TETRAMERIC STRUCTURE OF NUCLEOSIDE DIPHOSPHATE KINASE FROM PEA SEED

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1. Introduction

Nucleoside diphosphate kinase (NDP kinase), (EC 2.7.4.6), catalyzes a transphosphorylation reaction between a nucleoside triphosphate and a nucleoside diphosphate. The enzyme has recently been shown to be phosphorylated during its action [1–5]. The enzyme from bovine liver, baker's yeast and pea seed incorporates 3–4 moles of phosphate per mole of enzyme [6–9], suggesting that the enzyme is composed of four subunits, each with one active site. This view is further supported by the finding by Pedersen [3] that NDP kinase from bovine liver mitochondria contains eight titratable sulfhydryl groups, four of which are accessible in the native enzyme. For these reasons it was of considerable interest to investigate the subunit structure of NDP kinase. This enzyme may be isolated readily and in sufficient amounts from pea seed. The molecular weight of the enzyme is 70,000 [9].

2. Materials and methods

The pea seed enzyme was purified as described earlier [9]. Sephadex G-200 fine grain and Blue Dextran 2000 were purchased from Pharmacia, Sweden. Materials for preparing the polyacrylamide gels were obtained from Eastman Organic Chemicals, Coomassie Brilliant Blue R-250 was a product of Mann Research Laboratories, New York. α-Chymotrypsinogen, ovalbumin, albumin from bovine serum, trypsin from bovine pancreas, and pepsin were obtained from Sigma. Myoglobin of bovine heart was purchased from Calbiochem. Light chains from immunoglobulin G were prepared according to Fleischman et al. [10]. Human immunoglobulin G was a kind gift from Kabi, Sweden.

Polyacrylamide gel electrophoresis was performed as described by Shapiro, Vinuela and Maizel [11] using the modification by Dunker and Rueckert [12]. Purified NDP kinase and the proteins used as reference substances were dissolved or diluted in 4 M urea containing 1% (v/v) 2-mercaptoethanol and about 1% (w/v) sodium dodecyl sulfate (SDS). The final protein concentration was about 2 mg/ml, and the solutions were left for 30 to 45 min at 45°. The gels, containing 10% (w/v) polyacrylamide, were prepared in 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% (w/v) SDS. The electrophoretic mobilities were measured relative to that of α-chymotrypsinogen on the stained gels.

Chromatography on Sephadex G-200 of the ^{32}P-labelled enzyme and marker proteins was performed in the following way. The enzyme was ^{32}P-labelled by incubation with AT^{32}P and inactivated with alkali as described earlier [6]. A Sephadex G-200 column (2.1 × 73 cm) was equilibrated with 0.025 M triethanolamine–acetic acid buffer, pH 8.5, containing 0.25% (w/v) SDS. The molarity of the buffer is given with respect to added triethanolamine. The column was eluted with the same buffer by reversed flow at a rate of 7–10 ml per hr. Fractions of 4.5 ml were collected. About 10 mg of Blue Dextran 2000, 9 mg of light chains from immunoglobulin G and 3 mg of myoglobin were dissolved in the elution buffer as well as about 0.2 mg of $^{32}P_i$ and less than 0.2 mg of ^{32}P-labelled pea seed NDP kinase. Samples of about 7 ml were applied to the column. The ^{32}P-labelled enzyme and radioactive orthophosphate were traced by measuring the radioactivity in the eluate as previously described [6]. The concentration of the other substances in the effluent was estimated by measuring the ultraviolet absorbance at 280 nm in a Zeiss PMQ spectrophotometer. Blue dextran 2000 was traced by its ab-

