PURIFICATION AND QUARTERNARY STRUCTURE OF A CYCLIC NUCLEOTIDE-INDEPENDENT PROTEIN KINASE (NII) FROM PORCINE LIVER NUCLEI

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1. Introduction

The extensive phosphorylation of proteins in the cell nucleus of higher eukaryotes has evoked great interest in the protein kinases located in this cell organelle. From rat liver nuclei protein kinase activities were described which are cyclic nucleotide- independent and phosphorylate acidic proteins such as casein and phosvitin [1,2]. However, up to now there is a controversy on the number of protein kinases in the liver nucleus [3–5] and their quarternary structure [6], although two enzymes have been purified to homogeneity from rat liver nuclei [7,8]. In order to clarify this point, a protein kinase from porcine liver nuclei was purified to homogeneity which had very similar properties to that described from rat liver nuclei [7] (type NII according to the nomenclature in [1]), but has a quite different quarternary structure. The purification was based on the development of a large scale preparation of nuclei from porcine liver.

2. Experimental

2.1. *Large scale preparation of nuclei*

Buffers: 20 mM MES (pH 6.9), 5 mM mercaptoethanol and 0.02% NaN_3 (A); buffer A supplemented by 8 mM $MgCl_2$ (B); buffer B supplemented by 0.1 mM PMSF and 0.13 M NaCl (C); buffer C 0.13 M NaCl replaced by 2 M sucrose (D).

Porcine liver immediately cooled on ice after slaughtering was thoroughly perfused with tap water of 8°C (all the other procedures at 0–4°C) and cut into strips which were equilibrated for 1 h in several batches of buffer C and for a further 1 h in buffer D. The strips were homogenized by an electric mincer using a 1 mm diam. perforated disk, with continuous addition of buffer D in a ratio of 5 ml/g tissue, and thereafter by 3 pulses of 8 s duration with an Ultra-Turrax (Janke and Kunkel). The suspension was passed through a curtain cloth (30 mesh) by use of a straining rod; this was repeated with the residue on the cloth after homogenizing it with a 5-fold vol. of buffer D by short pulses of the Ultra-Turrax. The nuclei were collected by centrifugation in a continuous flow centrifuge (CEPA Padberg) at 47 000 × *g* and 6 l/h (using a 1 mm nozzle) with cooling at 4°C. Centrifugation was performed over a cushion of buffer D, pumped into the rotor at the beginning; at the end of the procedure buffer D was pumped in until the exit supernatant was clear. The nuclei which appeared undamaged and highly pure under the phase contrast light microscope were frozen in liquid nitrogen and stored at –20°C. Typical yields were 80 g nuclei (wet wt) from 1 kg liver.

2.2. *Purification of the protein kinase*

For lysis and extraction of the nuclei the procedure in [9] was modified which led to a significant increase in yield. After treatment with 0.3 M $(NH_4)_2SO_4$ in buffer A, homogenisation (Ultra-Turrax and Branson sonifier 4B) and centrifugation (Beckman rotor Ti 35 at 32 000 rev./min for 1 h), the dilution with buffer A to 0.05 M $(NH_4)_2SO_4$ was performed in several steps each of which was followed by sonification and centrifugation (Sorvall GSA rotor at 13 000 rev./min for 1 h). Chromatography on a DEAE-cellulose column was performed with buffer B using a 50–500 mM NaCl gradient. The active fractions were directly applied to a blue dextran column (fig.1). This column proved to be the

