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Properties of viruses of the potyvirus group. 2. Buoyant density, S value, particle morphology, and molecular weight of the coat protein subunit of bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y^N

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Abstract

The buoyant densities of bean yellow mosaic virus (BYMV) B25, pea mosaic virus (PMV) E198, lettuce mosaic virus (LMV), and potato virus Y^N (PVY^N) were 1.318, 1.321, 1.330, and 1.326 g/ml, respectively. Their S values were 143, 140, 143, and 145 S. The particle morphology of BYMV B25, PMV E198, and LMV could reversibly be changed by magnesium ions. PVY^N particles were broken in the presence of magnesium ions. The molecular weight of the coat protein subunit of the four viruses was 34,000 daltons. In many preparations also a 28,000 daltons component was present. This must be considered to be a breakdown product, derived from the 34,000 daltons component by proteolytic activity.

Introduction

Classification of viruses belonging to the potyvirus group has untill now been based primarily on morphology, transmissibility, and serology (Brandes and Wetter, 1959; Bercks, 1961; Bartels, 1964; Purcifull and Shepherd, 1964). However, the degree of relationship within the group could not be assessed properly on the basis of these properties alone (Taylor and Smith, 1968; Bos, 1970). The development of a simple method to purify some viruses of the potyvirus group (Huttinga, 1973) enabled us to study some of their chemical and physical properties, which can be of help in assessing relationships.

Materials and methods

Virus isolates and host plants. Bean yellow mosaic virus (BYMV) B25 (*/*:*/*:E/E:S/Ap), pea mosaic virus (PMV) E198 (*/*:*/*:E/E:S/Ap), lettuce mosaic virus (LMV) (*/*:*/*:E/E:S/Ap), and potato virus Y^N (PVY^N) (*/*:*/*:E/E:S/Ap) were the same as those used earlier (Huttinga, 1973). BYMV B25, PMV E198, and LMV were propagated on *Pisum sativum* 'Koroza', PVY^N on *Nicotiana tabacum* 'Samsun NN' (Huttinga, 1973).

Virus purification. The viruses were purified by differential centrifuging using relatively low centrifugal forces and a high pH buffer (Huttinga, 1973). Mostly the virus preparations were additionally purified by sucrose-gradient centrifuging in a zonal rotor (Huttinga, 1972).

Buoyant-density measurements. Buoyant densities were determined according to Szybalski (1968). For BYMV B25 and PMV E198 we used the first method in which the viruses have to be spun at two different densities of the initial solution. For PVY^N and LMV we used the second method working with BYMV B25 and PMV E198 as internal markers of known buoyant densities. Determinations were performed in CsCl solutions.

S-value measurements. Centrifugal analyses were done in a Spinco Model E ultracentrifuge using Schlieren optics. Sedimentation coefficients at infinite dilution were determined by the graphical method of Markham (1960).

Particle morphology. The particle morphology was studied from electron micrographs made of purified virus preparations which were in general negatively stained with 1% potassium phosphotungstate pH 6.5 in water. In a few cases the preparations were shadow-cast with palladium at an angle of 30°.

Determination of the molecular weight of the coat protein subunit. The viruses were degraded with sodium dodecylsulphate and the proteins reduced and carboxymethylated according to the method of Geelen et al. (1972). From such protein preparations the molecular weights were determined by polyacrylamide gel electrophoresis. Preparing the 10% polyacrylamide gels and the samples and performing the electrophoresis were done according to Weber and Osborn (1969). However, we added 0.1% 2-mercaptoethanol to the gels and the gel buffer. After the electrophoresis the gels were removed from the tubes by connecting these with a small piece of plastic tubing to a syringe filled with water, and then gently applying a steady pressure via the syringe (Fig. 1). The gels were stained for 2 h as described by Weber and Osborn (1969). Destaining was done by immersing the gels, after rinsing with deionized water, in a destaining solution (75 ml acetic acid, 50 ml ethanol, and 875 ml water). This solution was replaced regularly until the gels were sufficient clear to detect the protein bands. Gels were stored in the destaining solution. As internal markers we used bovine serum albumin, and the coat proteins of tobacco mosaic virus (TMV), cowpea chlorotic mottle virus (CCMV) and cowpea mosaic virus CPMV). All internal markers were

