Purification and Activity Gel Analysis of Polynucleotide Phosphorylase from the Cyanobacterium Nostoc sp. MAC

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Polynucleotide phosphorylase has been purified from the cyanobacterium Nostoc sp. MAC. The enzyme requires a divalent cation such as Mg²⁺, has a pH optimum of 10.5 and catalyses the polymerization of ADP into polynucleotide in a primer-independent reaction at a rate of 2 pmol min⁻¹ (mg protein)⁻¹. It has an apparent native \( M_r \) of 215000-240000. Non-denaturing polyacrylamide activity gels reveal a heterogeneous pattern of active bands similar to those previously observed with the corresponding enzyme from Micrococcus luteus, while SDS-denaturing activity gels reveal a single band of activity of \( M_r \), 91000 in both crude extracts and the most highly purified fraction. Activity has also been demonstrated in a cetyltrimethylammonium bromide non-denaturing activity gel. By analogy with other known polynucleotide phosphorylases, the Nostoc enzyme is probably a trimer of the 91000-\( M_r \), subunit.

INTRODUCTION

Polynucleotide phosphorylase (PNPase, polyribonucleotide orthophosphate nucleotidyltransferase; EC 2.7.7.8) was the first polynucleotide synthesizing enzyme to be identified, over 30 years ago (Grunberg-Manago & Ochoa, 1955). Surprisingly, however, a definitive physiological role has yet to be ascribed to this enzyme. PNPase catalyses both the phosphorolytic cleavage of polynucleotides and, in the reverse reaction, the synthesis of random polynucleotide from ribonucleoside diphosphate precursors. Synthesis of polynucleotide may occur de novo or by primer elongation, but the enzyme does not require a template and cannot copy one. It can also catalyse nucleoside diphosphate-phosphate exchange.

Recent studies with the Escherichia coli enzyme suggest that PNPase and RNAase II may share an interchangeable role in the degradation of mRNA in the cell, maintaining a balance between mRNA synthesis and breakdown during alterations in patterns of gene expression (Donovan & Kushner, 1986). Many other possible functions have been tentatively given to PNPase, including the degradation of foreign RNA in the cell (Donovan & Kushner, 1983) and the polyadenylation of mRNA (Littauer & Soreq, 1982). Moreover the conservation of this enzyme throughout evolution from E. coli to plants and animals would suggest an important role central to basic nucleic acid metabolism.

Comparatively little is known about PNPase in the cyanobacteria, which are the largest group of prokaryotes (Stanier & Cohen-Bazire, 1977); the only studies so far have involved the enzyme from Synechococcus sp. (Nolden & Richter, 1982). Certain cyanobacteria undergo complementary chromatic adaptation, during which rapid and extensive light-induced alterations in the patterns of gene expression occur (Tandeau de Marsac, 1977). A ready supply of nucleotides for this process could be made available at the energy-conserved diphosphate level by
phosphorolysis of redundant or storage polyribonucleotides by PNPase. Indeed, stable RNA species have been shown to accumulate in certain cyanobacteria under conditions of nutritional deprivation (Singer & Doolittle, 1974; Bonen et al., 1976) although their function has not yet been established (Suranyi et al., 1987). As a first step in the examination of the potential role of PNPase in cyanobacteria, we report here some studies of the purification and structure of PNPase from Nostoc sp. MAC.

METHODS

Materials. Actinomycin D was from Sigma. Calf intestinal alkaline phosphatase, gel filtration M, markers, ADP and poly(U) were from Boehringer. [2,8-3H]ADP (25 Ci mmol⁻¹; 925 GBq mmol⁻¹) was obtained from New England Nuclear. SDS-PAGE M, markers were from Bio-Rad. All other reagents were of analytical grade from BDH or Fisons.

