Acrylamide gel electrophoresis of proteins, acid phosphatases and RNases from three potato varieties

A. KUBICZ, E. WIECZOREK, B. MORAWIECKA

Department of Molecular Biochemistry, Institute of Botany and Biochemistry, University of
Wrocław, Kanonia 6/8, Wrocław, Poland

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Abstract

Studies on variety differences in the protein and acid phosphatase patterns as well as ribonuclease activity distribution were carried out by disc electrophoresis on saline extracts of three varieties of the potato *Solanum tuberosum* (L.). The protein bands varied in number, position and relative abundance. One main zone of the acid phosphatase activity was detected consisting of 2—3 electrophoretically different bands. Variety differences were concerned with the number and relative abundance of these bands.

RNase activity was detected in 4 main zones, in some of them additional subbands were visible. Differences between the three examined varieties were reflected in the occurrence of the particular activity zones or their subbands.

INTRODUCTION

In our previous studies on potato proteins we have shown that among other activities, also those of the acid phosphatase and ribonuclease were found (Morawiecka, Kubicz 1969). In further investigations Kubicz and Morawiecka (1970) observed that the acid phosphatase isolated from potatoes and partly purified appeared in multiple molecular forms.

Disc electrophoresis of proteins and enzymes has been already used to observe changes in protein and enzyme patterns of tissues in several developmental stages (Steward, Lindon, Barber 1965) as well as during germination and vegetation of plants (Markowski, Piskorski 1970). It was thus interesting for us to find out how variety differences between potatoes are reflected in the pattern of proteins and some enzymes after disc electrophoresis. In this study we investigated this problem on saline soluble proteins and acid phosphatases as well as on RNase of three varieties of the potato *Solanum tuberosum* (L.).

MATERIAL AND METHODS

Investigations have been carried out on three potato varieties: 'Pierwiosnek', 'Wulkan' and 'Uran'. The material was taken from between the 5th and 7th bud (eye). About 20 mm pieces were cut out radially and 1 g samples were homogenized with 3 ml of cold 0.9% Na Cl with addition of sand, in a Potter's homogenizer. The suspensions were centrifuged at 10 000 r.p.m. for 15 min. at 4°. Proteins were estimated in the supernatants by the tannin micromethod after Mejbaur-Katzenellenbogen (1955).

Disc electrophoresis was carried out after Ornstein (1964) and Davis (1964) at pH 9.5 on 7.5% polyacrylamide gels. Glass tubes, 0.5 × 6.5 cm, were filled to within 2 cm of the open end with the small pore gel solution containing 0.0005% riboflavin as initiator. The solution was photopolymerized for 20 minutes under water and 0.1 ml of the large pore solution containing 20% sucrose was layered over it and photopolymerized. 50–100 µl samples containing about 150 µg protein in 20% sucrose were used for electrophoresis.

Ribonuclease activity was detected on the gel slabs in 150–200 µg protein samples (except in variety 'Uran' where 400–500 µg protein samples were analyzed) as described by Wolf (1968).

Acid phosphatase activity was located at the gel by the diazo coupling technique. 50 µg samples containing 10–20 µg protein were subjected to electrophoresis. The enzyme activity was visualized after incubation of the gels in a mixture containing 5 mg alpha-naphtyl phosphate and 3 mg Fast Blue B in 5 ml 0.2 M acetate buffer, pH 5.0, at 37°.

RESULTS AND DISCUSSIONS

Analysis of potato proteins by disc electrophoresis allowed to get deeper into details of their composition and to show marked differences in protein and enzyme patterns between the 3 varieties of potatoes. The amount of protein in 0.9% sodium chloride extracts from potatoes ranged from 1.6 to 2.3 mg/ml. Variations in the composition of saline soluble proteins are shown in fig. 1. There were 9 protein bands in variety 'Pierwiosnek', 11 in 'Wulkan' and 13 in 'Uran'. In all three samples the most intense band appeared in the same position, in the middle of the gel. It seems to be of interest that the differences in protein patterns are more marked in the region of slower migrating proteins. The bands varied in number, position and relative abundance. These proteins may be those which give the particular variety its distinctive characteristics. This was further confirmed by the study of the acid phosphatase patterns. In the lower, anodic part of the gels, under the main protein component, 4

Fig. 1. Electrophoretic separation on polyacrylamide gels of saline soluble proteins of potatoes

a — cv. 'Pierwiosnek'; *b* — cv. 'Wulkan'; *c* — cv. 'Uran'

Disc electrophoresis was carried out at pH 9.5 on 7.5% gels, for 90 minutes at 4 mA per gel. Staining with Amido Black 10B, destaining with 7% acetic acid

Fig. 2. Zymograms of acid phosphatases of saline extracts of potatoes

a — cv. 'Pierwiosnek'; *b* — cv. 'Wulkan'; *c* — cv. 'Uran'

After disc electrophoresis at pH on 7.5% gels, for 90 minutes at 4 mA per gel at 4°, the gels were incubated two times for 20 minutes in 0.2 M acetate buffer, pH 5.0. The enzyme activity was visualized with alpha-naphthyl phosphate and Fast Blue B at pH 5.0

minor bands are visible, but no differences in their intensity as well as in their position occurred between the three samples.

