Heterogeneity of protein kinase NII

Multiple subunit-polypeptides

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Protein kinase NII has a $\alpha\alpha'\beta_2$ subunit structure, and consists of a chromatographically heterogeneous population. By two-dimensional polyacrylamide gel electrophoresis, each subunit was resolved into multiple polypeptides with various pI values: α subunit, 4 spots; α' subunit, 10 spots; and β subunit, 4 spots. NII underwent autophosphorylation on β subunits. Fractions of α and α' polypeptides also occurred as phosphoforms as shown by alkaline phosphatase treatment. In addition, α' subunit had another motif for heterogeneity, which separated α' polypeptides into two groups, and was exemplified by NIIa and NIIb that showed different enzyme kinetics and the nuclear localization. We interpret these results to account for the basis of the functional as well as molecular heterogeneities of protein kinase NII.

Protein kinase NII *Heterogeneity* *Subunit polypeptide* *2D Polyacrylamide gel electrophoresis* *Alkaline phosphatase* *Autophosphorylation*

1. INTRODUCTION

Protein kinase NII occurs as one of the major protein kinases in the cell nucleus [1–3]. In in vitro reactions, it phosphorylates a set of basic non-histone proteins [4], and HMG proteins 14 and 17 [5,6], or activates RNA polymerases I and II by phosphorylation [7,8]. Casein kinase II, which is identical or closely related to protein kinase NII, modulates topoisomerase II activity [9]. Thus, protein kinase NII seems to be involved in the modulation mechanisms for transcriptional activity in the cell nucleus.

Chromatography on DEAE-Sephadex revealed that protein kinase NII, isolated from various tissues of the rat, has heterogeneous populations [1]. For example, NII from rat liver nuclei has been separated and purified into two forms, NIIa and NIIb. They possessed the same molecular mass and subunit composition of $\alpha\alpha'\beta_2$, but revealed different reaction kinetics [10]. Here, the heterogeneity of protein kinase NII was studied by two-dimensional polyacrylamide gel electrophoresis using enzyme preparations purified from rat liver nuclei.

2. MATERIALS AND METHODS

2.1. *Purification, autophosphorylation and alkaline phosphatase treatment*

Protein kinase NII was extracted from the isolated nuclei of rat liver (210 g) and purified as described [10]. Enzyme fractions, eluted from heparin-Sepharose, were combined (3.75 ml) and dialyzed against 0.1 M NaCl in TG(25)MEMP buffer (50 mM Tris-HCl, pH 8.0, 25% (v/v) glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, and 0.2 mM PMSF). 1 ml of the enzyme solution was incubated at 30°C for

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15 min with [γ-^{32}P]ATP (20 μCi, New England Nuclear, NEG-002H). The preparation, after dialysis against 50 mM Tris-HCl, pH 8.3, 25% (v/v) glycerol, 3 mM $MgCl_2$, 5 μM $ZnCl_2$ and 0.2 mM PMSF, was divided into two portions, and treated at 30°C for 25 min with or without alkaline phosphatase from bovine intestine (Sigma) in 4 μl of 3.2 M $(NH_4)SO_4$, 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$. The samples were then dialyzed against 0.01% (w/v) SDS, lyophilized to dryness, and dissolved in 40 μl of the gel sample buffer [0.05% (w/v) SDS, 2.5 mM EDTA, 2.5% (v/v) 2-mercaptoethanol, 7 M ultrapure urea, 4% (w/v) Ampholyte, pH 3.0–10 (LKB), and 6% (v/v) NP-40]. 20 μl each of the samples was used in two-dimensional polyacrylamide gel electrophoresis [11], and the gels were stained using a silver stain kit from Diichi Chemicals. To analyze the samples in a lower pH range, Pharmacyte, pH 4–6.5 (Pharmacia), was substituted for the mixture of Ampholytes [11].