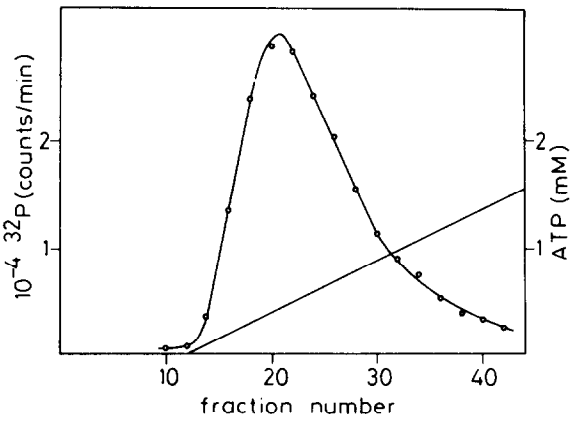

Fig.1. Chromatography on blue dextran–Sepharose. Blue dextran–Sepharose was prepared as in [12]. The active fractions of the DEAE-cellulose column were directly applied to a 2.5 X 35 cm column equilibrated with buffer B followed by an extensive wash with 0.4 M NaCl in buffer B. After the protein concentration in the wash had dropped to ~0, an ATP gradient from 0–2 mM in 0.4 M NaCl and buffer B was applied.

most efficient purification step (cf. table 1) due to the combination of a 0.4 M NaCl wash, which removed a lot of unspecifically bound protein, and an ATP gradient elution at this ionic strength. Thereafter the active fractions were concentrated to a small volume in a Berghof filtration cell (GN 10) using a BM 500 membrane. To avoid losses a high ionic strength was applied (1 M NaCl in buffer B) which was also used in the final gel filtration step on a Sephacryl S 200 column (1 X 100 cm).

Table 1
Purification of the nuclear protein kinase

Fraction	Total protein (mg)	Spec. act. (nmol . min^{-1} . mg^{-1})	Purification	Yield (%)
Supernatant				
After extraction of nuclei	1830	8	1	100
DEAE-cellulose	132	64	8	58
Blue dextran–Sepharose	1.5	4760	595	49
Sephacryl 200	0.7	8850	1070	41

The data are related to 120 g wet nuclei as starting material. Phosphotransferase activity was determined with casein (purified casein from Difco Labs)

3. Results

The DEAE-cellulose chromatography separated the protein kinase activity into two fractions. One fraction is not retained at the ionic strength applied

