differ from the environment in terms of electron density. In a dry state, starch did not

show such a difference, but when water was added the situation changed. Water is absorbed faster by the amorphous part (especially in amylopectin), which increases the difference in electron density in the structure of starch. A clear peak then appears on the SAXS curve, which originates from the part of starch of a lamellar structure (Fig. 1b). It is obvious that the rate of water absorption by different parts of the starch structure depends on many factors (e.g. temperature) and thus the SAXS measurements must be taken at a certain time; for temperatures around 20°C this time cannot exceed several hours. It affects the accuracy of the results obtained and the SAXS curve might show numerous „wild" oscillations. Due to the fact that the phenomenon of specific fast diffusion of water to the amorphous part of amylopectin and the impact of this phenomenon on SAXS curves are known, further in this paper the SAXS curves obtained for various starch suspensions are compared with the SAXS results for the suspension of starch in water. Figures 2, 3, 4, and 5 display SAXS curves for samples S2, S3, S4, and S5, respectively. Furthermore, every figure contains a curve of SAXS of the starch suspension in water (S1). Analyzing the SAXS curves attention should be paid to two phenomena: presence or absence of the diffraction peak and the intensity of scattering at different ranges of the q vector.
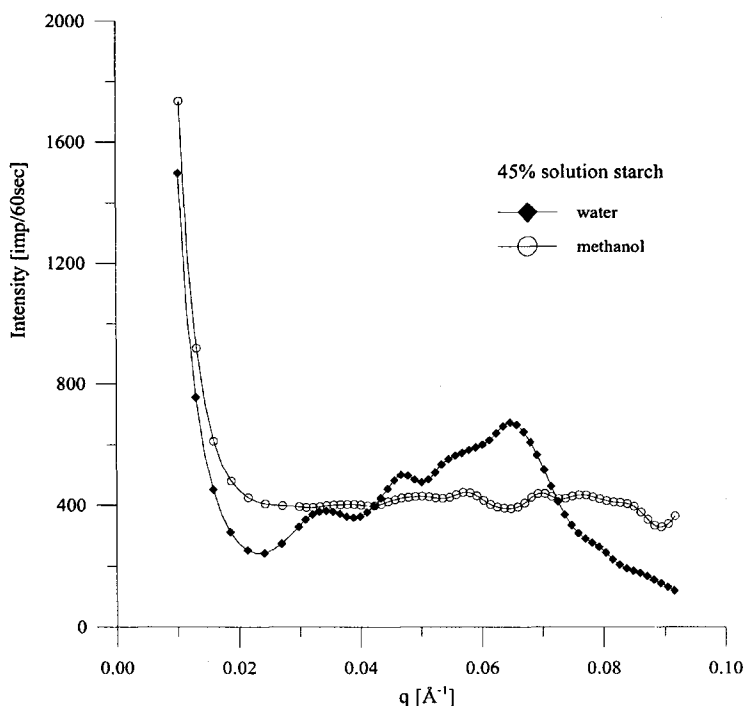

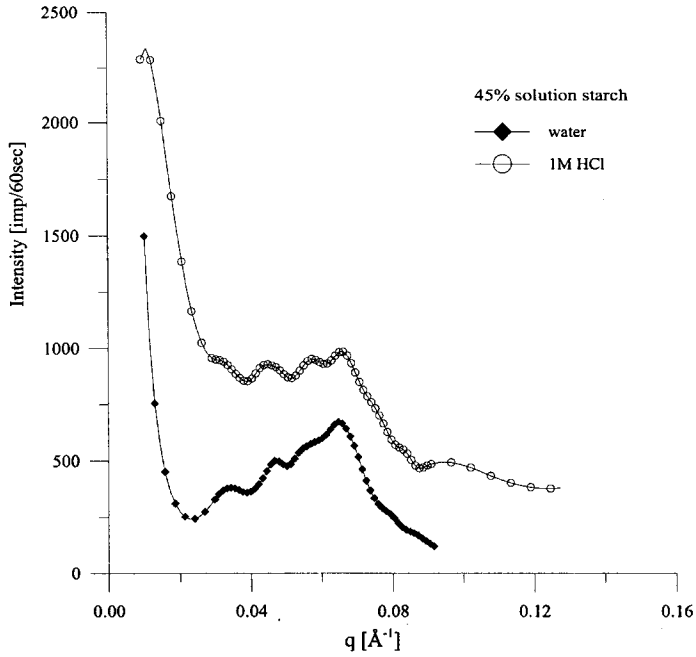Fig. 2.   Scattering curve of potato starch in water and methanol.