3. RESULTS AND DISCUSSION

Protein kinase NII was purified from rat liver nuclei. The enzyme contains α, α' and β subunits, whose molecular masses are 42, 38.5 and 24.5 kDa (fig.1). From the size of the enzyme (125–130 kDa [10]), it is deduced to possess a subunit structure of $\alpha\alpha'\beta_2$ or a mixture of $\alpha\alpha'\beta_2$, $\alpha_2\beta_2$ and $\alpha'_2\beta_2$ [10]. As shown by two-dimensional polyacrylamide gel electrophoresis, all of the subunits were resolved into multiple spots with various p*I* values (figs 2a and 3a). This indicates that each subunit consists of a mixture of polypeptides. The α subunit exhibited 4 spots (α1–α4) at p*I* values 7.69, 7.41, 7.21 and 7.04. The α' subunit revealed 6 spots (α'a–α'f) at p*I* values 8.76, 8.55, 8.32, 8.23, 8.15 and 7.86, in addition to 4 spots at the same p*I* values as α subunit polypeptides. The less sharply stained β subunit (fig.2a) was better resolved by the gel containing Pharmacyte, pH 4–6.5, in the first dimension; 4 spots (β1–β4) were seen at p*I* values 5.47, 5.38, 5.27 and 5.17 (fig.3a).

Protein kinase NII undergoes autophosphorylation [12]. Upon incubation of the purified enzyme with [γ-^{32}P]ATP, β subunit incorporated ^{32}P radioactivity. The β spots, except the one at p*I* 5.47, were labelled with ^{32}P radioactivity (fig.3c,d).

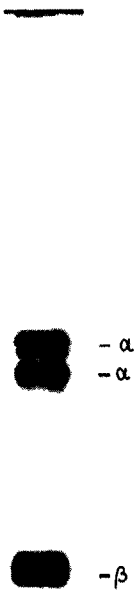

Fig.1. Subunit composition of protein kinase NII. Purified protein kinase NII from rat liver was electrophoresed on a polyacrylamide gel (13%, w/v) containing 0.1% (w/v) SDS, and visualized by silver-staining as described in section 2. The α subunit was often seen as split into two bands, the major upper band and the lower minor band. Sometimes, also α' subunit appeared as split bands. The relationship between these major and minor subbands was not elucidated in the present study.

When the ^{32}P-labelled protein kinase NII was treated with alkaline phosphatase, ^{32}P radioactivity was quantitatively removed from the enzyme. Gel electrophoresis followed by silver-staining indicated that β1 at p*I* 5.47 increased its intensity about 20-times, and the other β polypeptides diminished or disappeared (fig.3b). The nil incorporation of ^{32}P radioactivity into β1, and the increased abundance after dephosphorylation strongly suggest that β1 is the dephosphoform of β subunit. Furthermore, by phosphatase reaction, spots 3 and 4 of α subunit reduced their relative abundance with concomitant increases in the inten-

