CLOSTRIDIUM PERFRINGENS POLYNUCLEOTIDE PHOSPHORYLASE : TWO MOLECULAR SPECIES

G.W.Dietz,Jr.,and M.Grunberg-Manago
Institut de Biologie Physico-chimique,rue P.Curie,Paris,France

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Polynucleotide phosphorylase, an ubiquitous enzyme among bacteria, catalyzes the reversible polymerization of polynucleotides :

$$_{n}\text{ppN} \rightleftharpoons n\,p + (Np)_{n}$$

Although the general catalytic properties of this enzyme, isolated from several different bacteria, have been extensively examined, and the polynucleotides synthesized from nucleoside diphosphates have been carefully studied, most of the details of the structure and function of the enzyme remain unknown.

A most interesting form of this enzyme has been prepared from the anaerobic Clostridium perfringens (Dolin,1961,1962). With this polynucleotide phosphorylase the formation of poly A is stimulated to a large extent either by salt or by polybases such as polylysine. It was also observed during these studies that the extent of stimulation by polylysine or salt and the phosphorolysis activity towards poly A changed during the purification. This could have been due to the existence of the enzyme in more than one active form, or to the removal of some activator or inhibitor during the preparation. Data supporting the first alternative was given by Knight et al.(1963) who obtained two fractions from a stepwise elution of the enzyme from phosphate gel which differed quite markedly in their relative activity for phosphorolysis and polylysine stimulated polymerization, when tested with the adenine derivatives.

The following experiments demonstrate that the polynucleotide phosphorylase activity of Cl. perfringens does indeed exist in two different forms which have now been clearly separated.