Buffers. PNPase activity was assayed in buffer A (25 mM-Tris/HCl, pH 8.0, 10 mM-MgCl₂, 20 mM-(NH₄)₂SO₄, 7.5 mM-2-mercaptoethanol, 1 mM-EDTA) or CAPS buffer (25 mM-CAPS/KOH, pH 10.5, 10 mM-MgCl₂, 20 mM-(NH₄)₂SO₄, 7.5 mM-2-mercaptoethanol, 1 mM-EDTA, 10% (w/v) glycerol). Extraction buffer B contained 25 mM-Tris/HCl pH 8.0, 20 mM-(NH₄)₂SO₄, 7.5 mM-2-mercaptoethanol, 1 mM-EDTA and 10% (w/v) glycerol.

Cell cultures. Nostoc sp. MAC PCC 8009 was originally obtained from Dr A. J. Smith, University of Wales, Aberystwyth, UK. Organisms were grown in the liquid medium C of Kratz & Myers (1955) supplemented with 1 ml⁻¹ of the A6 microelements of Allen (1968). The cultures were routinely incubated at 31 °C at a light intensity of 220 μE m⁻² s⁻¹ provided by warm white fluorescent tubes in 9 l of medium in round-bottomed flasks. Air/CO₂ (95:5, v/v) was bubbled through the suspension and the cultures allowed to grow until they achieved an optical density of 0.2 at 750 nm. The cells were then harvested, yielding approximately 0.2 g wet weight l⁻¹, using an Alfa-Laval continuous flow centrifuge and used immediately for enzyme preparation.

Assay for PNPase. PNPase activity was determined by a modification of the polymerization assay described by LeRoy et al. (1977). The assay mixture consisted of buffer A, 7 μg actinomycin D, 0.2 μmol [3H]ADP (5 Ci μmol⁻¹; 185 GBq μmol⁻¹) and a variable amount of the PNPase preparation in a total volume of 100 μl; 20 μg poly(U) primer was also included in some assays. The mixture was incubated for 90 min at 37 °C and polynucleotide precipitated in 8% (w/v) TCA, 10 mM-sodium pyrophosphate on ice. The precipitate was transferred to a dry GF/C disc (Whatman) and washed repeatedly in 0.1 M-HCl, 10 mM-sodium pyrophosphate before dehydration in methanol and measurement of radioactivity in 4 ml 0.4% (w/v) PPO in toluene. A unit of enzyme activity is defined as that amount which catalyses the incorporation of 1 nmol AMP min⁻¹ into polynucleotide under standard assay conditions at pH 8.0.

Purification procedure. All procedures were conducted at 4 °C unless otherwise indicated. Cells were resuspended in 10 vols of buffer B, 2 mM-PMSF, 0.1% Triton X-100 before two passages through a French pressure cell at 83 MPa. The homogenate was then centrifuged at 199 000 g for 90 min. The supernatant was applied to a DEAE-cellulose column (DE52, Whatman), which had been pre-equilibrated in buffer B. Unbound material was removed by washing with two column volumes of buffer B, before eluting protein in two stages, firstly with 0.15 M-KCl in buffer B, then with 0.4 M-KCl in buffer B. Protein in the 0.4 M-KCl eluate, which contained the PNPase activity, was then concentrated by precipitation in 70% saturated (NH₄)₂SO₄ and redissolved in 5 ml buffer B. The resultant solution was passed through a GF/C disc (Whatman) and washed repeatedly in 0.1 M-HCl, 10 mM-sodium pyrophosphate before dehydration in methanol and measurement of radioactivity in 4 ml 0.4% (w/v) PPO in toluene. A unit of enzyme activity is defined as that amount which catalyses the incorporation of 1 nmol AMP min⁻¹ into polynucleotide under standard assay conditions at pH 8.0.

Activity gels. PNPase was identified following electrophoresis by its enzyme activity in situ according to the method of Thang et al. (1967). Electrophoresis of all gels for activity analysis was conducted at 4 °C and then native and CTAB gels were equilibrated in CAPS assay buffer for 90 min at 4 °C prior to assaying. Polypeptides separated in an SDS-denaturing gel were renatured prior to assay as described by Bertazzoni et al. (1986). All gels were separated in an SDS-denaturing gel were renatured prior to assay as described by Bertazzoni et al. (1986). All gels...
were sealed into plastic bags with 100 ml incubation mixtures consisting of CAPS buffer, 10 mM-ADP and, when required, 0.05 mg ml⁻¹ poly(U) as primer. The gels were then incubated with shaking at 37 °C for 21 h. Polynucleotide was visualized by staining in 1% (w/v) acridine orange, 1% (w/v) lanthanum acetate, 15% (v/v) acetic acid for 20 min at 20 °C.

