FOLIA PARASITOLOGICA (PRAHA) 31: 183–186, 1984.

SHORT COMMUNICATIONS

ISOLATION AND PROPERTIES OF ALPHA-AMYLASE FROM PERIENTERIC FLUID OF ASCARIS SUUM

J. ŽENKA and J. PROKOPIČ

Institute of Parasitology, Czechoslovak Academy of Sciences, Praha

Abstract. The properties of α-amylase from perienteric fluid of *Ascaris suum* were studied. The enzyme is strongly activated by chlorides at a simultaneous shift of pH optimum. It is fully activated already at 10^{-3} M concentration of chlorides. A method of α-amylase isolation was modified with respect to these properties to give the purification degree 278 and yield of 11 %.

The presence of amylase in the perienteric fluid of *A. suum* was reported by several authors (Cavier 1951, Monteoliva 1961, Hiraoka 1964, Fukushima 1966). Our preliminary studies revealed that amylase is very active in the perienteric fluid. Since there existed only few data on the properties of this enzyme from this source, we decided to study them in detail and to improve the isolation procedure on the basis of these studies. The papers by Dube and Nordim (1961) and Schwimmer and Balls (1949) dealing with the isolation of amylase from other sources on the basis of its adsorption to starch were considered in our experiments.

MATERIAL AND METHODS

Adult specimens of *A. suum* were recovered from pigs slaughtered at the abattoir in České Budějovice. They were immediately placed in saline and processed in the laboratory. After a thorough washing in saline they were suspended and cut at the lowest place of posterior end of body. The perienteric fluid was left to drop for 10 min and then centrifuged at 23 000 g for 10 min at 4 °C.

The activity of α-amylase was detected by Spofa test (Slovakofarma Hlohovec) using starch with covalently bound stain. The incubation lasted 15 min at 37 °C. In order to detect the effect of chlorides on α-amylase activity, kit tablets containing sodium chloride and phosphates were extracted 3 × by distilled water. The salts, particularly NaCl, were thus removed and a control by means of $AgNO_3$ was performed.

The amylase was isolated using the following procedure. It was first adsorbed on potatoe starch at 4 °C in the presence of ethanol (0—40 % solution: the concentration was optimalized — see Results). At the low temperature the α-amylase only adsorbed and the hydrolytic reaction speed was low. The ethanol prevented non-specific sorptions of ballast proteins. The desorption was performed in buffer, the composition of which offered optimal conditions for amylase activity.

The isolation procedure, including optimalization experiments, was as follows: 7 ml of perienteric fluid was mixed with 5 ml of 72 % ethanol (or saline or 96 % ethanol). All fluids were previously cooled to 4 °C. Potatoe starch (1 or 0.1 g) was added and the mixture was mixed at 4 °C in a magnetic mixer. In order to determine what time is sufficient for α-amylase adsorption, 0.2 ml samples were taken at 40 min intervals and α-amylase activity was demonstrated in them. The mixer had been stopped 5 min before every sample was taken. The starch sedimented during 5 min so that a pure sample of supernatant was obtained. After the adsorption had been terminated the starch was washed twice with 30 % ethanol (12 ml) and twice with 12 ml water at 4 °C. The enzyme preparation deprived of all impurities and salts was thus obtained. The washing solutions were removed by careful sucking off after the starch had sedimented. Then followed a double elution of α-amylase from the starch using 0.2 M phosphate buffer, pH 6.1, containing 0.02 M NaCl. The mixture was incubated at 37 °C in

water bath and thoroughly mixed at 5 min intervals. Samples of supernatant (0.2 ml) were taken at 10-min intervals (after sedimentation of starch) and α-amylase activity was determined in them. For the studies of the thermostability of α-amylase the control sample was left at room temperature and the other samples were incubated for 20 min at 45—75 °C using 0.2 M phosphate buffer, pH 6.1, as a medium. In order to optimalize the procedure, amylase was isolated in a larger scale.

RESULTS AND DISCUSSION

α-amylase activity and its adsorption to starch. The activity of α-amylase was 11 417 nkat per 1 liter of perienteric fluid which corresponds to 0.267 nkat/mg protein. The results of the determination of α-amylase activity in supernatant, i.e., of α-amylase which remained unadsorbed, are shown in Fig. 1. The time of 40 min is sufficient for the adsorption of amylase. Maximum ethanol concentration, which can be used without decreasing the amount of adsorbed α-amylase, is 30 %. It is suitable to use 1 g of starch, since 0.1 g is insufficient. When 30 % ethanol was used, 77 % α-amylase adsorbed to 1 g of starch within 40 min.