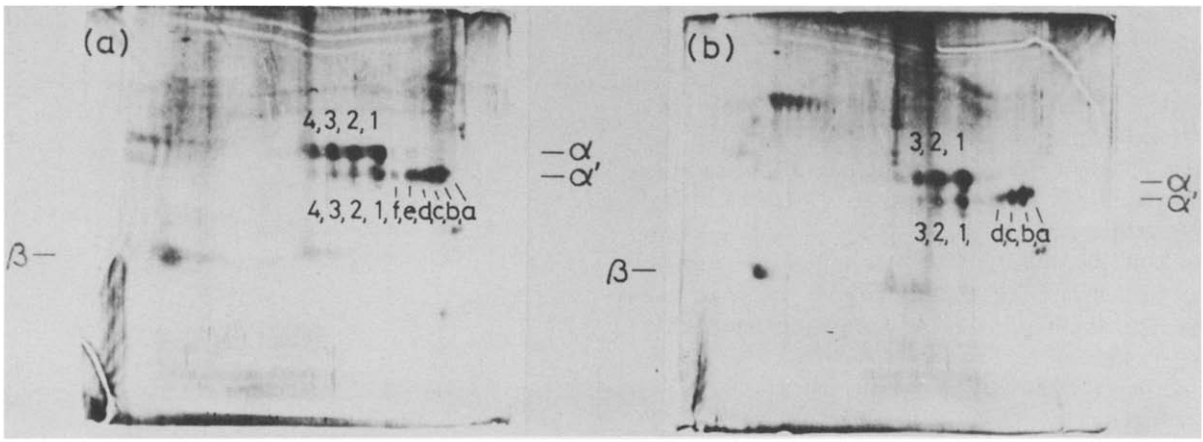

Fig.2. Two-dimensional gel electrophoresis of protein kinase NII. Purified protein kinase NII was divided into two portions, and treated with or without alkaline phosphatase as described in section 2. The samples were analyzed by two-dimensional gel electrophoresis. pH gradient formed in the first dimension was 4.6–8.7, and the gels were visualized by silver-staining. (a) Protein kinase NII; (b) protein kinase NII treated with alkaline phosphatase. Numbers and alphabets were used to designate each spot. Alkaline phosphatase is seen as an array of spots on the upper left corner in (b).

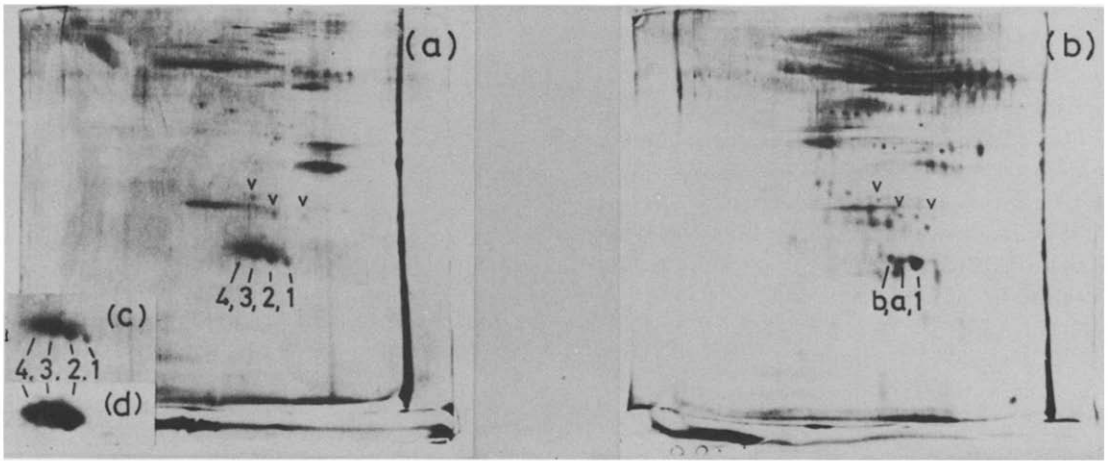

Fig.3. Subunit of protein kinase NII. Protein kinase NII was autophosphorylated with [γ-^{32}P]ATP as described in section 2, divided into two portions, and treated with or without alkaline phosphatase. Electrophoresis was carried out as in fig.2, except that Pharmacyte, pH 4–6.5, was substituted for Ampholytes. pH gradient formed was 4.0–6.2, and the gels were visualized by silver-staining. The spots marked by arrowheads were proteins co-purified with NII, and used as the internal markers to assign β subunit polypeptides. Note that dephosphorylation increased the intensity of β1 about 20-times. Since ^{32}P radioactivity of β subunit was completely removed by the phosphatase, βa and βb at the positions close to β2 and β3 are presumed additional dephospho-forms of β subunit. (a) Protein kinase NII; (b) protein kinase NII treated with alkaline phosphatase. Inset: (c) stained β subunit polypeptides; (d) their autoradiogram.

sity of spots 1 and 2 (fig.2). Similarly, polypeptides 3 and 4, and d–f of α' subunit decreased in their contents, resulting in reciprocal increases in the intensity of spots 1 and 2, and a–c (fig.2). These indicate that α and α' subunits were susceptible to the attack of alkaline phosphatase, or that fractions of α and α' polypeptides existed as phosphorylated forms. Since these subunits were

not modified by autophosphorylation, there should presumably be another protein kinase(s) that is responsible for this modification.

