Characterization of Ca^{2+} /calmodulin-dependent protein kinase in rat pancreatic islets

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We have attempted to identify islet Ca^{2+} /calmodulin-dependent protein kinase (CaM kinase) by comparing its activity with purified brain CaM kinase II. Islet CaM kinase, in the presence of calmodulin and Ca^{2+} , phosphorylated major endogenous substrates of 102, 57 and 53 kDa and also exogenous glycogen synthase; brain CaM kinase II phosphorylated glycogen synthase and peptides of 57 and 53 kDa. Alloxan (1 mM) inhibited the phosphorylation of glycogen synthase and the 102, 57 and 53 kDa islet peptides by islet CaM kinase; the phosphorylation of glycogen synthase and the 57 and 53 kDa substrates by brain CaM kinase II was also inhibited by alloxan. The Ca^{2+} and calmodulin-dependencies of phosphorylation of the endogenous islet substrates differed. In the presence of 400 nM calmodulin, half-maximal phosphorylation was attained at Ca^{2+} concentrations of 80 ± 9 , 401 ± 61 and

459 ± 59 nM for the 102, 57 and 53 kDa substrates respectively. In the presence of 10 μM Ca^{2+} , half-maximal phosphorylation was attained at calmodulin concentrations of 9 ± 2 , 38 ± 2.5 and 37 ± 2 nM for the 102, 57 and 53 kDa substrates respectively. Differential centrifugation located the 102 kDa substrate in the post-100000 *g* supernatant and the 57 and 53 kDa substrates in the particulate fraction. These data suggest that islet CaM kinase is similar to, if not identical with, brain CaM kinase II, but that phosphorylation of the endogenous 102 kDa substrate occurs by a distinct kinase which shows different sensitivities to Ca^{2+} and calmodulin. This kinase probably corresponds to CaM kinase III and the 102 kDa peptide to elongation factor 2 (EF-2), since the 102 kDa peptide was shown to undergo ADP-ribosylation in the presence of diphtheria toxin and NAD^+ .

INTRODUCTION

An influx of Ca^{2+} into the pancreatic β -cell causing an increase in intracellular Ca^{2+} is thought to be an important step in the mechanism of nutrient-induced insulin secretion (Wollheim and Sharp, 1981; Malaisse and Malaisse-Lagae, 1984; Prentki and Matschinsky, 1987). The increase in intracellular Ca^{2+} may activate Ca^{2+} -dependent protein kinases, including Ca^{2+} /calmodulin-dependent protein kinase (CaM kinase), which in turn phosphorylates key components in the secretory machinery (Colca et al., 1983b; Harrison et al., 1984). The nature and identity of these components is at present unclear. In addition, the identity of the islet CaM kinase is also unclear. The kinase could be any one of the family of Ca^{2+} /calmodulin-dependent protein kinases found in mammalian tissues, which include phosphorylase kinase, CaM kinases I, II and III and myosin light-chain kinase (Nairn et al., 1985a; Colbran et al., 1989).

An attempt to identify islet CaM kinase would conventionally include purification to homogeneity and subsequent kinetic and structural characterization of the kinase. However, owing to the availability of only limited quantities of islet material, this approach is difficult. In the present study we have attempted to identify islet CaM kinase by comparing its activity with purified CaM kinase II isolated from rat brain (McGuinness et al., 1985). This kinase has been extensively characterized and is thought to participate in neurotransmitter release from neuronal cells (Llinas et al., 1985; Bahler and Greengard, 1987). We have compared the substrate-phosphorylation patterns of the two kinases in islet homogenates and examined their sensitivity to inhibition by alloxan, a potent inhibitor of the islet enzyme (Colca et al., 1983a). In addition, we have further characterized the Ca^{2+} - and calmodulin-dependence of the islet CaM kinase activity.