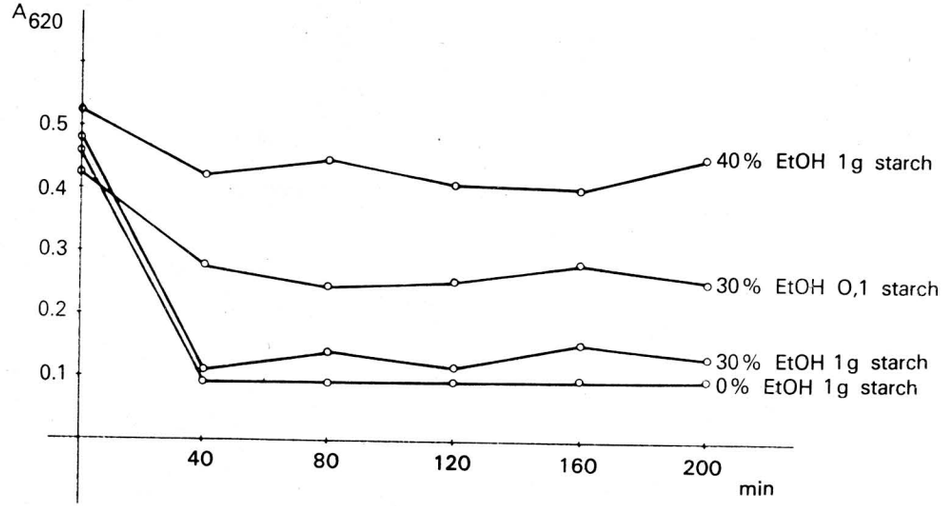

Fig. 1. Optimalization of conditions for α-amylase sorption on starch. Abscissa — time, ordinate — α-amylase activity expressed as absorbance measured at activity demonstration (it was unnecessary to detect absolute activity).

Properties of α-amylase. The effect of chorides on α-amylase activity is shown in Fig. 2. At the concentrations of 10^{-4}M—10^{-3} M the activity of amylase increases by 123 %. The pH optimum was detected both without chlorides and with 0.1 M concentration of chlorides in the reaction mixture. In both cases 0.2 M phosphate buffers were used. The results illustrated in Fig. 3 indicate that the chlorides affect not only the increase in amylase activity, but also the shift of pH optimum. In the absence of chlorides the pH optimum was 5.2, whereas in their presence at 0.1 M concentration the pH optimum was 6.1—6.4.

The results of α-amylase thermostability are shown in Fig. 4. α-amylase was found to be very sensitive to higher temperatures: at 45 °C the activity decreased to 43 %, at 50 °C to 4 % and at 55 °C no α-amylase activity could be detected.

Desorption of α-amylase on starch. Conditions corresponding to the detected maximum α-amylase activity were used for the desorption. Fig. 5 shows the result of a double desorption of α-amylase from starch. A greatest amount of α-amylase was released during the first elution after 20 min.

Isolation of α-amylase from a larger quantity of perienteric fluid using an optimalized procedure. α-amylase was isolated from 100 ml perienteric fluid. The adsorption was performed for 40 min in the medium with 30 % ethanol. α-amylase was eluted with 25 ml 0.2 M phosphate buffer at pH 6.1 containing 0.02 M NaCl. The elution was carried out at 37 °C for 20 min.

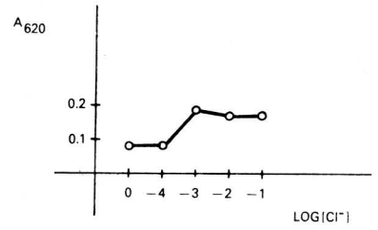

Fig. 2. Effect of chlorides on α-amylase activity. Abscissa — resulting chloride concentrations in reaction mixture, ordinate — see Fig. 1.

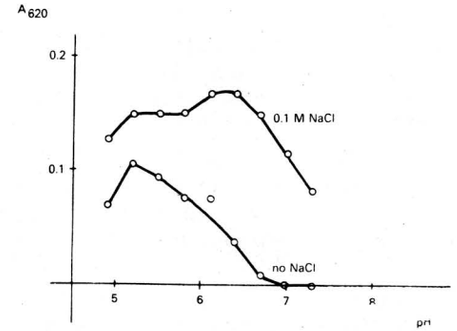

Fig. 3. Shift of optimum pH of α-amylase due to chloride effect.

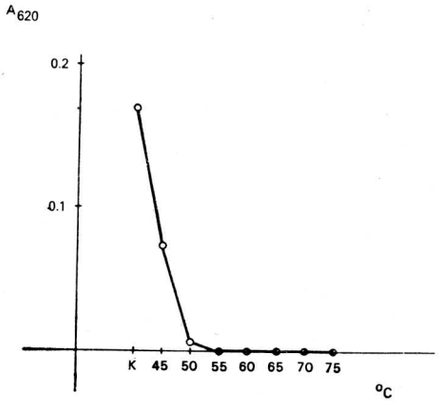

Fig. 4. α-amylase thermostability. Time of incubation 20 min at given temperatures.