RESULTS

Purification

Purification of PNPase activity from 90 l of mid-exponential phase culture was carried out as described in Methods. Stepwise batch chromatography of the crude homogenate (fraction I) on DEAE-cellulose removed the bulk of the nucleic acid contaminants and the major phycobiliproteins. Some 96% of the recovered PNPase activity eluted with 0.4 m-KCl along with chlorophyll a (fraction II). This activity was about three-fold higher than in the crude extract (Table 1), indicating the presence of inhibitors, probably nucleic acids, in fraction I.

When fraction II was chromatographed on Sephacryl S-300, two major peaks of activity were generally seen (Fig. 1). Calibration of the column with $M_r$ standards showed that the high-$M_r$ activity had an $M_r$ of 500000–520000 (fraction IIIa) while the second peak covered a range of $M_r$ from 90000–250000 (fraction IIIb) (fractions 14–17, Fig. 1). The proportions of these species varied from preparation to preparation from roughly equal amounts (as shown in the example in Fig. 1) to predominantly material of $M_r$ 210000–220000 (see Fig. 4). The two major PNPase activities thus isolated were then purified separately on a Mono-Q anion-exchange column. Both activities showed identical chromatographic profiles, eluting as sharp peaks at 225 mM-(NH₄)₂SO₄ (Fig. 2).

Fraction IVb (fraction IIIb after Mono Q) was further purified by affinity chromatography on poly(A)-Sepharose. Before application to the affinity column, fraction IVb was incubated at 37 °C with alkaline phosphatase followed by phosphate buffer to autolyse tightly bound nucleic acid fragments (Nolden & Richter, 1982). Without this treatment, the PNPase did not bind to the poly(A). After washing through the unbound proteins, PNPase was batch-eluted with 4 m-KCl (fraction V). After dialysis, volumes of this final fraction were stored at −70 °C.

When examined by native PAGE and silver staining, fraction V yielded one major and one minor protein band, suggesting that it was not completely homogeneous (Fig. 3). However only a single polypeptide band of $M_r$ 91000 was revealed by SDS-PAGE (Fig. 3b). Other minor bands including that of $M_r$ 105000 above the major band in lanes 2 and 4 also appeared consistently in lanes where no protein sample was loaded (e.g. Fig. 3b, lane 3), indicating that they were buffer contaminants and artefacts of the silver staining procedure. If the major band in Fig. 3(a) is indeed PNPase, it is possible that the band of higher mobility represents a form of enzyme with altered charge, e.g. due to deamidation of asparagine or glutamine. Nevertheless, in the absence of further demonstrations of homogeneity we will refer to our preparation as highly purified.

The purification scheme is summarized in Table 1. The estimated final purification factor of 14000 is several-fold higher than that reported for homogeneous preparations from other organisms (Littauer & Soreq, 1982) and is due mainly to the low initial specific activity of the