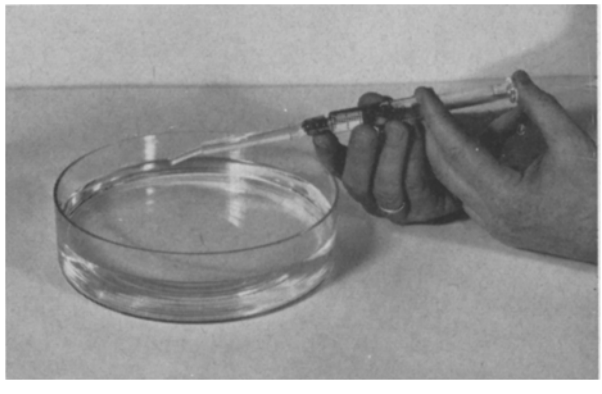

Fig. 1. Ejecting polyacrylamide gels from gel tubes with a syringe.

Fig. 1. Het verwijderen van polyacrylamide gels uit gelbuisjes met een injectiespuit.

Fig. 2. Purification of BYMV B25 on a sucrose gradient (10–40%), which was linear against volume, for 1 h at 119,000 *g*, in a Beckman Ti-14 rotor. The rotor was loaded with about 25 mg virus. Overlayer 100 ml 0.1 M tris-HCl pH 9. Temperature 5 °C. From left to right: the meniscus peak, consisting of material of low molecular weight, with on the right a shoulder caused by somewhat larger material, and the virus peak.

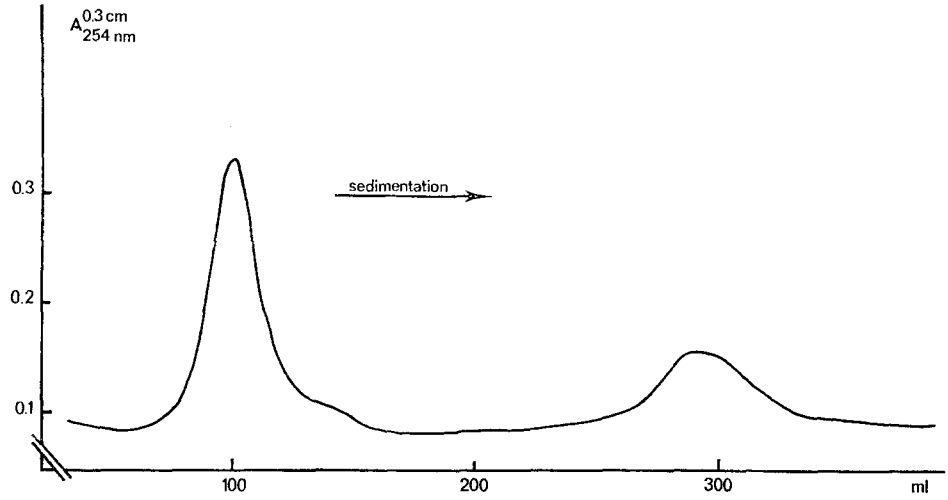

Fig. 2. Zuivering van BYMV B25 op een suikergradiënt (10–40% en lineair met volume) gedurende 1 uur bij 119.000 g in een Beckman Ti-14 rotor. De rotor was beladen met ongeveer 25 mg virus, afgedekt met 100 ml 0,1 M tris-HCl pH 9. De temperatuur bedroeg 5 °C. Van links naar rechts: de meniscuspiek veroorzaakt door laag-moleculair materiaal met rechts daarvan een schouder veroorzaakt door wat zwaarder materiaal, en de viruspiek.

reduced and carboxymethylated in the same way as the proteins of the viruses under investigation.

Results

Virus purification. Virus preparations purified by the method using moderate centrifugal forces and a high pH, still contained a lot of material of low molecular weight as can be seen from Fig. 2, presenting a recording of the contents of a zonal rotor after a run of 1 h at 119,000 *g*. The rotor was loaded with 10 ml of virus suspension

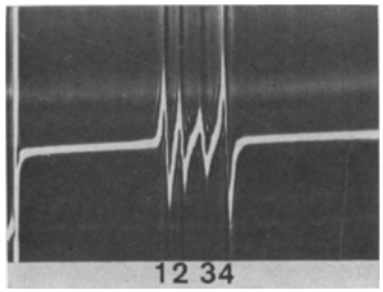

Fig. 3. Equilibrium-centrifuging pattern of BYMV B25 (1), PMV E198 (2), PVY^N (3), and LMV (4) in CsCl after 17 h at 117,600 *g*. Bar angle 70°. Temperature 20 °C.