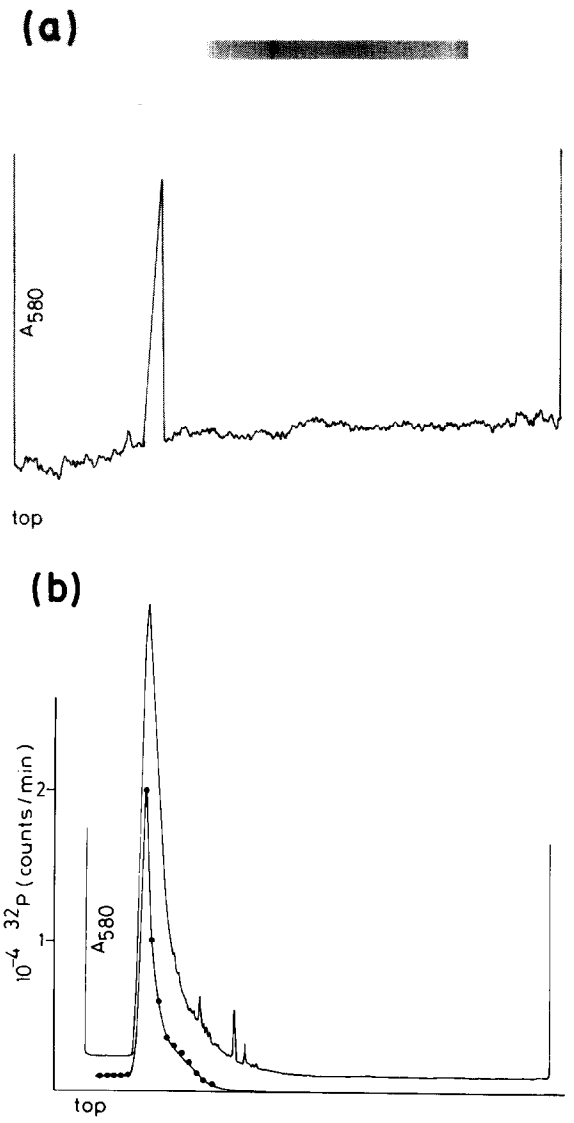

Fig.2. Gel electrophoresis of the purified protein kinase. (a) 9% discontinuous SDS slab gel according to [13]. The protein band was recorded at 580 nm after staining with Coomassie blue. (b) Correlation of protein kinase activity with the protein band in non-denaturing gel electrophoresis (6.5% polyacrylamide gel with 50 mM Tris/glycine (pH 8.4)). Scan at 580 nm after staining with Coomassie blue (—); phosphotransferase activity in cpm of ^{32}P incorporated into casein from ATP (-•-).

and amounts to 30–40% of the total protein kinase activity. This behavior is very similar to that of the protein kinase activity from rat liver nuclei [1,6,7]. The fraction obtained after chromatography on Sephacryl S 200 was subjected to gel electrophoresis under non-denaturing conditions and to discontinuous SDS gel electrophoresis. Fig.2a shows the SDS gel; a heavily stained band, which comprises >95%, is observed. This polypeptide was estimated to be ~105 000 M_r.

To correlate the protein band identified in the electrophoretic gel with kinase activity, the non-denaturing gel was cut into slices, extracted with buffer and probed for phosphotransferase activity. It was found that the activity corresponded to the protein band detected on the gel (fig.2b). An aliquot of the extracted active protein was further subjected to SDS gel electrophoresis which indicated a protein band with the same R_F-value corresponding to M_r 105 000 as that determined by the direct application as above.

M_r 200 000 for the native enzyme was determined by gel permeation chromatography on Sephacryl S 300 at 1 M NaCl in buffer A. This suggests that the protein kinase consists of two probably identical subunits with M_r ~105 000.

For activity the enzyme requires Mg^{2+} and is stimulated by NaCl, maximum activity was found at 8 mM $MgCl_2$ and 0.2 M NaCl with casein as substrate. These results are very similar to those reported for the enzyme from rat liver nuclei [6–8]. With casein as substrate optimum activity was found at pH 7, with phosvitin at pH 6.5 and with mixed histones a broad region of pH 6–7 was observed.

K_m = 1.8 μM for ATP was determined with casein as phosphoryl acceptor. Phosvitin and casein appeared to be very good substrates with V_{max} 14 and 12 μmol/min . mg, respectively. Mixed histones were phosphorylated at a reduced rate (4 μmol/min . mg). Using M_r 200 000 for the enzyme the specific V_{max} values can be transformed into turnover numbers. For phosvitin a turnover number of ~50 s^{-1} is obtained; such high rates for a protein kinase had only been described for cyclic nucleotide-dependent enzymes using short synthetic peptide [10]. This is a further criterion for the high degree of purification of the present enzyme.

4. Discussion

These data suggest that the protein kinase NII from porcine liver nuclei has M_r 200 000 and consists of 2 probably identical subunits. This quarternary structure is quite distinct from that of the corresponding enzyme from rat liver nuclei. The latter enzyme was reported to consist of 4 subunits (α,α,β_2) with M_r < 50 000 and with total M_r 123 000 [6,7]. This discrepancy is rather astonishing, as the 2 enzymes are strikingly related concerning their other properties such as substrate specificity, dependency on ions and that they can utilize both ATP and GTP [6] (H. B., unpublished). The quarternary structure of the present protein kinase can, however, be related to that of a partially purified enzyme recently described from the chromatin of murine erythroleukemia cells [11]. This protein kinase has properties of a type NII enzyme and is reported to have total M_r 200 000, also determined at higher ionic strength. It remains to be clarified whether nuclear cyclic nucleotide-independent protein kinases of the type NII have different quarternary structures in different higher animals or whether the different quarternary structures reported partially arise from protease degradation during preparation.

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