MATERIALS AND METHODS

Materials

Glycogen synthase was kindly provided by Ms. Sara Nakielný and Dr. D. G. Hardie (University of Dundee, U.K.). [γ - ^{32}P]ATP, [^{32}P]NAD and Rainbow [^{14}C]methylated protein molecular-mass markers (14.3–200 kDa) were supplied by Amersham International, Amersham, Bucks., U.K. Alloxan, ampholines (pH 5–7), benzamidine, dithiothreitol, Nonidet P-40, Pharmalyte 2D (pH 3–10), phenylmethanesulphonyl fluoride, protein molecular-mass standards (29–200 kDa), SDS and β -mercaptoethanol were supplied by Sigma Chemical Co., Poole, Dorset, U.K. X-OMAT AR film was supplied by Kodak, Hemel Hempstead, Herts., U.K. BSA (fraction 5) was supplied by Boehringer Mannheim, Lewes, East Sussex, U.K. Acrylamide (Electran grade) and urea (Aristar grade) were supplied by BDH, Eastleigh, Hants., U.K.

Purification of CaM kinase II from rat brain

For preliminary experiments, purified rat brain CaM kinase II was kindly provided by Dr. Grahame Hardie (University of Dundee). For additional studies, the enzyme was purified from 10 rat brains by a protocol based on the method of McGuinness et al. (1985).

Isolation of islets of Langerhans

Islets of Langerhans were prepared by collagenase digestion of pancreases from male Wistar rats (Sutton et al., 1986). Free islets were collected under a dissecting microscope with a wire hoop into Hepes-buffered Hanks medium containing 0.5% BSA. Islets

were washed three times in 1.5 ml volumes of incubation buffer (0.25 M sucrose, 50 mM glycerophosphate buffer, pH 7.0, 5 mM benzamidine, 1 mM dithiothreitol, 1 mM phenylmethanesulphonyl fluoride) and then homogenized in 0.1–0.2 ml of buffer with a hand-held small glass homogenizer.

Phosphorylation of peptides

The phosphorylation of peptides in islet homogenates was carried out by the procedure of Woodgett et al. (1982) in a final volume of 35 μ l. Islet homogenate corresponding to 10–20 islets was mixed with 400 nM calmodulin, 10 μ M Ca^{2+} and, where indicated, glycogen synthase (1 μ g) and brain CaM kinase II (0.2 μ g). The mixture was preincubated for 5 min in the absence and presence of 1 mM alloxan, which had been freshly dissolved in glycerophosphate buffer. In some experiments the preincubation with alloxan was omitted and the free Ca^{2+} or calmodulin concentrations were varied. Free Ca^{2+} concentrations were calculated as previously described (Hughes et al., 1987) using 0.5 mM EGTA in the buffer. Reactions were started by addition of 1 μ Ci of [γ - 32 P]ATP (final concentration 50 μ M) and terminated after various incubation times (up to 3 min) at 37 °C by addition of 35 μ l of SDS buffer (100 mM Tris, pH 6.8, 2% SDS, 20% glycerol, 300 mM β -mercaptoethanol). Reactions were also terminated by cooling on ice before subcellular fractionation (see below).

Subcellular fractionation

Incubation mixtures were centrifuged at 900 *g* for 10 min and the resulting supernatants were centrifuged at 24000 *g* for 20 min. The supernatant from this step was centrifuged at 100000 *g* for 60 min to yield the final 100000 *g* pellet and supernatant fractions. All the centrifugation steps were carried out at 4 °C. The fractions were either resuspended or mixed with SDS buffer for gel electrophoresis. For comparison of islet peptide phosphorylation and ADP-ribosylation, a 100000 *g* supernatant fraction was prepared first from islet homogenate and then used in the phosphorylation assay described above.