Fig. 3. Patroon van een evenwichtscentrifugering van BYMV B25 (1), PMV E198 (2), PVY^N (3) en LMV (4) in CsCl na 17 uren bij 117.600 g. Spleethoek 70°. De temperatuur bedroeg 20 °C.

containing about 25 mg of virus. From left to right there is a meniscus peak, consisting of material of low molecular weight, with on the right a small shoulder, and the virus peak.

Buoyant densities. All viruses reached equilibrium in a single band. However, BYMV B25, PMV E198, LMV, and PVY^N differed considerably in buoyant density as can be seen from Fig. 3 presenting the results of an equilibrium density-gradient centrifuging in CsCl of a mixture of the four viruses. The buoyant densities determined for BYMV B25, PMV E198, LMV, and PVY^N were 1.318, 1.321, 1.330, and 145 1.326, respectively.

S values. The viruses each sedimented in a single peak. The S values at infinite dilution in 0.1 M tris-HCl pH 9 buffer and 20 °C were 143, 140, 143, and 145 for BYMV B25, PMV E198, LMV, and PVY^N, respectively.

Particle morphology. In the electron microscope the four viruses generally appeared as flexuous rods. Their appearance, however, could easily be influenced by environ-

Fig. 4. Effect of Mg ions on BYMV B25. A: a preparation after dialyzing overnight against 0.05 M EDTA. B: the same preparation after addition of 0.05 M $MgCl_2$. The particles in B are straight.

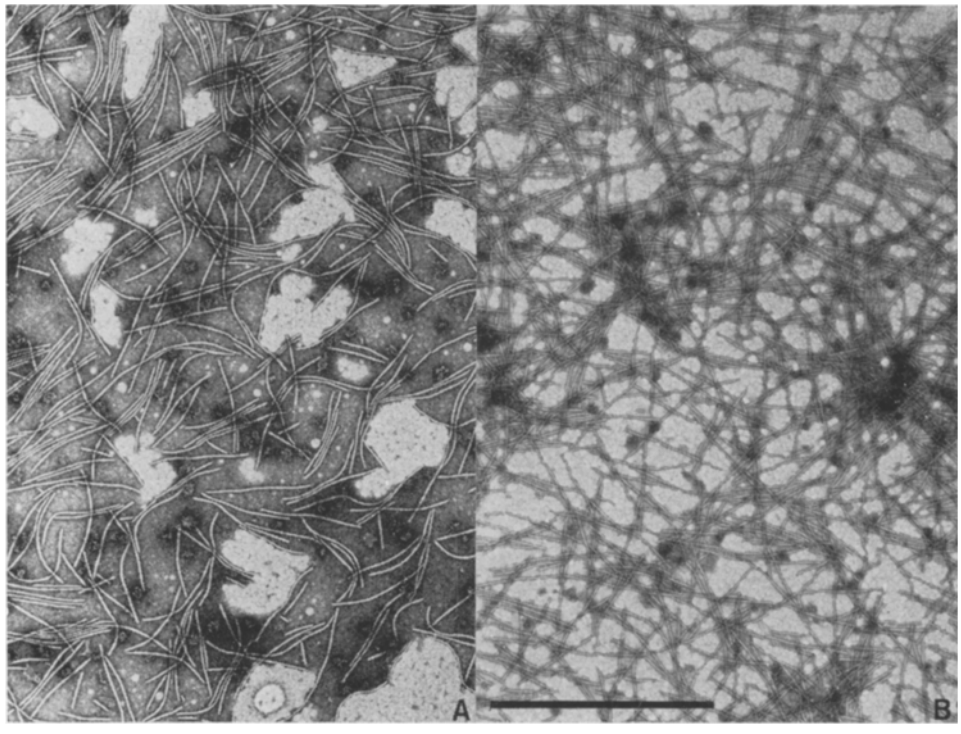

Fig. 4. Het effect van magnesium-ionen op BYMV B25. A: een preparaat na 1 nacht dialyseren tegen 0,05 M EDTA. B: hetzelfde preparaat na toevoegen van 0,05 M $MgCl_2$. De deeltjes bij B zijn veel strakker dan die bij A.