As seen with α' subunit in fig.2a, spots 1 and a showed a pI difference as large as 1.1 units, and after phosphatase treatment spots 3 and 4 seemed to degenerate to 1 and 2, and spots d–f to a–c (fig.2b). Most probably, there are two distinct molecular structures for α' subunit, which can be grouped into 1–4 and a–f. It is unlikely that they simply have different degrees of phosphorylation modification on the same polypeptide. Rather, they may possess different primary amino acid sequences, or one is a modified form of the other, derived by modification other than phosphorylation.

This motif for the heterogeneity occurring on α' polypeptides is actually seen with NIIa and NIIb. These enzymes are from rat liver nuclei, and separated on DEAE-Sephadex. NIIa exists in the nuclei as chromatin-bound form, while NIIb occurs in the nucleoplasmic soluble fraction as well as in the chromatin-bound fraction. Furthermore, they follow distinct enzyme kinetics: the turnover number of NIIb for casein substrate is 5-fold larger than that of NIIa [10]. As seen in fig.4, they have different α' polypeptides separated by the motif discussed above: NIIa was abundant in the polypeptides a–d, while NIIb in 1 and 2. These results suggest that the different polypeptide compositions confer on protein kinase NII varied enzyme activities and the nuclear localizations. To support this notion, after dephosphorylation with alkaline phosphatase, V_{max} of protein kinase NII increased about 2-fold due to the lowered K_m for ATP (the K_m changed from 2.31 to 1.3 μM); although, it is not clear at the present time whether this effect resulted from dephosphorylation of β subunit or of α (α') subunit.

In summary, this study examined the subunit structure of protein kinase NII by two-dimensional gel electrophoresis, and demonstrated that all of the subunits, α, α' and β, consist of multiple polypeptides differing in pI values. The enzyme undergoes phosphorylation on α, α' and β subunits, seemingly at various degrees: the first motif that gives rise to a heterogeneous mixture of the enzyme. The molecular heterogeneity becomes more complex by the second motif for α' polypeptides, which was seen with NIIa and NIIb. These motives seem to confer on protein kinase NII varied functional states such as enzyme activity and the nuclear localization.

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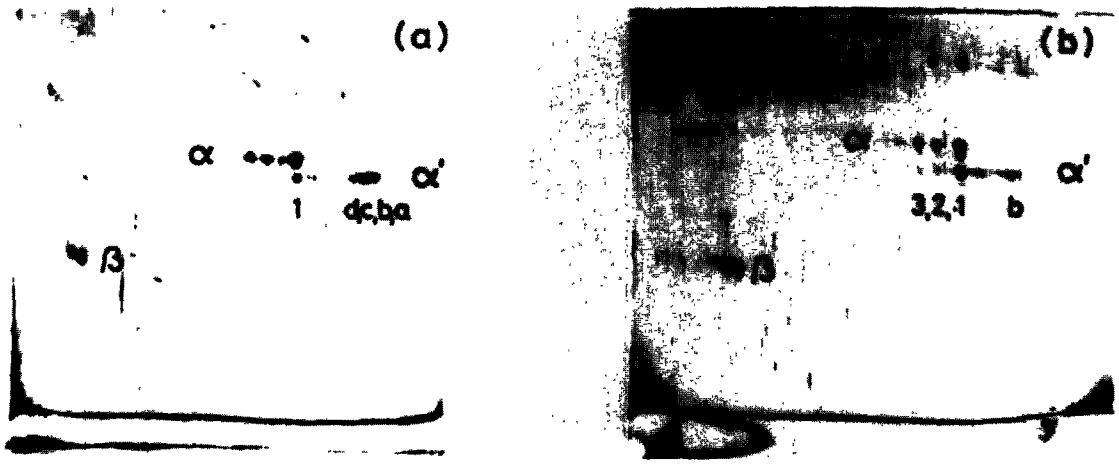

Fig.4. Protein kinase NIIa and NIIb. Protein kinase NIIa and NIIb were purified as described [10], and analyzed by two-dimensional gel electrophoresis as in fig.2.

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