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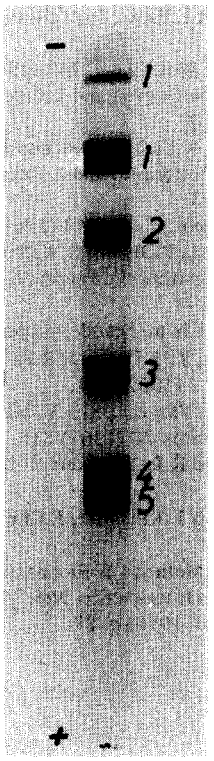

Fig. 1. Electrophoretogram of NDP kinase from pea seed and marker proteins on a 10% (w/v) polyacrylamide gel containing 0.1% (w/v) SDS. The electrophoresis was run as described in the text (cathodic migration). The bands are assigned as follows: (1) bovine serum albumin (also giving a sharp band at the top of the gel), (2) ovalbumin, (3) α-chymotrypsinogen, (4) myoglobin, and (5) NDP kinase from pea seed. The cathode was placed below the gel.

sorbance at 630 nm. The K_{av} value [13] of each substance chromatographed was estimated, assuming the elution volumes of Blue Dextran 2000 and ^{32}P-orthophosphate to correspond to V_0 and V_t, respectively.

3. Results and discussion

An electrophoretogram of NDP kinase and several reference proteins is shown in fig. 1. From separate experiments NDP kinase was shown to migrate as a single band. When the relative electrophoretic mobilities of the proteins were plotted versus the logarithm of their molecular weights, compiled in reference 12, a straight

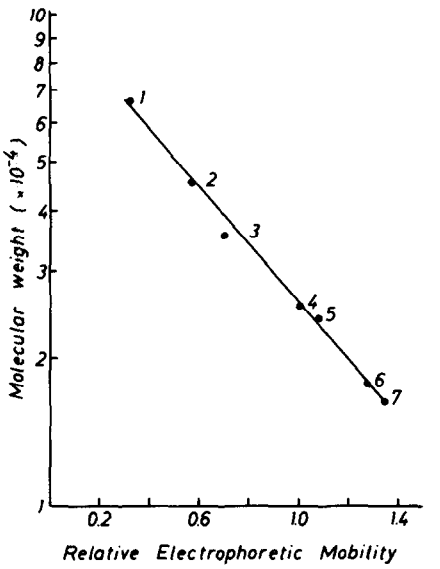

Fig. 2. The electrophoretic mobilities of NDP kinase from pea seed and marker proteins on a 10% (w/v) polyacrylamide gel containing 0.1% SDS plotted against the logarithm of their molecular weights [12]. The bands are assigned as follows: (1) bovine serum albumin (molecular weight 66,000), (2) ovalbumin (46,000), (3) pepsin (35,500), (4) α-chymotrypsinogen (25,700), (5) and trypsin (23,800), (6) myoglobin (17,600), and (7) NDP kinase from pea seeds, correspondent to a molecular weight of 16,000. The electrophoretic mobilities are given relative to that of α-chymotrypsinogen. The straight line is fitted to the values by the least squares method.

line was obtained as predicted [12]. These results are shown in fig. 2. The apparent molecular weight of pea seed NDP kinase in the system used is about 16,000.

Chromatography on Sephadex G-200 gave a K_{av}-value of 0.40 for the ^{32}P-labelled enzyme. Treatment with 2-mercaptoethanol and iodoacetamide according to Fleischman et al. [10] did not change this value. The corresponding values for myoglobin (molecular weight 17,600) and light chains (molecular weight 22,500) were 0.40 and 0.33, respectively. Therefore, it can be concluded that the molecular weight of each ^{32}P-labelled subunit is approximately equal to that of myoglobin (17,600) [14].

The results obtained in this report indicate that NDP kinase from pea seed is a tetrameric protein. Each subunit has a molecular weight of about 17,000 and accomodates one active site.

The fact that the enzyme could easily be dissociated into subunits by treatment with alkali and detergent

or by a hydrogen bond disrupting agent, such as urea, indicates that the bonds holding the subunits together in the native enzyme are noncovalent.

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