### Table 1. Summary of the purification procedure for Nostoc PNPase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Activity (units)</th>
<th>Specific activity (units mg⁻¹)</th>
<th>Yield (%)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Crude extract</td>
<td>6510</td>
<td>130</td>
<td>0.02</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>Concentrated DEAE-cellulose</td>
<td>290</td>
<td>403</td>
<td>1.4</td>
<td>310</td>
<td>70</td>
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<td>Sephacryl S-300</td>
<td>110</td>
<td>66</td>
<td>0.6</td>
<td>51</td>
<td>30</td>
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<tr>
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<td>23</td>
<td>51</td>
<td>2.2</td>
<td>39</td>
<td>110</td>
</tr>
<tr>
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<td>25</td>
<td>3.1</td>
<td>19</td>
<td>155</td>
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<td>12</td>
<td>8.1</td>
<td>9.2</td>
<td>405</td>
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<tr>
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<td>Poly(A)-Sepharose</td>
<td>0.016</td>
<td>4.5</td>
<td>280</td>
<td>4</td>
<td>14000</td>
</tr>
</tbody>
</table>
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Fig. 1. Chromatography of Nostoc PNPase fraction II on Sephacryl S-300. Fractions of 12.7 ml were collected and 50 μl samples assayed with poly(U) primer as described in Methods. Fractions 10–12 (fraction IIIa) and 14–17 (fraction IIIb) were pooled separately for further purification. M, markers were thyroglobulin (670000), ferritin (450000), catalase (240000), aldolase (158000), bovine serum albumin (68000), ovalbumin (43000) chymotrypsinogen (25000) and horse heart cytochrome c (12000).

, PNPase activity; □, log₁₀M,; ——, A₂₈₀.

Fig. 2. Chromatography of Nostoc PNPase fraction IIIb on Mono-Q. Fractions (1 ml) were collected and 50 μl samples were assayed with poly(U) primer as described in Methods. PNPase activity of fraction IIIa eluted in a position identical to that shown here. ●, PNPase activity; ——, ([NH₄]₂SO₄); ——, A₂₈₀.

Assay requirements

Polymerization of nucleoside diphosphates was totally dependent on the presence of a divalent cation, both with and without added primer. Activity with Mn²⁺ was only 7–9% of that with Mg²⁺. The pH optimum was 10.5, with 14% and 70% of the activity being obtained at pH values of 9-5 and 11.5, respectively. The most highly purified preparation had an absolute

crude homogenate, which contains large amounts of phycobiliproteins and solubilized membranes. When assayed at pH 8-0, the specific activity of the final fraction was 280 nmol AMP incorporated min⁻¹ (mg protein)⁻¹. However when the pH optimum of the PNPase was later examined it was found to be 10-5, giving an activity in the final preparation of 2 μmol AMP incorporated min⁻¹ (mg protein⁻¹). Therefore PNPase isolated from Nostoc sp. MAC by this procedure is 10-fold more active than the corresponding enzyme from Synechococcus sp. (Nolden & Richter, 1982), even though the latter organism has a much higher maximum growth rate.
requirement for nucleoside diphosphate substrates, e.g. ADP or UDP. However, material which eluted from the Sephacryl S-300 column was able to use ATP for polymerization due to the presence of contaminating ATPase activity; under standard assay conditions in the presence of the polymerization inhibitor acridine orange (10 mM), 30% of the ATP was converted to ADP as evidenced by HPLC on Partisil 10-SAX (data not shown).

The primer-dependence of the PNPase preparations was monitored throughout the purification. PNPase activity in crude extracts of Nostoc is wholly primer-dependent. However, it was found that as purification proceeded, PNPase became less dependent on a primer for activity. Following fractionation on Mono-Q, the enzyme had become virtually primer-independent, the activity in the absence of primer being 90% of that with primer. However, after autolysis of nucleic acid and affinity chromatography, the enzyme returned to a degree of primer-dependence (activity without primer was only 22% of that with primer). When supplied with [$^{32}$P]pU6 as primer and ADP as substrate, the primer was incorporated efficiently into a poly(A) product of high uniform chain length (approx. 5000) (data not shown). The criteria outlined above permit classification of this enzyme as a PNPase.

\[ M_c \text{ and subunit composition} \]

Data from the purification procedure yielded an approximate \( M_c \) of 90000–250000 for the smaller forms of PNPase and 500000–520000 for the larger species. More accurate estimations of the size of the former were made with a preparation containing predominantly the smaller forms by chromatography on an analytical column of Sephacryl S-300 (Fig. 4a) and by sucrose
Fig. 4. $M_r$ determination of Nostoc PNPase. (a) Fraction II (1-4 ml) was applied to a 95 × 1.6 cm column of Sephacryl S-300 and eluted at 5 ml h$^{-1}$ with buffer B. Fractions (2-3 ml) were collected and 50 µl samples assayed. $M_r$ markers were as for Fig. 1. PNPase; ×, log$_{10} M_r$. (b) A 200 µl sample of fraction II was sedimented for 18 h at 140000 g through a 4.6 ml, 5-10% (w/v) sucrose gradient prepared in buffer B containing 0-6 M-KCl in an SW40.1 rotor. Sedimentation was from left to right and fractions were assayed for PNPase (Φ) and alkaline phosphatase (arrow, $M_r = 140000$). The refractive index of each fraction $(\Delta)$ was determined with an Abbe refractometer. The extract used for these determinations was different from the one for which the large-scale purification is described.