Comparison of zymograms of the acid phosphatase system also showed very interesting differences between the 3 examined varieties. From Fig. 2 it can be seen that there is one zone of acid phosphatase activity containing 2—3 electrophoretically different bands. This zone of activity corresponds to the main protein zone. Variations in the zymogram patterns are concerned with the number and relative abundance of these bands. Figure 2a shows that in variety 'Pierwiosnek' there are 3 bands of phosphatase activity, with marked predominance of the intermediate one. The other two varieties showed 2 bands of enzyme activity of different intensity but in variety 'Wulkan' the faster migrating band is considerably more intense, whereas in variety 'Uran' the pattern showed opposite distribution.

Wolf (1968) detected a number of zones of RNase activity in several plants after disc electrophoresis. Björk (1965) and Morawiecka (1969) have shown nuclease activity to be present in potato extracts. We thus tried to search for RNase activities in our material after disc electrophoresis. Our results suggest the existence of a number of probably different RNases in the saline extracts of potatoes. Also the patterns of this enzyme showed marked variety differences. The activity was detected in 4 zones, designated as A, B, C₁, C₂ and D from cathode to anode (Fig. 3). The

Fig. 3. Electrophoretic separation of ribonucleases of saline extracts of potatoes

a — cv. 'Pierwiosnek'; b — cv. 'Wulkan'; c — cv. 'Uran'

After disc electrophoresis at pH 9.5 on 7.5% gels, for 90 minutes at 4 mA per tube at 4°, the gels were incubated two times for 20 minutes in 0.2 M acetate buffer, pH 5.0. The enzyme activity was visualized after Wolf (1958).

slowest migrating RNase A occurred mainly in the variety 'Pierwiosnek'. RNase B has been detected in all 3 samples in form of 2—3 bands of different intensity and relative abundance, depending on the variety. RNase C is rather characteristic for the variety 'Pierwiosnek' where this zone of activity is predominating. Here the ribonuclease again occurred in multiple forms: 2 bands of activity can be distinguished (C_1 and C_2), but only the faster migrating band C_2 is also present in the two other potato varieties. The fastest migrating towards the anode RNase D is present only in 2 varieties ('Wulkan' and 'Uran') and only traces of this activity can be visible in variety 'Pierwiosnek'. It has to be emphasized that the total RNase activity in the 'Uran' variety is much lower than in the other samples, as to visualize this enzyme on the gel a 2—3 times higher load of protein was used for electrophoresis. The results of studying RNase activities in the gels suggest that RNase C_2 and D from potato extracts might have high specific activities as they appeared in regions where only minor or no protein bands were detected.

The differences in zymogram patterns of the acid phosphatase presented in this paper are concerned with the predominating, fastest migrating activity (Kubicz, Morawiecka 1970) as the other ones are so small that they can be detected only after purification of the crude potato extract.

The RNase activity which exists in a number of probably different enzymes, shows variety differences concerned with the occurrence and distribution not only of the main activity zones, but also of their sub-bands.

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*Elektroforeza na żelu poliakryloamidowym białek kwaśnej fosfatazy
i rybonukleazy 3 odmian ziemniaków*

Streszczenie

Przebadano różnice międzyodmianowe w wyciągach solnych ziemniaków odmiany 'Pierwiosnek', 'Uran' i 'Wulkan' uwidocznione po elektroforezie na żelu poliakryloamidowym białek, kwaśnej fosfatazy oraz rybonukleaz. Różnice w obrazie elektroforetycznym białek dotyczą ilości, rozmieszczenia oraz intensywności poszczególnych frakcji. We wszystkich 3-ch odmianach wykazano obecność jednej głównej strefy aktywności kwaśnej fosfatazy, która, w zależności od odmiany, składa się z dwóch do trzech frakcji.

Aktywność rybonukleazową wykryto w 4 głównych strefach, niektóre z nich wykazały obecność dodatkowych pasm aktywności; różnice międzyodmianowe odzwierciedlały się w głównych strefach aktywności lub ich składnikach.