ALTERED CELLULAR RATIO OF TYPE I AND TYPE II CYCLIC AMP-DEPENDENT PROTEIN KINASE IN HUMAN MAMMARY TUMORS

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1. Introduction

The molecular mechanism of a great number of non-steroid hormones involves the direct participation of cAMP and cAMP-regulated protein kinases in eukaryotic cells [1,2]. Through the action of cAMP-dependent protein kinases non-steroid hormones achieve the phosphorylation and functional modification of specific cellular proteins [3,4]. The potential significance of cAMP-mediated nuclear protein phosphorylation in rat mammary tumors has become evident through studies on the regression of DMBA-induced rat mammary tumors [5,6] giving evidence that hormone-dependent tumor regression might be due to an antagonistic relationship between steroid hormones and cAMP. The involvement, however, of cyclic nucleotides in the action of steroid hormones is not clear, but there is a considerable amount of evidence that an interrelationship may exist between steroid hormones and the cAMP-sensitive protein kinase system [7–9].

An increasing number of experimental findings show that the amount of type I and type II protein kinase isozyme activity determines the response of specific stimuli in a given tissue [10,11] as well as a selective, biological role for the two types of protein kinase isozymes. Ontogenetic studies suggest that type II isozyme may be involved in the process of cellular differentiation [12,13], whereas type I isozyme is related to cell proliferation [14].

In view of a possible involvement of cAMP-dependent protein kinase in cellular proliferation and differentiation, and in view of the reported decreased cellular levels of cAMP-dependent protein kinase and cAMP-binding activities as well as lower levels of cAMP in human mammary tumors in comparison to normal mammary tissue [15,16], we have characterized the major fractions of cAMP-dependent protein kinase activities in normal and neoplastic human breast tissue by ion exchange chromatography. Two major fractions have been identified and referred to as isozyme type I and type II cAMP-dependent protein kinase [17]. These two isozymes differ mainly in their regulatory subunits [19]; their catalytic subunits are similar [20].

We have found that the isozyme ratio type I versus type II protein kinase is 2-fold higher in neoplastic human mammatry tissue as compared to normal breast tissue. With respect to the increased cellular densities of neoplastic mammary tissue [15] the total amount of cAMP-dependent protein kinase activity is not lower in neoplastic as compared to normal mammary tissue. A relative cellular increase of cAMP-dependent protein kinase isozyme type I in human mammary tumors as compared to normal breast tissue is reported.

Abbreviation: cAMP, adenosine 3′:5′-monophosphate

Enzyme: cAMP-dependent protein kinase or ATP: protein phosphotransferase (EC 2.7.1.37)

This is paper 3 in a series on the role of the adenylyl cyclase–protein kinase system in human mammary tumors from the Laboratory of Experimental Endocrinology, Dept. of Gynecology, University of Basel

2. Materials and methods

All biochemical reagents were obtained from Sigma Chemical Co. Protamine sulfate was from Merck. [γ-^{32}P]ATP (2–10 Ci/mmol) was purchased from New England Nuclear and DEAE-cellulose from Whatman (DE-52).

Breast tissue specimens were obtained from cancer patients during mastectomy, from biopsies (1–3 g tissue) and from control patients with simple dysplasia. Specimens were kept at 4°C on ice after excision and stored at −70°C. Morphological characterization of the tissue was carried out by microscopic examination of a pathologist within 1–2 h after surgery. For the protein kinase determination the breast tissue was pulverized in liquid nitrogen. The powdered tissue was then homogenized in 4 parts (w/v) of ice-cold 10 mM Tris–HCl buffer containing 1.5 mM EDTA, 6 mM 2-mercaptoethanol and 10% glycerol at pH 7.4 with a Polytron PT-20 (Brinkmann Instruments) at low speed (setting 4). The homogenate was centrifuged for 60 min at 105 000 × g. The supernatant fraction was recovered and chromatographed on DEAE-cellulose. cAMP-sensitive protein kinase activity was measured as in [21] using protamine sulfate as substrate in the presence of 3.3 μM cAMP with and without heat-stable protein kinase inhibitor. The inhibitor was isolated from rabbit skeletal muscle by the method in [22]. Partial purification of the inhibitor was carried out by heat-treatment of the muscle extract followed by trichloroacetic acid precipitation. Protein was determined by the Lowry method [23].