density gradient sedimentation (Fig. 4b). Gel filtration showed the predominant species to have an $M_r$ of 215000 ± 15000 while sedimentation through sucrose revealed a major peak of $M_r$ 240000 ± 20000 with a shoulder of $M_r$ around 200000. Estimates of the $M_r$ of the E. coli PNPase range between 200000 and 250000. This enzyme is known to be a trimer with a subunit $M_r$ variously reported as being between 84000 and 95000 (Littauer & Soreq, 1982). Since SDS-PAGE of fraction V from our studies suggests a subunit $M_r$ for the Nostoc PNPase of 91000, it is not unreasonable to propose that this enzyme is also a trimer with an $\alpha_3$ structure like those of E. coli, Micrococcus luteus, Clostridium perfringens and Azotobacter vinelandii (Littauer & Soreq, 1982).

Activity gel analysis

Enzyme assay in situ in polyacrylamide gels is a useful means of correlating enzyme activity with individual protein species and for detecting heterogeneity. In this case it was employed to determine whether the bands observed on stained gels comprised PNPase and to investigate the relationship between the high and low $M_r$ forms (Fig. 5). When samples of crude extract (fraction I, lane 1), fraction IVa (lane 2), fraction IVb (lane 3) and fraction V (lane 6) were examined in this way, the same cluster of bands comprising two major and several minor species was observed in each case. This shows that the purified PNPase is the only such enzyme in the crude extract and that the high and low $M_r$ forms are indeed identical. When fraction IVb was assayed in a gel without added primer the same pattern appeared (lane 4), showing that there were no further primer-dependent activities at this stage of purification. This also confirmed that the polynucleotide in the gel was the result of enzyme-catalysed synthesis and not due to the concentrative binding of poly(U) primer by an RNA-binding protein. Further bands were visible when the gel was overloaded with PNPase activity (lane 5).

A very similar zone of PNPase activity comprising two major and several minor bands has been observed with the purified enzyme from M. luteus (Barbehenn & Klee, 1978). This pattern has been attributed to the existence of 'size isomers' with the major bands differing in size ($M_r = 220000$ and 200000) rather than charge. A similar heterogeneity was observed in protein and activity patterns after isoelectric focusing (Barbehenn et al., 1982). However, when the Nostoc activity profiles in Fig. 5 were compared to the silver-stained protein profiles (lanes 7–9), none of the active bands corresponded to the major protein(s) present even in the final fraction.
Fig. 5. Non-denaturing polyacrylamide gel electrophoresis of purified Nostoc PNPase. Samples of various fractions were electrophoresed in a 7.5% gel and either assayed for activity (with added primer, unless otherwise stated) (lanes 1–6) or stained with silver (lanes 7–9). Lane 1, 50 µg fraction I; lane 2, 27 µg fraction IVa; lane 3, 18 µg fraction IVb; lane 4, 18 µg fraction IVb assayed without primer; lane 5, 42 µg fraction IVb; lane 6, 0.2 µg fraction V; lane 7, 4 µg fraction IVb; lane 8, 3 µg fraction IVa; lane 9, 0.2 µg fraction V.