ADP-ribosylation of 100000 *g* supernatant fraction of islet homogenate

The ADP-ribosylation of peptides in the 100000 *g* supernatant fraction prepared from islet homogenates was carried out as described by Nairn and Palfrey (1987) in a final volume of 35 μ l. Supernatant fractions (10 μ l) were preincubated with 5 mM dithiothreitol/1 mM EGTA in the presence and absence of 17.5 ng of diphtheria toxin for 5 min. Reactions were then started by addition of 1 μ Ci of [32 P]NAD (final concn. 0.75 μ M) and terminated after 3 min incubation at 37 °C by addition of 35 μ l of SDS buffer or 10 μ l of 150 mM dithiothreitol/10% SDS for analysis by two-dimensional gel electrophoresis.

Peptides were separated by SDS/PAGE on 8.5% gels, and phosphorylation was quantified by densitometric scanning of autoradiographs (Christie and Ashcroft, 1985). In some experiments, peptides were separated by two-dimensional gel electrophoresis (Hochstrasser et al., 1988) by using isoelectric-focusing tube gels in the first dimension and SDS/polyacrylamide gels in the second dimension.

Data are presented as means $\pm$ S.E.M. for the numbers of experiments indicated. Statistical analysis was carried out by Student's *t* test or, where appropriate, a paired *t* test.

RESULTS

Effect of alloxan on islet CaM kinase and brain CaM kinase II activity

The time course for the phosphorylation of exogenous glycogen synthase by islet homogenate and brain CaM kinase II is shown in Figure 1. For islets, incorporation reached a plateau after 1 min, whereas the incorporation catalysed by brain CaM kinase II continued to increase for 3 min. Preincubation with 1 mM alloxan markedly inhibited the phosphorylation of glycogen synthase by both islet homogenate and brain CaM kinase II.

The effect of preincubation with alloxan on the phosphorylation of endogenous peptides and exogenous glycogen synthase by rat islet homogenates and brain CaM kinase II is shown in Figure 2. Activation of endogenous islet CaM kinase resulted in the phosphorylation of several islet peptides of molecular mass 102 kDa, 57 kDa, 53 kDa and, less consistently, 200 kDa and 76 kDa (lane 1) and the phosphorylation of exogenous glycogen synthase (lane 3). Phosphorylation of these peptides was inhibited by preincubation with 1 mM alloxan (lanes 2 and 4). Inclusion of brain CaM kinase II with the islet homogenate (lanes 5 and 6) resulted in additional

Figure 1 Effect of alloxan on phosphorylation of glycogen synthase by islet homogenate and brain CaM kinase II

Islet homogenates (a) or brain CaM kinase II (b) were mixed with 10 μ M Ca^{2+} , 400 nM calmodulin and glycogen synthase and preincubated in the presence (●) or absence (○) of 1 mM alloxan for 5 min. Reactions were then started by addition of [γ - 32 P]ATP (final concn. 50 μ M) and stopped after the times indicated by addition of SDS buffer. Proteins were separated by SDS/PAGE. The resulting gel was dried and autoradiographed. The extent of phosphorylation of glycogen synthase was quantified by densitometry. The data are representative of three experiments.

Figure 2 Effect of alloxan on islet CaM kinase and brain CaM kinase II activity

Islet homogenates (lanes 1–6) and brain CaM kinase II (lanes 5–8) and, where appropriate, glycogen synthase (GS; lanes 3 and 4) were mixed with $10 \mu\text{M}$ Ca^{2+} and 400 nM calmodulin and preincubated in the absence or presence of 1 mM alloxan for 5 min . Reactions were then started by addition of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (final concn. $50 \mu\text{M}$) and stopped after 3 min by addition of SDS buffer. Proteins were separated by SDS/PAGE. The resulting gel was dried and autoradiographed. The positions of the endogenous islet substrates and GS are shown at the left of the autoradiograph. The data are representative of four experiments.

phosphorylation of the 57 kDa and 53 kDa peptides, but not of the 200 kDa and 102 kDa peptides (compare lanes 1 and 5). On densitometric analysis, phosphorylation of the 57 kDa and 53 kDa peptides was increased by $378 \pm 77\%$ and $362 \pm 80\%$ respectively ($P < 0.05$, paired t test, $n = 4$), whereas phosphorylation of the 200 kDa and 102 kDa peptides remained unchanged at $123 \pm 10\%$ and $116 \pm 9\%$ respectively (not significant, paired t test, $n = 4$). These phosphorylations by brain CaM kinase II were also inhibited by alloxan (lane 6). In assays incorporating brain CaM kinase II alone, the major band phosphorylated was of approx. 50 kDa (lane 7); this autophosphorylation was also inhibited by preincubation with alloxan (lane 8).

