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Biochemical characterization of an effective substrate and potent activators of CK2 copurified with Bowman-Birk-type proteinase inhibitor from soybean seeds in vitro

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Abstract

By means of Mono P column chromatography, an effective phosphate acceptor (EPA) of casein kinase 2 (CK2) was purified from the Bowman-Birk-type proteinase inhibitor (BBI) fraction of soybean seeds. The most acidic EPA (aEPA, p*I*=approx. 3.7) was heavily phosphorylated when incubated with CK2 and 5 μM [γ-^{32}P]ATP in the presence of poly-Arg (a CK2 activator) in vitro. However, aEPA was slightly phosphorylated by casein kinase 1 (CK1) as effective as C-kinase and not at all by A-kinase in vitro. The 13 N-terminal amino acid residues (SDHSSSDDESSKP) of aEPA were 100% homologous to the corresponding sequence of soybean BBI-type proteinase inhibitor CII (SBI CII). Polyamine at 3 mM stimulated 4.6-fold the CK2-mediated phosphorylation of aEPA, and this phosphorylation was sensitive to quercetin (ID_{50}=approx. 0.1 μM) in vitro. Furthermore, two basic proteins [Mr=29,000 (p29) and 17,000 (p17)] copurified with BBI were identified as proteolytic cleavage products of basic 7S globulin and functioned as potent CK2 activators in vitro. aEPA fully phosphorylated by CK2 in the presence of poly-Arg or basic proteins formed a complex with trypsin, whereas unphosphorylated aEPA was digested by trypsin in vitro. These results suggest that (i) aEPA (a BBI isoform) may coexist with two basic proteins (p29 and p17) generated from basic 7S globulin; and (ii) the physiological interaction between aEPA and its binding trypsin-like proteinases may be regulated through specific phosphorylation of aEPA by CK2 activated with the two basic proteins in legume seeds.

Keywords: Casein kinase 2; Effective CK2 substrate; Bowman-Birk-type proteinase inhibitor; Potent CK2 activator; Soybean trypsin inhibitor

1. Introduction

The seeds of many plants belonging to the legume family are rich sources of serine proteinase inhibitors [1,2], of which two are widely distributed in legume seeds: Kunitz-type soybean trypsin inhibitor (STI, Mr=approx. 21,000), containing four cysteine residues; and Bowman-Birk-type proteinase inhibitor (BBI), with a relatively low molecular weight (Mr=7000–9000), 14 cysteine residues forming seven disulfide bridges, and two reactive sites (so-called double-headed inhibitors), enabling interaction with two molecules of proteinase [1,2]. Several BBI isomers have been isolated and characterized from various legume seeds, such as soybean [3–5], peanut [6,7], azuki [8], pea [9], and broad beans [10]. These isoinhibitors behave similarly, inhibiting bovine chymotrypsin at a ratio of 1 to 1 and bovine trypsin at a ratio of 1 to 2 [1,2]. Pinna's research group has demonstrated that

Abbreviations: A-kinase, cAMP-dependent protein kinase; aEPA, acidic effective phosphate acceptor; CK1, casein kinase 1; CK2, casein kinase 2; C-kinase, Ca^{2+}- and phospholipid-dependent protein kinase; BBI, Bowman-Birk-type proteinase inhibitor; DTT, dithiothreitol; EGCG, epigallocatechin gallate; HPLC, high-performance liquid chromatography; SBI, soybean BBI-type proteinase inhibitor; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; STI, soybean trypsin inhibitor

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rat liver casein kinase TS (CK-TS) greatly phosphorylates the low molecular weight soybean BBI-type proteinase inhibitor CII (SBI CII [4,5]), whereas the high molecular weight Kunitz-type inhibitor (Mr=21,500) acts as a poor substrate for CK-TS in vitro [11,12]. However, the physiological significance of the CK-TS-mediated phosphorylation of SBI CII (a BBI isoform) on its proteinase inhibitory activity and the physiological interaction of SBI CII with trypsin or chymotrypsin in vitro remain to be elucidated.