mental conditions. If purified preparations were dialyzed overnight against 0.05 M ethylenediaminetetra-acetic acid (EDTA), flexuous particles were found in the electron microscope (Fig. 4A). When then 0.05 M $MgCl_2$ was added to such preparations the particles became straight (Fig. 4B). When the $MgCl_2$ was removed by dialyzing against EDTA the particles became flexuous again. This effect could be demonstrated for BYMV B25, PMV E198, and LMV, but till now not for PVY^N. Addition of 0.05 M $MgCl_2$ to the latter caused breakage of the particles.

An accurate method to measure the length of flexuous particles still is not available. Hence nothing can be said about the difference in length between straight and flexuous particles.

Coat protein subunit. In our first attempts to determine the molecular weight of the coat protein subunit we found that the coat protein preparations of the four viruses in most cases caused two bands in the polyacrylamide gels. The molecular weight of the two proteins had to be the same for the four viruses because in co-electrophoresis experiments their migration was identical (Fig. 5). The amounts of heavy and light components varied in the four viruses, but varied also in one virus between preparations, suggesting that the light component originates from the heavy one. We were indeed able to induce the formation of the light component by incubating virus predominantly containing the heavy component for 60 h at 37 °C in a solution with proteolytic activity. This solution was obtained from an old virus preparation contaminated with bacteria. The bacteria were removed by centrifuging for 10 min at 8,000 *g*. The supernatant was spun for 1 h at 198,000 *g*. The supernatant of the high-speed centrifuging was used as the source of proteolytic activity. After incubation of the virus preparations, protein was removed, reduced, and carboxymethylated as described in Materials and methods. The effect of the proteolytic incubation, which

Fig. 5. Electrophoretic patterns in 10% polyacrylamide gels of: BYMV B25 (1), BYMV B25 + PMV E198 (2), PMV E198 (3), PMV E198 + LMV (4), LMV (5), LMV + PVY^N (6), PVY^N (7), and PVY^N + BYMV B25 (8). Electrophoresis was performed at room temperature during 4 h with a constant current of 7 mA per gel.

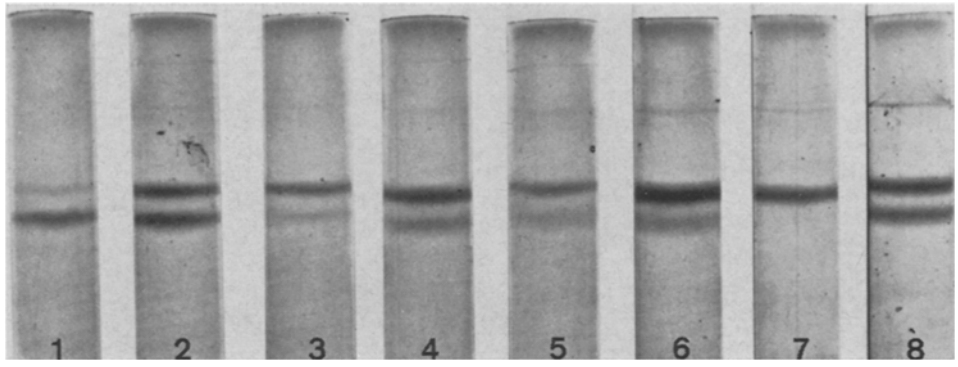

Fig. 5. Elektroforese-patronen in 10% polyacrylamide gels van: BYMV B25 (1), BYMV B25 + PMV E198 (2), PMV E198 (3), PMV E198 + LMV (4), LMV (5), LMV + PVY^N (6), PVY^N (7) en PVY^N + BYMV B25 (8). De elektroforese werd uitgevoerd bij kamertemperatuur gedurende 4 uren met een constante stroomsterkte van 7 mA per gel.