Characterization of the Ca^{2+} - and calmodulin-dependence of islet CaM kinase phosphorylation

In order to gain further insight into the nature of the islet CaM kinase, we characterized the Ca^{2+} - and calmodulin-dependence of phosphorylation of the three main endogenous islet substrates. The effect of increasing the Ca^{2+} concentration in the incubation mixture in the presence and absence of calmodulin on peptide phosphorylation is shown in Figure 3. For all of the endogenous substrates, increasing the free Ca^{2+} concentration caused a dose-dependent increase in phosphorylation, although the apparent sensitivity to the Ca^{2+} concentration varied for the different peptides. For the 57 kDa and 53 kDa peptides, half-maximal phosphorylation in the presence of calmodulin was achieved at Ca^{2+} concentrations of 401 nM and 458 nM respectively. In contrast, phosphorylation of the 102 kDa peptide was enhanced by lower Ca^{2+} concentrations; half-maximal phosphorylation was obtained at the significantly lower Ca^{2+} concentration of 80 nM (Table 1). In addition, phosphorylation of the 102 kDa peptide was apparently less dependent on the presence of

Figure 3 Ca^{2+} -dependency of Ca^{2+} /calmodulin-dependent protein phosphorylation in islet homogenates

Islet homogenates were incubated with $50 \mu\text{M}$ $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at various Ca^{2+} concentrations in the absence (○) or presence (●) of 400 nM calmodulin for 3 min . Reactions were stopped by addition of SDS buffer and proteins were separated by SDS/PAGE. The resulting gels were dried and autoradiographed. Phosphorylation of the islet peptides was quantified by densitometric scanning and was expressed relative to the maximal phosphorylation observed at $10 \mu\text{M}$ Ca^{2+} . Data are expressed as means $\pm$ S.E.M. of four experiments.

calmodulin. In the absence of calmodulin, 80% of maximal phosphorylation was obtained, compared with $30\text{--}40\%$ maximal phosphorylation of the 57 kDa and 53 kDa peptides (Figure 3). This observation was confirmed in experiments to examine the effects of increasing calmodulin concentration on peptide phosphorylation in islet homogenates (Figure 4). Whereas both the 57 kDa and 53 kDa peptides were half-maximally phosphorylated at a calmodulin concentration of approx. 40 nM , the 102 kDa peptide was half-maximally phosphorylated at the significantly lower calmodulin concentration of 9 nM (Table 1).

Subcellular fractionation of the islet CaM kinase substrates after the phosphorylation assay located 75% of the 53 kDa and 57 kDa peptides in the particulate fractions (23000 g pellet and

Table 1 Phosphorylation of endogenous substrates by islet CaM kinase

Experiments were carried out as detailed in the legends to Figs. 3 and 4. Half-maximal Ca^{2+} activation was calculated from incubations containing 400 nM calmodulin. Subcellular fractionation of the islet peptides was carried out as described in the Materials and methods section. Distribution of peptides was assessed by densitometric scanning of autoradiographs. For quantification of maximal phosphorylation of the 102 kDa and 53/57 kDa peptides, incubation conditions were chosen containing 0.3 μM and 0.1 μM Ca^{2+} respectively. Data are means of two experiments. * $P < 0.001$ compared with the half-maximal phosphorylation of the 57 kDa and 53 kDa peptides.