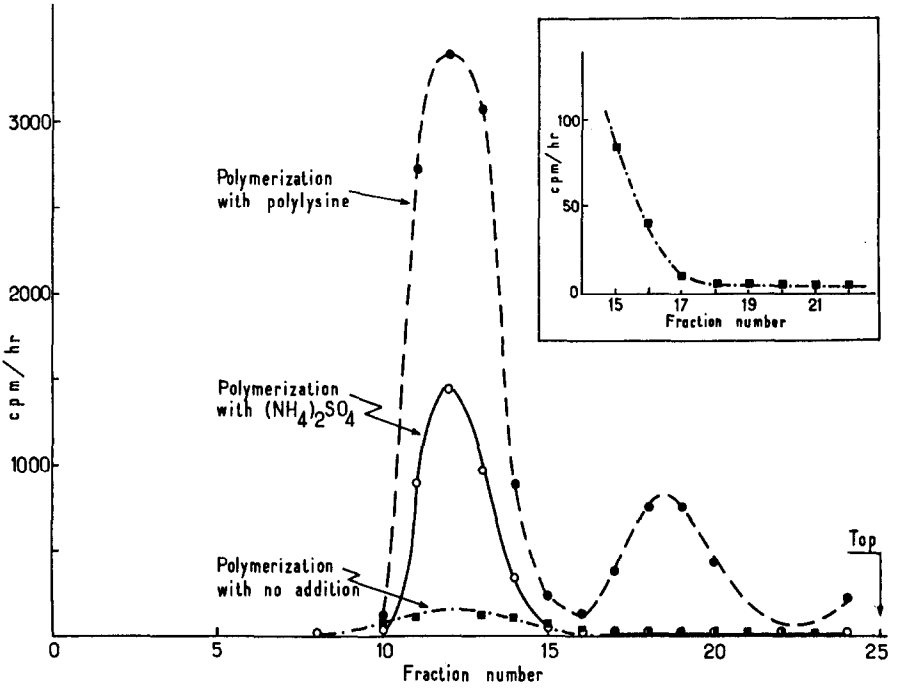

Figure 1

Activity profiles for poly A synthesis after sucrose gradient centrifugation

0.1 ml of the enzyme preparation was layered on a 5-20% sucrose density gradient (Martin and Ames, 1961) made up in a buffer containing 0.02 M Tris-HCl, 0.001 M EDTA, and 0.007 M β-mercaptoethanol, pH 8.3 at room temperature, and centrifuged for 12 hours at 36,000 rpm in a Spinco SW 39 rotor at 0-5°C. The assay for the enzyme activity by the incorporation of radioactive ADP into acid insoluble precipitate was essentially that of Knight et al. (1963). The complete system including 0.05 ml of the enzyme fraction from the gradient (final volume 0.150 ml) contained the following reagents in mMolar concentrations ; Tris-HCl, pH 8.3 at room temperature, 100; $MgCl_2$, 5; ADP-^{14}C (specific activity 23,000 cpm/μmole), 4; and β-mercaptoethanol, 1. When included, the polylysine (MW 3,000) concentration was 280 μg/ml, the ammonium sulfate 0.02 M. Insert shows magnified view of low molecular weight region for polymerization with no addition.

Fig. 1 presents the results of a sucrose density gradient centrifugation of the enzyme (purified through the DEAE-cellulose column step, Fitt et al. (1967)) assayed for the synthesis of poly A in the presence of polylysine. There are two peaks of activity. When the enzyme was assayed for ADP polymerization in the absence of polylysine, or in the presence of salt instead of polylysine, only the more rapidly sedimenting material was active.

In addition, as can be seen in fig.2, only the more rapidly sedimenting component is active in the phosphorolysis of poly A.

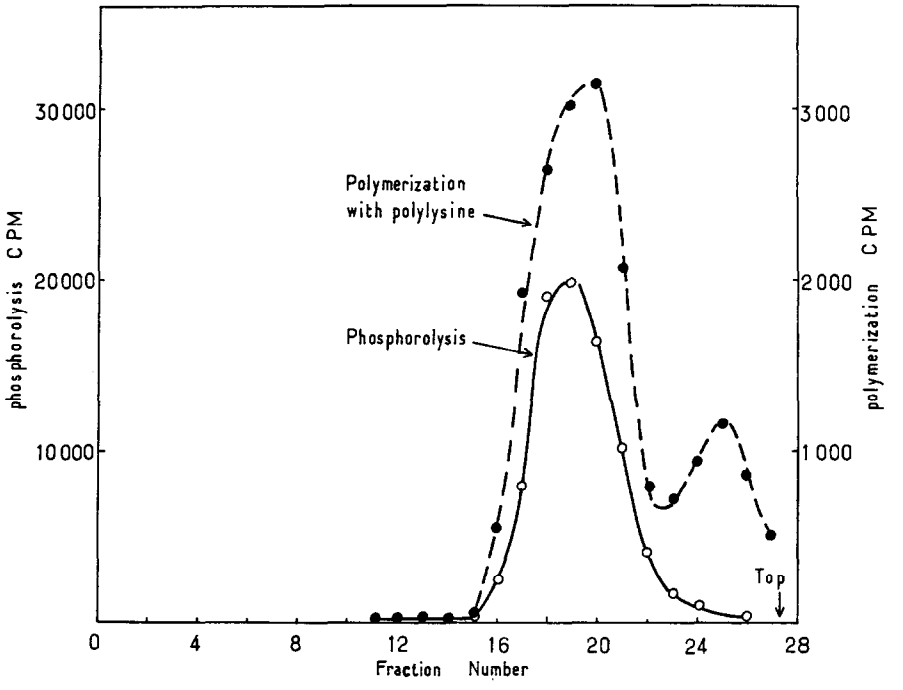

Figure 2

Activity profiles for the synthesis and phosphorolysis of poly A after sucrose gradient centrifugation

Sucrose density centrifugation as in fig.1 for 12 hours, 30,000 rpm. Phosphorolysis activity was assayed by the standard procedure as reported by Knight et al. (1963)

The light material, therefore, has an absolute requirement for polylysine -- which cannot be replaced by salt -- for the polymerization of ADP, and does not show any phosphorolysis activity. This component also requires β-mercaptoethanol: when a gradient was run in the absence of this compound and tested, only the heavy peak appeared in the activity profile. Addition of β-mercaptoethanol to the incubation mixture restored the activity of the light peak. The dependence on this SH compound was further investigated using pooled fraction from β-mercaptoethanol-free gradients, as show in fig.3. While the activity of the light enzyme is highly dependent upon the presence of β-mercaptoethanol, the heavy material is not and, if anything, is slightly inhibited.