Fig. 3.   Scattering curve of potato starch in water and 1 M HCl.
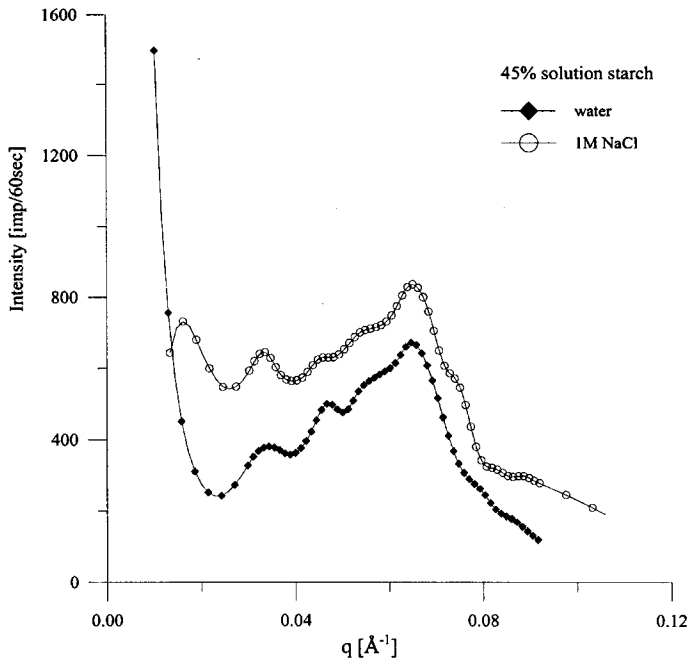

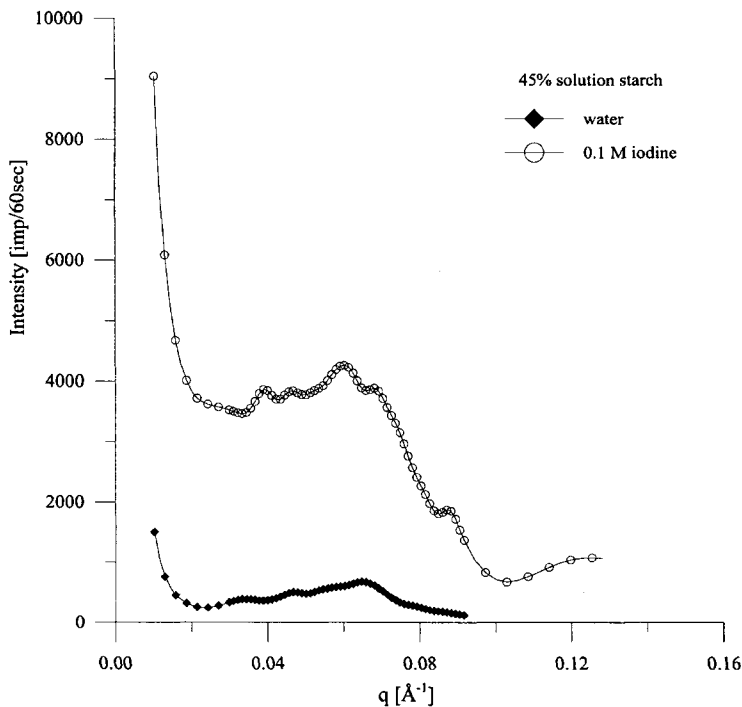Fig. 4.   Scattering curve of potato starch in water and 1 M NaCl.