Parameter	Peptide		
	102 kDa	57 kDa	53 kDa
Concn. for half-maximal activation (nM)			
Ca^{2+}	$80 \pm 9^*$	401 ± 61	459 ± 59
Calmodulin	$9 \pm 2^*$	38 ± 2.5	37 ± 2
Distribution in subcellular fractions (%):			
Homogenate	100	100	100
24 000 g pellet	6.7	59.4	52.8
100 000 g pellet	13.6	20.3	22.6
100 000 g supernatant	79.7	20.3	24.5

Figure 4 Calmodulin-dependency of Ca^{2+} /calmodulin-dependent protein phosphorylation in islet homogenates

Islet homogenates were incubated in the presence of 10 μM Ca^{2+} and 50 μM [γ - ^{32}P]ATP at various calmodulin concentrations for 3 min. Reactions were stopped by addition of SDS buffer and proteins were separated by SDS/PAGE. The resulting gels were dried and autoradiographed. Phosphorylation of the islet peptides (102 kDa, $\circ$; 57 kDa, $\bullet$; 53 kDa, $\blacksquare$) was quantified by densitometric scanning and was expressed relative to the maximal phosphorylation observed at 400 nM calmodulin. Data are presented as means $\pm$ S.E.M. of four experiments.

100 000 g pellet), whereas the 102 kDa peptide was found mainly (80 %) in the 100 000 g supernatant (Table 1). Additional studies revealed that both the kinase activity and its 102 kDa substrate were present in this subcellular fraction of islet homogenates (results not shown).

ADP-ribosylation of 100 000 g supernatant fraction of islet homogenate

The 100 kDa CaM kinase substrate in rat liver and pancreas has been identified as elongation factor 2 (EF-2), since it undergoes ADP-ribosylation in the presence of NAD^+ and diphtheria toxin (Ryazanof, 1987; Nairn and Palfrey, 1987). We examined the possibility that the 102 kDa islet peptide corresponded to EF-2 by comparing the migration of the phosphopeptide with labelled peptides from islet fractions incubated with diphtheria toxin and [^{32}P]NAD $^+$ (Figure 5). Phosphorylation of the 100 000 g super-

Figure 5 Comparison of CaM kinase-catalysed phosphorylation and diphtheria-toxin-induced ribosylation of peptides in a 100 000 g supernatant fraction of islet homogenate

(a) CaM kinase-dependent phosphorylation of endogenous peptides in the islet supernatant fraction was carried out in the presence of 50 μM [γ - ^{32}P]ATP and 400 nM calmodulin and in the absence (lane 1) or presence (lane 2) of 0.3 μM Ca^{2+} for 3 min at 37 $^{\circ}\text{C}$. In parallel incubations, islet supernatant fractions containing 5 mM dithiothreitol were preincubated in the absence (lane 3) or presence (lane 4) of 17.5 ng of diphtheria toxin for 5 min. Reactions were then started by addition of 0.75 mM [^{32}P]NAD $^+$, and incubations continued for 3 min at 37 $^{\circ}\text{C}$. Reactions were stopped by addition of SDS buffer, and peptides were separated by SDS/PAGE. For (b) and (c), reactions were stopped by addition of 150 mM dithiothreitol/10% SDS, and peptides were separated by two-dimensional gel electrophoresis as indicated (IEF isoelectric focusing). (b) Ca^{2+} /calmodulin-dependent protein phosphorylation in the presence of 0.3 μM Ca^{2+} and 400 nM calmodulin. (c) ADP-ribosylation in the presence of diphtheria toxin. The autoradiographs are representative of two similar experiments. The arrow marks the position of the 102 kDa bands.