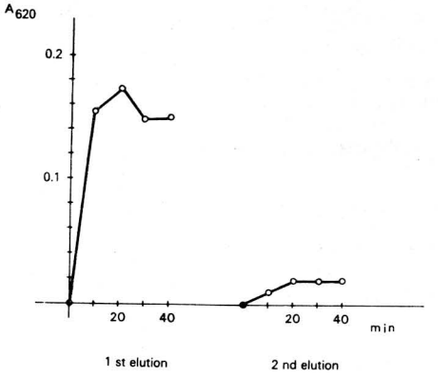

Fig. 5. α-amylase desorption from starch using 0.2 M phosphate buffer at pH 6.1 containing 0.02 M NaCl at the temperature of 37 °C.

It was found that α-amylase from perienteric fluid of *Ascaris suum* is markedly affected by chlorides which induce the increase in activity (by 123 %) and shift of pH optimum from 5.2 to 6.1—6.4. α-amylase differs in its properties from the enzyme found in the intestine of *A. suum*, in which optimum pH 9.3—9.4 (Rogers 1940, Carpenter 1952) and 30 % activation with chlorides at pH 9.4 (Carpenter 1952) were reported.

Results of isolation procedure:

Indices of purification procedure	Initial perienteric fluid	Resulting enzyme preparation
Volume	100 ml	25 ml
Activity	531 nkat*	58.3 nkat
Protein amount	4500 mg	1.775 mg
Specific activity	0.118 nkat/mg	32.8 nkat/mg
Purification degree	1	278
Yield	—	11 %

* Lower initial activity of perienteric fluid (compared to mean value — see point 1) was due to the decrease in activity during storing. The initial amount of perienteric fluid was accumulated during several collections of ascarids at the abattoir.

For the studies of chloride effect on α-amylase activity it was necessary to use a preparation without salts and impurities. The starch with adsorbed α-amylase was found to be suitable for this purpose as it could be perfectly washed. The necessity of this was confirmed during the studies when α-amylase was fully activated already at 10^{-3} M NaCl. It was ascertained that the knowledge of the properties of the enzyme is a prerequisite of the second step of isolation — desorption of α-amylase from the starch. The enzyme could not be released from starch (without affecting its activity) by any of the following procedures: treating with 1 M and 2 M NaCl, treating with concentrated solutions of ethanol, eluating with buffers at low pH. The elution at maximum enzyme activity was found to be the only possible solution of this problem. The following papers will deal with antigenic properties of α-amylase.

ВЫДЕЛЕНИЕ И ИЗУЧЕНИЕ СВОЙСТВ α-АМИЛАЗЫ ИЗ ОКОЛОКИШЕЧНОЙ ЖИДКОСТИ *ASCARIS SUUM*

Й. Женка и Я. Прокопич

Резюме. Изучали свойства α-амилазы из околокишечной жидкости *Ascaris suum*. Фермент сильно активируется при помощи хлоридов и одновременно перемещается оптимум pH. Фермент вполне активирован уже при 10^{-3} M концентрации хлоридов. Метод выделения α-амилазы модифицирован с учетом этих свойств, получая степень очищения 278 и выход 11 %.

REFERENCES

CARPENTER M. F. P., The digestive enzymes of *Ascaris lumbricoides* var. *suis;* their properties and distribution in the alimentary canal. Dissertation, Univ. Michigan. Univ. Microfilms, Publ. no. 3729. Ann Arbor. Mich., 183 pp., 1952.

CAVIER R., L'équipement enzymatique du liquide coelomique de L'Ascaris du porc, *Ascaris lumbricoides* Linné 1758. Bull. Soc. Chim. Biol. 33: 1391—1399, 1951.

DUBE S. K., NORDIM P., Isolation and properties of sorghum alfa-amylase. ABB 94: 121—127, 1961.

FUKUSHIMA T., A reinvestigation of glycogen and amylase, and a description of a glycoprotein in the hemolymph of *Ascaris lumbricoides*. Exp. Parasitol. 19: 227—236, 1966.

HIRAOKA Y., On the enzymatic activities in *Ascaris* body fluid. Jap. J. Parasitol. 13: 143—148, 1964.

MONTEOLIVA M., Estudio enzimático del liquido perivisceral de *Ascaris lumbricoides* del cerdo mediante electrophoresis en papel. Rev. Ibér. Parasitol. 21: 339—344, 1961.

ROGERS W. P., Digestion in parasitic nematodes. I. The digestion of carbohydrates. J. Helminthol. 18: 143—154, 1940.

SCHWIMMER S., BALLS A. K., Isolation and properties of crystalline alfa-amylase from germinated barley. J. Biol. Chem. 179: 1063—1074, 1949.

Received 26 January 1983.
Translated by: M. Dašková

J. Ž., Parasitologický ústav ČSAV,
Na sádkách 702, 370 05 České Budějovice,
ČSSR