Fig. 5.   Scattering curve of potato starch in water and 0.1 M I_2 in KI.

The curve of SAX scattering of the starch suspension in water presented in Figs. 2, 3, 4, and 5 shows a clear wide peak with a maximum at q = 0.063-0.065Å^{-1}. Analyzing the shape of this peak one should remember that the presented data are slit smeared type and they have not been mathematically smoothed. The curve of scattering of the starch suspension in methanol (waterless) does not show any peak. Its course is very similar to the curve of dry starch scattering. This proves the lack of the specific diffusion of methanol to the starch structure. The SAXS curve of the starch suspension in 1 M HCl in water has a very interesting course. Theoretically one may suspect that along with the diffusion of water, there will be a diffusion of Cl⁻ ions (they are the only ones that affect the change in electron density). Introducing Cl⁻ ions should increase SAXS. As shown on the figure, the intensity of SAX scattering is greater. The scope of the diffraction peak is also clear, although it is less distinct than in the case of starch suspension in pure water. However, interesting is an especially high growth of scattering in the initial part of the SAXS curve. It proves that Cl⁻ ion diffusion reached not only the amorphous part of amylopectin, but also the C region (Fig. 1), which caused scattering to greater structure objects and an increase of scattering in the initial part of the SAXS curve.

The course of SAXS curves of starch suspensions in 1 M NaCl and water (Fig. 4) is very similar: both curves show clear diffraction peaks. Therefore, it can be assumed that Na^+ and Cl^- ions diffuse only in places of water diffusion. The comparison of the course of SAXS curves of starch suspensions in HCl and NaCl shows the impact of pH on the way of water diffusion to the starch structures [8]. Data presented in Fig. 5 indicate that iodine ions diffuse together with water to some parts of starch. The visible diffraction peak proves it. Scattering is much more intense that in the suspension of starch in water. It is clear, however, that in the first part of the SAXS curve, scattering is greater than for water, which can be interpreted that the solution of I_2, KI, and water do not diffuse exactly in the same way as pure water. A significant growth of SAX scattering after the introduction of iodine ions to the starch structure suggests that those ions could play the role of indicators that would better exhibit the differences in starch structure. The results presented above clearly prove that the SAXS method can be used to investigate the process of various media diffusion to the starch structure. Through investigating the shape of SAXS curves, it allows us to identify those parts of the starch structure to which specific media diffuse. At the same time it allows us to investigate the impact of various factors (pH for example) on the diffusion. Use of intense radiation from a synchrotron for obtaining SAXS curves, which means shortening the time of measurement, will allow us to investigate the kinetics of this diffusion.

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BADANIA SKROBI W CIEKŁYM OŚRODKU METODĄ NISKOKĄTOWEGO ROZPRASZANIA PROMIENIOWANIA RENTGENOWSKIEGO (SAXS)

Streszczenie

Metoda niskokątowego rozpraszania promieniowania rentgenowskiego jest jedną z metod badania skrobi. Pozwala ona zobaczyć budowę skrobi, ujawniającą się jako zmiana rozkładu gęstości elektronowej. Gdy obserwacje prowadzi się w ciekłym ośrodku, zmiany rozkładu gęstości elektronowej są łatwiej-

sze do zaobserwowania. Dotychczas stosowanym do tego celu ośrodkiem była jedynie woda. W niniejszych badaniach użyto też innych ośrodków. Skrobie zawieszono w wodzie, metanolu, 1 M kwasie solnym, 1 M wodnym roztworze NaCI, i 0,1 M roztworze J_2 w KJ. Analiza krzywych SAXS pokazała, że różnią się one między sobą. Różnice te były szczególnie widoczne w przypadku zastosowania 1 M kwasu solnego i 1 M roztworu NaCl. Szczególnie duże rozproszenie promieniowania przez skrobię w kwasie solnym w początkowym przebiegu krzywej wskazuje na dyfuzję anionu Cl^- do różnych regionów gałeczki, przy czym dyfuzja ta zależy od pH roztworu. Metoda może być przydatna w badaniach strukturalnych skrobi. ▨