natant fraction from islet homogenate in the presence of $0.3 \mu\text{M}$ Ca^{2+} and 400 nM calmodulin increased the phosphorylation of only one peptide band of molecular mass 102 kDa (Figure 5a, lanes 1 and 2). In the absence of Ca^{2+} , a small but significant level of phosphorylation of this peptide was detected, in contrast with that seen in incubations containing islet homogenates (see Figure 3, top panel). In parallel incubations containing [^{32}P]NAD $^{+}$, diphtheria toxin stimulated the ADP-ribosylation of a single peptide band of molecular mass 102 kDa which migrated to the same position as the CaM kinase substrate (lanes 3 and 4). On two-dimensional gel analysis, the 102 kDa substrate migrated to a position corresponding to a peptide of slightly lower molecular mass (approx. $90\text{--}95 \text{ kDa}$) and separated into a series of phosphopeptide spots. These migrated to the same positions as the series of ADP-ribosylated peptide spots resulting from the treatment of the islet fraction with diphtheria toxin (compare Figures 5b and 5c).

DISCUSSION

Endogenous substrates for islet CaM kinase have been characterized by researchers from several laboratories, including our own, and have been shown to include three major species of molecular mass 100 kDa , 57 kDa and 53 kDa . These peptide substrates have been reported to be phosphorylated under conditions of enhanced islet CaM kinase activity in islet subcellular fractions (Harrison and Ashcroft, 1982; Brocklehurst and Hutton, 1983; Colca et al., 1983b; Thams et al., 1984), permeabilized islets (Jones et al., 1988) or in intact islets prelabelled with [^{32}P]phosphate (Colca et al., 1983b; Kowluru and MacDonald, 1984; Dunlop and Larkins, 1986). It has been suggested that the 57 kDa and 53 kDa peptides correspond to the α - and β -subunits of tubulin (Colca et al., 1983b) or alternatively that phosphorylation of the 53 kDa peptide corresponds to autophosphorylation of the kinase itself (Harrison et al., 1984). Although pancreatic islets have been shown to contain myosin light-chain kinase activity (MacDonald and Kowluru, 1982), the nature of the kinase which phosphorylates the three major peptide substrates characterized here is unknown. This kinase could potentially be any one of a number of Ca^{2+} /calmodulin-dependent protein kinases found in mammalian cells (Nairn et al., 1985a; Colbran et al., 1989). In the present study we attempted to identify the islet CaM kinase by comparing it with CaM kinase II purified from rat brain. This enzyme seemed a suitable kinase for comparison, since it has been shown to participate in secretory processes in neuronal cells (Llinas et al., 1985; Bahler and Greengard, 1987; Colbran et al., 1989) and because it is thought to undergo autophosphorylation (Colbran et al., 1989; Soderling, 1990), which may also be a feature of the islet enzyme (Harrison et al., 1984). Our approach was to compare the substrate-phosphorylation pattern of the two kinases in islet homogenates and to examine their sensitivity to inhibition by alloxan with respect to phosphorylation of endogenous islet substrates and exogenous glycogen synthase. Our data show that, although both islet CaM kinase and brain CaM kinase II phosphorylate exogenous glycogen synthase (not necessarily at the same site) and peptides of $50\text{--}57 \text{ kDa}$, the brain CaM kinase II does not phosphorylate the endogenous islet substrate of 102 kDa .

It has previously been shown that CaM kinase activity in homogenates of brain and pancreatic islets is susceptible to inhibition by alloxan (Norling et al., 1984). The mechanism has been suggested to involve alkylation by alloxan of specific thiol groups in the enzyme (Kloepper et al., 1991). In the present study, the use of purified brain CaM kinase II has allowed us to

compare the effect of alloxan inhibition on the two enzymic activities under conditions in which they utilize common substrates (islet proteins and exogenous glycogen synthase). Although it would be desirable to compare the effects of alloxan on enzymes purified from both tissues, this is difficult given the limited amount of islet material available. It was found that phosphorylation of exogenous glycogen synthase and endogenous islet peptides by both islet CaM kinase and brain CaM kinase II was subject to inhibition by alloxan. These observations suggest that islet CaM kinase is similar to CaM kinase II. It has been suggested that phosphorylation of the islet 53 kDa phosphopeptide represents autophosphorylation of the kinase (Harrison et al., 1984). Since the main autophosphorylated species in the brain CaM kinase II, although inhibitable by alloxan, had a slightly lower molecular mass (50 kDa), the islet form may not be identical with the brain enzyme.