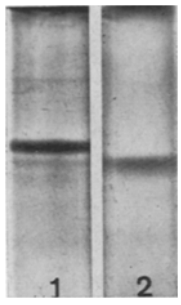

Fig. 6. Conversion of the heavy protein component into the light one. 1: LMV with predominantly heavy component, incubated for 60 h at 37 °C without proteolytic activity in the presence of 4.5×10^{-4}M NaN_3. 2: Comparable LMV preparation incubated for 60 h at 37 °C with proteolytic activity in the presence of 4.5×10^{-4}M NaN_3. The heavy component is conversed into the light one.

Fig. 6. Omzetting van de zware eiwitcomponent in de lichte. 1: LMV met voornamelijk de zware component na incubatie gedurende 60 uren bij 37 °C zonder proteolytische activiteit en in de aanwezigheid van 4,5 × 10^{-4} M NaN_3. 2: Vergelijkbaar LMV preparaat na incubatie onder dezelfde omstandigheden, echter nu in de aanwezigheid van proteolytische activiteit. De zware eiwitcomponent is omgezet in de lichte.

always occurred in the presence of 4.5×10^{-4} M NaN_3 to prevent growth of bacteria, is demonstrated in Fig. 6. On the left is an untreated preparation of LMV with predominantly the heavy component, on the right a treated preparation of LMV with the light component only. Similar effects were found for BYMV B25, PMV E198, and PVY^N.

Sometimes a freshly purified virus preparation during incubation at 37 °C without adding proteolytic activity and in the presence of 4.5×10^{-4} M NaN_3, showed a spontaneous change from heavy to light protein subunits, indicating that purified virus preparations that had not been given the additional sucrose-gradient centrifuging sometimes contained proteolytic enzymes.

The logarithmic plot of molecular weight of the heavy and the light component and that of five internal markers against relative mobility is shown in Fig. 7. Molecular weights of 34,000 and 28,000 daltons were estimated for the heavy and the light protein component, respectively.

Discussion

Earlier we reported that the S values of the viruses did not differ significantly (Huttinga 1973). However, further research, in which mixtures of two viruses were centrifuged, revealed that the differences really exist.

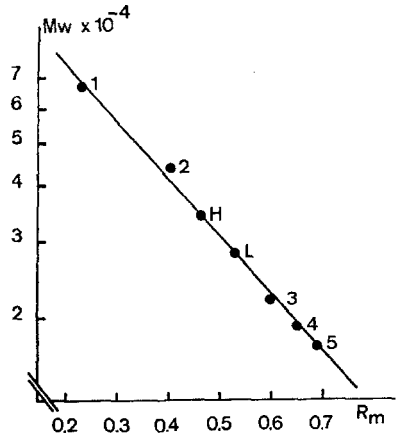

Fig. 7. Determination of the molecular weight of the heavy (H) and light (L) coat proteins of BYMV B25, PMV E198, LMV, and PVY^N. Relationship between the logarithm of the molecular weight (Mw) and the electrophoretic mobility (R_m) of the protein markers: 1. bovine serum albumin (68,000), 2. CPMV coat protein I (44,000), 3. CPMV coat protein II (22,000), 4. CCMV coat protein (19,400), and 5. TMV coat protein (17,400).

Fig. 7. Bepaling van de molecuulgewichten van de zware (H) en de lichte (L) manteleiwitten van BYMV B25, PMV E198, LMV en PVY^N. Relatie tussen de logarithme van de molecuulgewichten (Mw) en de elektroforetische mobiliteit (R_m) van de ijk-eiwitten: 1. runderserum-albumine (68.000), 2. CPMV manteleiwit I (44.000), 3. CPMV manteleiwit II (22.000), CCMV manteleiwit (19.400) en 5. TMV manteleiwit (17.400).

There is a considerable difference in buoyant densities of BYMV B25, PMV E198, LMV, and PVY^N which is not reflected in the S values. BYMV B25 and LMV, for example, which have buoyant densities of 1.318 and 1.330 g/ml, respectively, have the same sedimentation coefficient. We have no explanation for this, no more than for the fact that if equilibrium centrifuging was done with PMV E198 in a swinging bucket rotor, we found a band if centrifuged at 153,000 *g*, but not if centrifuged at 208,000 *g*. Research on this aspect of these and other viruses of the group will be continued. The buoyant densities we found for BYMV B25, PMV E198, LMV, and PVY^N are somewhat lower than those reported for tobacco etch virus (1.332g/ml) and turnip mosaic virus (1.336 g/ml) (Damirdagh and Shepherd, 1970).