To try to resolve this curiosity it seemed relevant to ask whether the single polypeptide observed in SDS-PAGE coincided with PNPase activity. Many enzymes can be renatured and assayed in situ after SDS-PAGE upon removal of the SDS (Bertazzoni et al., 1986). This technique also appears to be successful with PNPase. Crude extract and partially purified fraction IVb showed a single band of activity at \( M_r = 91000 \) corresponding in position to the previously detected single polypeptide of fraction V when assayed with primer (Fig. 6) or without primer (not shown). An identical band was observed when \(^{3}H\)ADP was used as substrate and the gel fluorographed (not shown). To our knowledge, this is the first time that the SDS-activity gel technique has been applied to PNPase.

Thus the data from native and SDS-denatured activity gels combined with the information obtained from corresponding silver-stained gels present a difficulty with regard to their interpretation. SDS gels suggest a correspondence between the only protein band visible and the activity, whilst native gels appear to imply that activity resides in proteins which are not readily visible above the background staining rather than in the major protein(s). The only likely situations that could lead to such a result are (i) that the major protein in fraction V is not PNPase but a contaminant which has a polypeptide, or subunit polypeptide, size identical to that of the PNPase subunit, or (ii) that the bulk of the PNPase protein is, for some reason, catalytically inactive, with the activity residing in a series of minor forms with net charges different from the bulk protein. At present we cannot distinguish between these two possibilities.

Finally, a recent development in PAGE is the CTAB gel, which allows the separation of native proteins under non-denaturing conditions according to their \( M_r \) (Akin et al., 1985). After removal of the CTAB by washing, PNPase activity was readily detected when a sample of fraction IVb was assayed in situ with or without primer (Fig. 7). Only a single, broad band of activity was observed (lanes 1 and 2), which could not be resolved into multiple species and
Fig. 6. SDS-PAGE of \textit{Nosroc} PNPase. Lanes 1 and 2 were assayed for activity with added primer; lane 3 was stained with silver. Lane 1, 400 \( \mu \)g fraction I; lane 2, 20 \( \mu \)g fraction IV \( b \); lane 3, \( M_r \), standards as for Fig. 3(b). The portions of the gel containing lanes 1 and 2 and lane 3 were separated, stained separately, then re-assembled for photography after equilibration of both parts in water.

Fig. 7. CTAB non-denaturing gel electrophoresis of \textit{Nosroc} PNPase. 20 \( \mu \)g fraction IV \( b \) were assayed with (lane 1) or without (lane 2) added primer. 4 \( \mu \)g fraction IV \( b \) were stained with silver (lane 3). \( M_r \), standards (not shown) as for Fig. 3(b).

which corresponded to one of the major protein bands in fraction IV \( b \) (lane 3). This would tend to suggest that the differences observed in the native gels were due to charge differences. The \( M_r \) of the active band was estimated to be about 150000–180000, somewhat lower than that estimated by other techniques.

\textbf{DISCUSSION}

In order to extract PNPase from \textit{Nostoc} homogenates, the inclusion of Triton X-100 at 0-1\% was found to be essential. This suggests that the \textit{Nostoc} enzyme may be associated with the cell membrane as has been reported for \textit{Streptococcus faecalis} and \textit{Halobacterium cutirubrum} (Godefroy-Colburn & Grunberg-Manago, 1972). The high pH optimum observed for the synthetic activity is not without precedent. The optimum pH for the \textit{Vibrio costicola} enzyme is greater than 9-5 (Harry \textit{et al.}, 1985). Primer-dependence tends to be more pronounced at lower pH (Godefroy-Colburn & Grunberg-Manago, 1972) but this was not seen with the \textit{Nostoc} enzyme. The increase in apparent primer-independence during purification may reflect the purification of the enzyme away from inhibitory 3'-phosphate-terminated oligonucleotide fragments which are probably present in the extracts. Most purified PNPases are wholly primer-independent. Proteolysis increases the primer-dependence of most PNPases but although fraction V appeared to have regained a degree of primer-dependence, this may not be due to
proteolysis in this case since it binds avidly to poly(A)-Sepharose. Proteolysis is usually associated with the loss of polynucleotide binding.