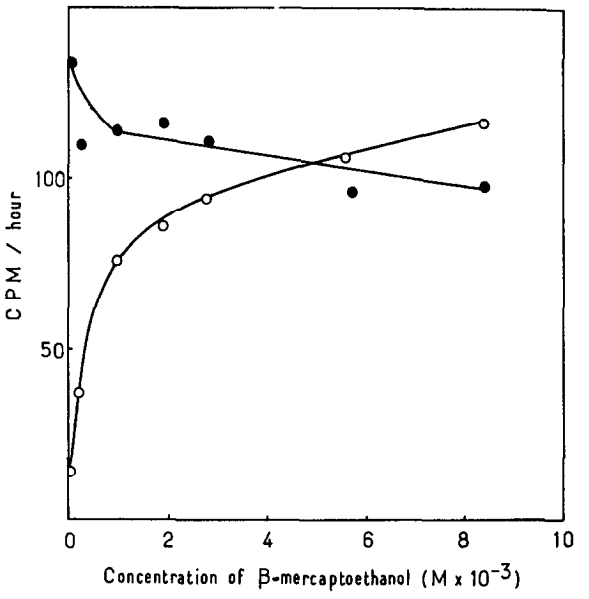

Figure 3

Effect of β-mercaptoethanol on the activity of the two enzyme fractions

The enzyme preparation was fractionated on a sucrose density gradient as in fig. 1, except that the buffer in the gradient lacked β-mercaptoethanol. The light and heavy fractions were separately pooled and tested in the presence of polylysine, 280 μg/ml, for poly A synthetic activity.

—●—●—● heavy fraction

—○—○—○ light fraction

These two peaks of polylysine-stimulated incorporation of ADP into poly A have also been found when the crude extract is run on the sucrose density gradient, suggesting that the phenomenon is not an artefact resulting from the purification procedure. Furthermore, these two peaks have been shown in two different enzyme preparations originating from different batch cultures of the bacteria.

The molecular weights of the two peaks have been calculated from a gradient centrifugation with alcohol dehydrogenase included as marker with the enzyme (Martin and Ames, 1961). The heavy species gives a value of 192,000 while the light material has a molecular weight of 62,000. The value for the heavy species is quite similar to those calculated for Escherichia coli enzyme (Williams and Grunberg-Manago, 1964) and for the enzyme

isolated from Micrococcus lysodeikticus (Singer and O'Brien, 1963). Although there has been no previous report of a low molecular weight polynucleotide phosphorylase corresponding to the light material found here, studies with the enzyme from E.coli suggest the possibility that this enzyme may exist as the aggregate of sub-units, with some of the smaller species retaining activity (Thang, Godefroy, Schenkein and Grunberg-Manago, unpublished). Most recently, a study of a mutant of E.coli has lead to the discovery of an altered enzyme which has a molecular weight of about 100,000, but which in this case catalyzes only the phosphorolysis reaction (Thang, Thang and Grunberg-Manago 1967).

Seen in the light of the above results, it becomes obvious that the properties of a polynucleotide phosphorylase preparation from Cl.perfringens may vary depending on the proportions of the two molecular species present. After starch block electrophoresis, Dolin (1961, 1962) obtained a preparation which had catalytic properties similar to the light peak found here, and it is probable that his procedure preferentially yielded this component.

It is also possible that these two components differ in their relative affinity for phosphate gel, which would explain the two fractions observed by Knight et al. (1963) and mentioned above.

Whether the low molecular weight species represents a sub-unit of the heavier molecule remains an open point. Preliminary experiments in which the heavy material was pooled, reconcentrated and run again on the gradient, gave only one peak of activity, corresponding to the heavy material. However, the light material is rather unstable in dilute solution and any sub-unit derived from the heavy material may therefore have been inactivated during handling, or alternatively, the optimal conditions for dissociation have not as yet been found. If the small component is a sub-unit of the 200 000 molecular weight enzyme, it will be important to show whether it is active as such or whether it aggregates during the reaction to form the heavier component. Although the difference in the effect of β-mercaptoethanol on the two components might be taken as evidence against the one enzyme sub-unit hypothesis, it is possible that the dissocation of the large molecule into sub-units exposes a sensitive group previously protected by the tertiary structure of the multimer form.

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