The behaviour of BYMV B25, PMV E198, and LMV with respect to Mg ions is very similar to that reported for henbane mosaic virus, pepper veinal mottle virus and bean yellow mosaic virus by Govier and Woods (1971). In our experiments, however, from each virus each form of particle could be converted into the other by changing the medium. Mg ions had no effect on PVY^N, apart from inducing breakage. Till now we could not confirm the differences in length between virus particles with and without Mg ions, as reported by Govier and Woods (1971), because we were not able yet to measure lengths of flexuous particles accurately.

In all coat protein preparations two proteins were found with molecular weights of 34,000 and 28,000 daltons, respectively. The light component could be derived from the heavy one by proteolytic treatment. There are earlier reports of changes in chemical composition of viruses due to enzyme action. For TMV 'Vulgare' Rees and Short (1965) found that one amino acid residue could be split off. For CPMV and bean pod mottle virus a difference of a few amino acid residues was found by Niblett and Semancik (1969). Koenig et al. (1970) reported a change in molecular weight of the same magnitude as we found. They estimated molecular weights for the protein of potato virus X of 29,800 and 24,000 daltons. This change could also be induced by proteolytic activity. A somewhat smaller change in molecular weight, induced by storage without bacteriostatic additive, was reported for tobacco rattle virus (Mayo and Cooper, 1973).

Our data show that the molecular weight of the protein subunit of BYMV B25, PMV E198, LMV, and PVY^N is 34,000 daltons. The 28,000 daltons component must be considered a breakdown product. The value of 34,000 daltons is in rather close agreement with that of pepper veinal mottle virus (32,000–33,000 daltons) (Brunt and Kenten, 1971), that of henbane mosaic virus (32,000 daltons) (Govier and Plumb, 1972), and that of BYMV and bean common mosaic virus (35,000 daltons) (Uyemoto et al., 1972). However, it is considerably higher than that reported for turnip mosaic virus (26,000 daltons) and tobacco etch virus (28,600 daltons) (Hill and Shepherd, 1972a and b). Damirdagh and Shepherd (1970) found two components in polyacrylamide gels of protein preparations of tobacco etch virus. The slowest-moving component disappeared after reduction and carboxymethylation and they considered the fastest-moving one as the coat protein subunit. However, the reduction and carboxymethylation method included incubation for 10–16 h at 37 °C and during this period proteolytic enzymes may have been active. Occurrence of proteolytic activity may also explain why Hill and Shepherd (1972a and b) found such low molecular weights for the protein subunits of turnip mosaic virus and tobacco etch virus, because they used Damirdagh and Shepherd's method. We therefore have the opinion that it is

premature to distinguish two chemical groups of coat protein subunits within the potyvirus group as Uyemoto et al. (1972) did.

Our conclusion is that viruses of the potyvirus group differ considerably in some properties but are very similar in others. It will be necessary to investigate the intrinsic properties of more representatives of the group under strictly specified conditions, before they can be used as reliable tools for the classification of the viruses within the group.

Samenvatting

Eigenschappen van virussen van de potyvirusgroep. 2. Zweefdichtheid, S-waarde, deeltjesvorm en molecuulgewicht van de manteleiwiteenheid van het bonescherpmozaïekvirus, het erwtemozaïekvirus, het slamozaïekvirus en het aardappelvirus Y^N

De zweefdichtheden van het bonescherpmozaïekvirus (BYMV) B25, het erwtemozaiekvirus (PMV) E198, het slamozaïekvirus (LMV) en het aardappelvirus Y^N (PVY^N) bedroegen respectievelijk 1,318, 1,321, 1,330 en 1,326 g/ml. Hun sedimentatiecoëfficiënten waren 143, 140, 143 en 145 S. De deeltjesvorm van BYMV B25, PMV E198 en LMV kon reversibel worden veranderd door toevoeging en verwijdering van magnesium-ionen. Deze induceerden het optreden van breuken in de deeltjes van PVY^N. Het molecuulgewicht van de manteleiwiteenheid van de vier virussen bedroeg 34.000 daltons. In veel preparaten was bovendien een component van 28.000 daltons aanwezig. Deze moet worden beschouwd als een proteolytisch afbraakprodukt dat ontstaat uit de component van 34.000 daltons.

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