We find that the Ca^{2+} - and calmodulin-dependent phosphorylation of the endogenous substrates by islet CaM kinase exhibits a complex pattern. Phosphorylation of the 102 kDa peptide was more sensitive to activation by Ca^{2+} and calmodulin than was that of the 57 kDa and 53 kDa peptides, which exhibited similar sensitivities. It is possible that Ca^{2+} may exert a direct effect on the 102 kDa peptide (altering its conformation and exposing the phosphorylation site), so that increased phosphorylation is obtained at lower Ca^{2+} concentrations. This might account for the altered Ca^{2+} -activation curve for this peptide, but seems unlikely to account for the increased sensitivity of phosphorylation of the 102 kDa peptide to calmodulin activation, which is mediated by the kinase. The simplest interpretation of these data is that a different Ca^{2+} /calmodulin-dependent kinase phosphorylates the 102 kDa peptide. This kinase exhibits an increased sensitivity to Ca^{2+} and calmodulin activation, which distinguishes it from CaM kinase, which phosphorylates the 53 kDa and 57 kDa peptides. (This 102 kDa kinase may alternatively bind calmodulin tightly, requiring only low concentrations for maximal activity.) Indeed, convincing evidence has been presented that a distinct CaM kinase (CaM kinase III) specifically phosphorylates a 100 kDa cytosolic peptide substrate in mammalian tissues (Nairn et al., 1985b). This process is apparently specific for CaM kinase III, since various other Ca^{2+} /calmodulin-dependent protein kinases tested (including CaM kinase II) were unable to utilize the 100 kDa peptide as a substrate. In the present study, brain CaM kinase II did not phosphorylate the 102 kDa islet peptide. The appearance of the endogenous islet 102 kDa peptide mainly in the $100000 g$ -supernatant fraction of islet homogenates is consistent with this peptide being a CaM kinase III substrate. Indeed, we have found that both the kinase and the 102 kDa peptide are present in this subcellular fraction of islet homogenates (results not shown) which is also consistent with this proposal.

The 100 kDa peptide substrate for CaM kinase III was subsequently identified as EF-2 (Ryzanof, 1987; Nairn and Palfrey, 1987), since the N-terminal amino acid sequence of the purified peptide was found to be identical with the N-terminal sequence of EF-2 deduced from the nucleic acid sequence of cDNA (Kohno et al., 1986) and because the 100 kDa peptide was shown to be ADP-ribosylated in the presence of diphtheria toxin. In the present study we have found that the slightly larger 102 kDa islet CaM kinase substrate (molecular mass $102 \pm 0.5 \text{ kDa}$, $n = 14$ experiments) corresponds to EF-2, since it also undergoes ADP-ribosylation in the presence of diphtheria toxin. This finding was confirmed on two-dimensional gel analysis, where the 102 kDa phosphopeptides were coincident with the labelled ADP-ribosylated peptides. The multiple spots observed may correspond to different phosphorylation states of EF-2.

In summary, we have characterized the CaM kinase activity in rat islets by comparing it with purified brain CaM kinase II. Both kinases phosphorylate glycogen synthase and islet peptides of molecular masses 53 kDa and 57 kDa. Both kinases are also sensitive to inhibition by alloxan. These findings suggest that pancreatic islets contain a form of CaM kinase II activity similar to, although not necessarily identical with, that characterized in brain. Our subsequent studies indicate that CaM kinase III, which specifically phosphorylates EF-2, is present in rat pancreatic islets and that this kinase appears particularly sensitive to activation by Ca^{2+} and calmodulin. This is apparently the first report that CaM kinase III exhibits different activation kinetics to other CaM kinases. Furthermore, the present study raises the question of a possible role for Ca^{2+} /calmodulin-mediated regulation of protein synthesis via CaM kinase III in the pancreatic B-cell.

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