Rat Pancreatic Nucleoside Diphosphate Kinase, a Novel Regulator of Cholecystokinin Receptor Affinity

Cloning and Expression

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We previously demonstrated that the existence of two affinity states of the pancreatic cholecystokinin (CCK) receptor depends on the presence of ATP.[1] Subsequently, we established that the effect of ATP to induce two CCK binding affinity states was biochemically mediated by the enzyme nucleoside diphosphate kinase (NDPK) (manuscript in preparation). Nucleoside diphosphate kinase catalyzes the transfer of high energy gamma-phosphate groups from nucleoside triphosphates to nucleoside diphosphates. In that study the ability of an assortment of nucleoside triphosphates to serve as substrate for NDPK was compared with their ability to induce two binding affinity states of the CCK receptor on rat pancreatic membranes for which previous studies have observed only a single binding affinity state. Nucleoside triphosphates capable of serving as a substrate for NDPK could also induce two CCK binding affinity states on pancreatic membranes, whereas those that could not serve as substrate for NDPK could not induce two CCK binding affinity states. Furthermore, GDP potentiated the effect of ATP on binding (manuscript in preparation). To conclusively identify NDPK we found it necessary to clone and functionally express this enzyme. We report here that the characteristics of the cloned and expressed enzyme are similar to those of pancreatic membrane NDPK and consistent with the ability of this enzyme to induce two CCK binding affinity states.

METHODS

To identify pancreatic NDPK molecularly, it was amplified by the polymerase chain reaction (PCR) from a λgt11 rat pancreatic cDNA library, cloned, expressed, and functionally characterized. Polymerase chain reaction primers were prepared based on the published sequence of rat skeletal muscle NDPK.[2] The 5′ primer was a 25-mer (CGGGATCCATGGCCAACAGCGAGCG) which incorporated a *Bam*HI restriction site to facilitate subcloning, and the primer for the complementary strand was a 33-mer (CGGAATTCCTCACTCATACATCCAGTTCTGCGC) incorporating an *Eco*RI restriction site. These primers allowed amplification of a single band of

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about 465 bp. The cloned PCR product was sequenced using the Sanger dideoxy sequencing protocol. The cloned NDPK was ligated into the expression vector pGEX-KT,[3] which expresses cloned proteins in frame with *Schistosomal* glutathione S-transferase, under the control of the IPTG-inducible *tac* promoter. Transformed bacteria were grown and fusion protein synthesis was initiated by adding IPTG. Cells were lysed by two passes through a French press, and fusion protein was purified by affinity chromatography using glutathione-agarose beads. Fusion protein was used to immunize New Zealand white rabbits, and the rabbits received boosters three times at 5-week intervals, blood was drawn, and immune serum obtained.

RESULTS

The coding sequence of the PCR product was identical to that of rat skeletal muscle NDPK. The apparent molecular weight of the purified fusion protein was 42 kD, consistent with the combined molecular weight of NDPK (18 kD) and GST (26 kD) (FIG. 1). The fusion protein exhibited the activity and kinetic parameters

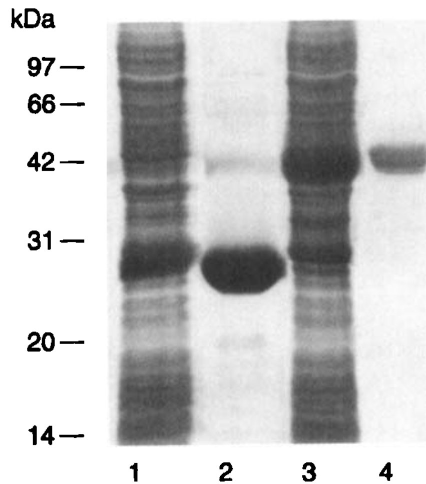

FIGURE 1. Coomassie blue stained 12% SDS-PAGE of recombinant bacterial lysates and purified recombinant proteins. Total bacterial lysate and purified GST (1,2); total lysate from recombinant bacteria and the GST-NDPK fusion protein (3,4). The apparent molecular weight of the purified fusion protein (42 kDa) is consistent with the combined molecular weight of NDPK (18 kDa) and GST (26 kDa). The migration distance and sizes of molecular weight markers are indicated on the *left*.

expected of an NDPK. In typical experiments ATP was utilized with a K_m of 455 μM and GDP with a K_m of 127 μM; the maximal velocity of these reactions averaged 3,400 times that measured in total pancreatic membranes per unit protein. Immunoblots of total pancreatic membranes, performed using the antiserum produced against the recombinant fusion protein, identified a single band with an apparent molecular weight of 18 kD in AR4-2J cell membranes, total, and enriched pancreatic membranes (FIG. 2). The antiserum only weakly recognized NDPK in CHO cells.

DISCUSSION

These findings demonstrate the presence of NDPK in rat pancreas at both the nucleic acid and protein level. The purified recombinant enzyme exhibited kinetic

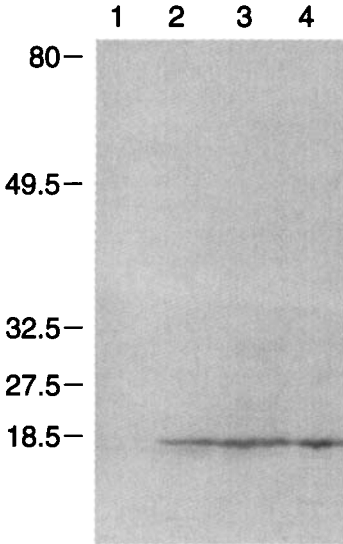

FIGURE 2. Immunoblot of (1) total CHO cell membranes, (2) total AR4-2J cell membranes, (3) total pancreatic membranes, and (4) enriched pancreatic membranes. Membranes (50 μg) were run on a 5–15% gradient SDS-PAGE, transferred to nitrocellulose, and probed with antiserum 553 raised against the GST-NDPK fusion protein.

characteristics consistent with our earlier findings on rat pancreatic membranes. These results provide further support for a role of NDPK in regulating CCK receptor affinity.

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