The tissue was classified according to the criteria given by the World Health Organization 1968 [24]. The cellular density was evaluated by counting no. nuclei/1000 μm^2 tissue in 4 different tissue areas per biopsy [15].

3. Results

In view of the multiplicity of cAMP-dependent protein kinase isozyme pattern in a given tissue, we analyzed the cytosol fraction of normal (simple dysplasia) and neoplastic human mammary tissue by DEAE-cellulose chromatography (fig.1).

In all of the elution profiles protein kinase activity was resolved into two major peaks of cAMP-depen-

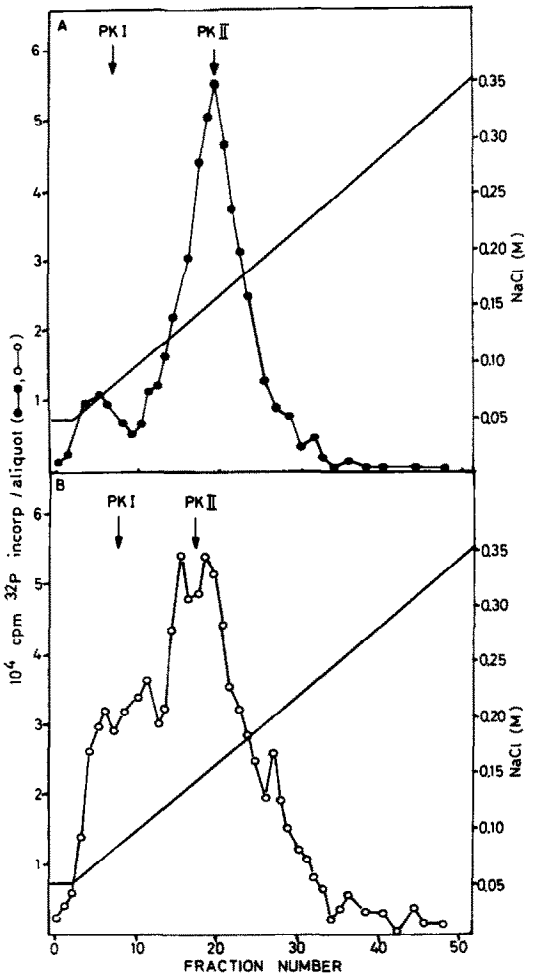

Fig.1. Comparative DEAE-cellulose elution profiles of cAMP-dependent protein kinase obtained from the cytosols of normal (A) and neoplastic (B) human breast tissue. Pooled cytosols of equal amounts of soluble protein (normal tissue: 30 mg protein; neoplastic tissue: 15 mg protein) were applied to a DEAE-cellulose column (1.2 × 4.0 cm). After extensive washing with 0.05 M NaCl in 10 mM Tris–HCl buffer (pH 7.4) containing 6 mM 2-mercaptoethanol, elution was carried out with a linear 0.05–0.50 M NaCl gradient (80 ml) in the same buffer. Fractions (1 ml) were collected and 0.1 ml aliquots were assayed for protein kinase activity in the presence of 3.3 μM cAMP with and without 80 μg heat-stable inhibitor. The profiles represent the difference of protein kinase activities obtained without and with the heat-stable inhibitor.

dent protein kinase activity eluted at 0.08–0.11 M NaCl, designated as peak PK-I, and protein kinase activity eluted at 0.14–0.22 M NaCl, designated as peak PK-II (fig.1). According to their relative elution position from DEAE-cellulose, which is known to separate type I and type II isozymes [17], based on the different stimulation by 0.5 M NaCl [18], due to the finding that fractions PK-I and PK-II bound cAMP specifically (data not shown) and were inhibited by the heat-stable rabbit skeletal muscle inhibitor, we conclude that type I cAMP-dependent protein kinase isozyme eluted with the PK-I fraction and type II isozyme with the PK-II fraction. The elution profiles shown in fig.1A,B represent the protein kinase activities calculated from the difference of enzyme activity stimulated by 3.3 μM cAMP in the absence and presence of the heat-stable inhibitor. About 25–30% of the protein kinase activity of normal and neoplastic tissue is not inhibited and can be considered as cAMP-insensitive.