Recently, we characterized (i) casein kinase 2 (CK2) as a protein kinase responsible for the physiological regulation of glycyrrhizin (GL)-binding functional proteins, such as glucocorticoid receptor [13], soybean lipoxygenase 3 [14], secretory type IIA phospholipase A_2 [15], and human immunodeficiency virus type I (HIV-1) enzymes (reverse transcriptase [16] and protease [17]) in vitro; and (ii) plant polyphenol-containing antioxidant compounds, such as quercetin and epigallocatechin gallate (EGCG [18]) and galloyl pedunculagin [19], as potent CK2 inhibitors in vitro. During the identification and characterization of the native substrate proteins copurified with CK2 from various animal organs, such as liver and brain [13], and from plant sources [14,20,21], we detected an effective phosphate acceptor (EPA) of CK2 in the commercial STI and BBI fractions when incubated with [γ-^{32}P]ATP in the presence of poly-Arg (a CK2 activator) in vitro. Therefore, the present study was carried out to purify and characterize an Mr=approx. 13,000 acidic EPA (aEPA, p*I*=approx. 3.7) from the commercial purified BBI preparations as an effective CK2 phosphate acceptor and two basic proteins [Mr=29,000 (p29) and Mr=17,000 (p17)] copurified with BBI from soybean seeds. Here, we describe (i) the purification of aEPA from the commercial BBI fraction by Mono P column chromatography (HPLC); (ii) the biochemical characterization of purified aEPA as an effective CK2 phosphate acceptor, and two basic proteins (p29 and p17) copurified with BBI as potent CK2 activators in vitro; (iii) the identification of aEPA as SBI CII and two basic proteins as proteolytic cleavage products of basic 7S globulin; and (iv) the physiological significance of the CK2-mediated phosphorylation of aEPA on its physiological interaction (a complex forming ability) with trypsin in vitro.

2. Materials and methods

2.1. Chemicals

[γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Amersham Pharmacia Biotech (Arlington Heights, USA); dithiothreitol (DTT), 2-mercaptoethanol (2-ME), quercetin, EGCG, dephosphorylated α-casein (a CK2 substrate), poly-Arg (approx. 100 residues), and purified trypsin (bovine pancreas) were from Sigma Chemicals (St. Louis, USA); anti-serum (rabbit) against STI (soybean) was from ICN Pharmaceutical Inc. (Aurora, Ohio, USA); and anti-trypsin antiserum (rabbit) to trypsin (bovine pancreas) was from Rockland Inc. (Gilbertsville, Pennsylvania, USA).

2.2. BBI and STI preparations

A BBI preparation (1 mg protein inhibits approx. 2.8 mg trypsin with an activity of 10,000 units/mg protein) was obtained from Sigma Chemicals (St. Louis, USA). Three STI preparations were obtained: (i) specific activity: 9000–18,500 units/mg from Wako Pure Chemical Industries, Ltd (Tokyo); (ii) specific activity: approx. 10,000 units/mg from Sigma Chemicals (St. Louis, USA); and (iii) specific activity: approx. 10,000 units/mg from ICN Chemical Industries, Ltd (Tokyo).

2.3. Protein kinases

Recombinant human CK2 (rhCK2) (a heterodimer of $\alpha_2\beta_2$ [α-subunit (Mr=44,000) and β-subunit (Mr=26,000)]; specific activity 400 kunits/mg) was obtained from Biomol Research Laboratories (Plymouth Meeting, PA, USA), and rat liver CK1 (specific activity: 17,500 units/mg) was obtained from Promega Co. (Madison, USA). Rat brain C-kinase (specific activity: 1–2 μmol phosphate/min/mg protein transferred to histone) was from Molecular Probes (Oregon, USA), and bovine heart A-kinase (specific activity: 83 units/μg protein) was from Sigma Chemicals (St. Louis, USA).

2.4. Detection of the CK2-mediated phosphorylation of aEPA in vitro by autoradiography

To detect ^{32}P-labeled aEPA phosphorylated by CK2 in vitro, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography was performed, as reported previously [13–19]. To phosphorylate aEPA by CK2, the indicated fractions (purified aEPA, BBI, and STI fractions) were separately incubated for 30 min at 30 °C in reaction mixtures (50 μl) composed of 40 mM Tris–HCl (pH 7.6), 2 mM DTT, 3 mM Mn^{2+}, poly-Arg (0.75 μg), 5 μM [γ-^{32}P]ATP (500 cpm/pmol), and CK2 (approx. 20 ng). The phosphorylation of aEPA by CK2 was arrested by the addition of an equal volume of SDS-PAGE sample buffer [2% SDS, 10% 2-ME, and a trace of Bromophenol Blue (BPB)]. After boiling for 5 min, aliquots (10 μl) of the mixtures were applied on a polyacrylamide gel containing 0.1% SDS. After electrophoresis, polypeptides on the gel were detected by staining with Coomassie Brilliant Blue R-250 (CBB R-250) and dried. The ^{32}P-labeled aEPA on the gel was detected by exposing to an X-ray film (Fuji photo film Co. LTD., Tokyo), as reported previously [13–19].