During the course of purification of PNPase from \textit{Nostoc}, gel filtration on Sephacryl S-300 revealed the existence of multiple size forms with predominant species of \textit{M}, 215000–240000 and 500000–520000. PNPases from \textit{E. coli}, \textit{M. luteus} and several other micro-organisms also display this heterogeneity, which has been attributed to the association of additional polypeptide subunits or the presence of bound nucleic acids, which induce a conformational change in the enzyme (Littauer & Soreq, 1982; Barbehenn \textit{et al.}, 1982). An \( \alpha_3 \beta_2 \) form of PNPase has been isolated from \textit{E. coli} (Portier, 1975) with an \( M_r \) of 365000. The \( \beta \) subunit (\( M_r = 48000 \)) has no known function and dissociates from the complex at \( I < 0.2 \). An analogous structure should not therefore be present in our gel filtration profiles owing to the low ionic strength of buffer B. A detailed study of the \textit{M. luteus} enzyme has revealed at least ten oligomeric forms of PNPase differing in charge and in size from \( M_r \), 180000 to 530000 (Barbehenn \textit{et al.}, 1982). The relative amounts of these forms change during purification, with dissociation to the predominant \( \alpha_3 \) trimer occurring upon electrophoresis and dilution. All appear to have the common catalytic subunit of \( M_r \), 92000. Our studies with \textit{Nostoc}, which show identical Mono-Q elution profiles and activity gel patterns for the two major forms of PNPase, would suggest that there is also a single enzyme in \textit{Nostoc} which can display a heterogeneity similar to that of \textit{M. luteus}.

The \( \alpha_3 \) structure of the \textit{E. coli} and \textit{M. luteus} PNPases has been proposed to adopt a triangular conformation with the catalytic centre protected from proteolysis in the central 'hole' (Valentine \textit{et al.}, 1969). The question of whether each individual subunit is catalytically active or whether the catalytic centre is integral to the quaternary structure of the enzyme has not yet been answered. Our finding that substantial activity can be generated in an SDS-activity gel may support the former possibility. On removal of the SDS, the denatured polypeptides must refold within the confines of the gel matrix. Large polypeptides, such as the full-size catalytic subunit of the eukaryotic DNA polymerase-\( \alpha \) of \textit{M}, 200000, are sterically hindered from refolding properly (Karawya \textit{et al.}, 1984). The same difficulty may be encountered by oligomeric enzymes, which additionally require the proper spatial orientation of the refolded subunits.

Although we found them soft and difficult to handle, CTAB gels would appear to be a useful addition to the activity gel technique. The \( M_r \) determined in this way for PNPase may not be entirely reliable owing to the short distance which the enzyme migrates in a 7-5\% gel (lower percentage gels were extremely sticky) and to the fact that some proteins may show anomalous migration rates depending on their structure (e.g. bovine serum albumin; Akin \textit{et al.}, 1985). Nevertheless both the CTAB and SDS-activity gels show that PNPase catalytic activity resides in a core protein structure of uniform size. The heterogeneity observed on ordinary activity gels must therefore be the result of charge differences or size differences caused by readily dissociable, non-covalently bound factors, such as oligonucleotides.

A major difference between our observations and those of Barbehenn & Klee (1978) with the \textit{M. luteus} enzyme is that their activity gel profiles exactly matched the pattern of bands stained for protein whereas our active bands do not correspond to the protein observed on silver staining the purified enzyme. The specific activity of our final fraction V of 2 \( \mu \)mol min\(^{-1} \) mg\(^{-1} \) is tenfold lower than the reported specific activities of several purified and partially purified PNPases (Godefroy-Colburn & Grunberg-Manago, 1972; Littauer & Soreq, 1982) and 70-fold lower than that of \textit{Azotobacter vinelandii} (Gajda \textit{et al.}, 1971), which might support the conclusion that the preparation is not homogeneous.

In conclusion, our studies have demonstrated the existence of a PNPase activity in the cyanobacterium \textit{Nostoc} sp. MAC which has a structure very similar to the well-characterized enzymes from \textit{E. coli} and \textit{M. luteus}. In addition we have demonstrated the utility of SDS and CTAB activity gels in studies of this enzyme. We are now in a position to examine the proposed role of PNPase in cells undergoing chromatic adaptation.

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