A quantitative relationship between the specific activities of normal and neoplastic tissue can be established by calculating the specific activities of each isozyme peak for each DEAE-cellulose profile. The estimated specific activities were calculated by obtaining the sum of the cAMP-dependent protein kinase activities present in each fraction. Table 1 shows that the estimated specific activity of neoplastic tissue is 4-fold higher as compared to normal tissue.

Although both isozymes were present and identified in normal as well as in neoplastic tissue, a 2-fold increase of the type I/type II ratio is obtained for the neoplastic tissue (table 1). Such a ratio increase appears to be due to an increase of type I isozyme (PK-I) in neoplastic tissue (fig.1B). Type II isozyme is the predominant protein kinase in normal tissue, where only small amounts of type I can be resolved after DEAE-cellulose chromatography (fig.1A).

The specific activities of type I and type II isozymes measured in the cytosol fractions do not take into account the marked variations of cellular densities in normal (1.81 ± 0.64 cells/1000 μm^2) and neoplastic tissue (5.60 ± 2.29 cells/1000 μm^2). When the protein kinase activity is expressed as relative enzyme levels per cell, the neoplastic tissue is not characterized by a decrease of cAMP-dependent protein kinase activity per cell (table 1), as reported [15]. This difference can be explained by the preferential loss of type I isozyme in frozen cytosols stored over longer periods of time (unpublished result).

4. Discussion

Our results indicate that the specific activities of type I and type II isozyme are significantly higher in human breast cancer tissue than in normal mammary tissue provided the protein kinase is compared per unit weight of cellular protein. If the 3-fold increase of cellular density in neoplastic tissue is taken into account, the relative cellular level of type I isozyme in neoplastic mammary tissue is 2-fold higher as compared to the relative cellular level in normal tissue, whereas the relative cellular activity of type II isozyme is the same in neoplastic as in normal tissue. This finding is in contrast to [25] where no type I protein kinase could be shown in the growing and regressing DMBA-induced rat mammary tumor but is in accordance with [14] where type I protein kinase

Table 1
Cyclic AMP-dependent protein kinase type I and type II in human breast tissue

Tissue	Protein kinase act. (pmol ^{32}P incorp./min × mg prot.)			Rel. act./cell	Isozyme ratio type I/type II
		Type I	Type II		
Simple dysplasia (8)	91.8 ± 43.9	21.9 ± 10.7	69.9 ± 33.2	5.1 ± 2.4	0.32 ± 0.13
Carcinoma (9)	369.2 ± 66.8	141.8 ± 25.5	227.5 ± 41.3	6.6 ± 1.2	0.63 ± 0.17

Each value is the mean ± SD. The number of tissue biopsies examined is given in parentheses. The protein kinase represents the difference between the activity resolved after DEAE-cellulose elution in the presence of 3.3 μM cAMP without and with 80 μg heat-stable rabbit muscle inhibitor. The protein kinase activity per cell was calculated by dividing the total specific activity through the respective cellular densities (simple dysplasia 1.81 ± 0.64; carcinoma 5.60 ± 2.29)

was found to be augmented during mitosis. This suggests a positive correlation between the isozyme ratio of type I/type II and cell proliferation rate.

Our data, considered in context with previous findings, imply a possible role of the interrelationship between cAMP-dependent protein kinase type I and type II in the growth regulation of human breast tumors. Further studies on the various types of protein kinases and their role in human mammary tumor growth are underway.

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