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Secretory Immunoglobulin A from Human Milk Catalyzes Milk Protein Phosphorylation

GEORGY A. NEVINSKY,* YURY YA. KIT, DMITRY V. SEMENOV, DENIS YU. KHLIMANKOV,[1] AND VALENTINA N. BUNEVA

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk 630090, Lavrentieva Ave. 8, Russia, E-mail: Nevinsky@niboch.nsc.ru

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ABSTRACT

This article presents evidence that protein kinase activity is an intrinsic property of secretory immunoglobulin A (sIgA) from milk of healthy human mothers. Polyclonal sIgA was purified by sequential chromatography on protein A-Sepharose, DEAE-cellulose, and gel filtration on Toyopearl HW-55 and Sepharose 4B columns. Its purity was established by one- and two-dimensional SDS-PAGE. The protein kinase activity was inhibited by specific antibodies (Abs) against sIgA, and was stable to acidic and alkaline conditions. Catalytic sIgA showed optimal reaction conditions (pH and $MgCl_2$ concentration) and substrate specificity different from those of known protein kinases; i.e., sIgA phosphorylated the serine residues of various milk proteins in the presence of different γ-[^{32}P]nucleoside- and deoxynucleoside-5′-triphosphates. The homogeneous Fab fragment of sIgA also showed kinase activity. An ATP-binding activity of fractions of sIgA was demonstrated by affinity chromatography on ATP-Sepharose and by covalent binding of an affinity analog of ATP; this activity was mediated by the L chain of sIgA. The authors believe these observations are the first example of the catalytic activity of IgA Abs and of natural catalytic Abs with synthetic activity. In addition, the findings suggest the likelihood that catalytic Abs are generated by the immune system of healthy mothers.

Index Entries: Catalytic antibodies; protein kinases; human mother's milk.

*Author to whom all correspondence and reprint requests should be addressed.

Nomenclature: Abzymes, catalytically active antibodies; Abs, antibodies; DTT, dithiothreitol; oxATP, 2',3'-dialdehyde derivative of ATP; PAGE, polyacrylamide gel electrophoresis.

INTRODUCTION

The field of catalytic antibodies (Abs), or abzymes, has been amply reviewed recently *(1–6)*. The artificial catalytic Abs may be generated through transition-state stabilization by Abs, as suggested by Jencks *(7)*. Protein-hydrolyzing *(8,9)*, DNA-hydrolyzing *(10)*, and RNA-hydrolyzing *(11)* natural Abs have been detected in the sera of patients with various autoimmune pathologies. Patients with autoimmune diseases produce Abs to nucleoprotein complexes *(12)*, to DNA, and to enzymes that participate in nucleic acid metabolism *(13)*. In autoimmune diseases, there can be spontaneous induction of anti-idiotypic Abs, which are Abs directed against the unique epitopes of other Abs to various primary antigens (Ags). These anti-idiotypic Abs may have the characteristics of the primary Ag, including catalytic activity *(10,14)*. This idea is now supported by evidence that an anti-idiotypic Abs to acetylcholinesterase displays a catalytic function *(14)*. Thus, in autoimmune diseases, the presence of abzymes may be associated with autoimmunization.

All mammalian neonates are essentially agammaglobulinemic at their mucosal surfaces at birth *(15)*. Human milk contains Abs to bacterial, viral, and protozoal Ags, which reach mucosal surfaces in the respiratory and gastrointestinal tracts of breast-fed infants *(15–17)*. Passive immunity may also be acquired by transfer of mother's milk IgG across the epithelium of the intestinal surface to the circulatory system of the newborn *(18)*.

The authors have previously reported that Abs with DNase and RNase activity are present in human milk *(19,20)*, and have investigated whether catalytic Abs possessing protein kinase activity can be detected in milk, because the enzymatic phosphorylation of proteins plays a fundamental role in the regulation of many key physiological processes *(21)*. And the authors recently reported that secretory immunoglobulin A (sIgA), purified by chromatography on protein A-Sepharose, possesses casein kinase activity *(22,23)*. This observation could be criticized as being caused by copurifying protein kinases, because other proteins can form complexes with IgA and sIgA *(22,25)*, and Ab-mediated catalysis is usually characterized by a relatively low reaction rate. The present report provides evidence and arguments that the protein kinase activity is an intrinsic property of sIgA.

MATERIALS AND METHODS

Materials

Reagents used in this work were obtained chiefly from Merck (Darmstadt, Germany) and Euromedex. We also used protein A-Sepharose, NTP, and dNTP (Sigma, St. Louis, MO), diethylaminoethyl (DEAE)-cellulose DE-52 (Whatman, Mardstone, UK), Sepharose 4B (Pharmacia, Uppsala, Sweden), Toyopearl HW-55 fine (Toyo Soda, Japan), Triton X-100 (Ferak, Berlin, Germany). Radioisotopes were purchased from Amersham (Little Chalfont, UK) (3000 Ci/mmol).

Adenosine triphosphate (ATP)-Sepharose (4 μmol of ligand/mL of Sepharose) was obtained by immobilizing the 8-aminooctyl-γ-amide of ATP, and casein-Sepharose (6 mg casein/mL adsorbent), by immobilizing of casein on BrCN-activated Sepharose, according to the standard protocol of Pharmacia. Anti-IgA-Sepharose (20 mg anti-IgA antibodies/mL adsorbent) was prepared in the same way.

Chromatographic Purification of sIgA

Milk (100 mL), taken between 1 wk and 4 mo of lactation from 30 healthy human mothers (19–35 yr old), was centrifuged for 1 h at 6000*g*. The lipid phase was removed, the solution was filtered through paper *(23)*, and the filtrate was loaded on a 7-mL anti-IgA-Sepharose column (or on a 7-mL protein A-Sepharose column) equilibrated with TBS buffer (150 m*M* NaCl, 20 m*M* Tris-HCl, pH 7.4). The column was washed with TBS (200 mL) and proteins adsorbed nonspecifically were eluted with TBS containing 1% Triton X-100 and 0.3 *M* NaCl (200 mL), followed by 100 m*M* citrate buffer, pH 4.6 (100 mL). sIgA (or Abs containing sIgA and IgG, in the case of protein A-Sepharose) was eluted in 100 m*M* glycine-HCl, pH 2.6, and fractions containing Abs were immediately neutralized with 1 *M* Tris-HCl, pH 9.0, and dialyzed against 1 m*M* Tris-HCl, pH 7.0.

In control experiments, purified sIgA from the anti-IgA-Sepharose column fraction was purified further on protein A-Sepharose, and Abs purified on the protein A-Sepharose column were purified further on anti-IgA-Sepharose (*see* Results), using the protocol described above.

In the authors' standard procedure of sIgA purification, IgG is removed from the sIgA by chromatography on DEAE-cellulose according to Kit et al. *(23)*. The latter column (3 mL) was equilibrated with 5 m*M* potassium phosphate, pH 7.5, and protein was applied in the same buffer and eluted with a concentration gradient (5–50 m*M*) of the same buffer. The eluted sIgA was precipitated by addition of polyethylene glycol to 140 g/L,

dissolved in a minimal volume of TBS, and gel-filtered on Toyopearl HW-55 column (15 x 570 mm) equilibrated with TBS. The eluted sIgA fraction was precipitated with polyethylene glycol, dissolved in buffer A (10 m*M* Tris-HCl, pH 6.8, and 3.0 m*M* $MgCl_2$), and gel-filtered on a column of Sepharose 4B equilibrated with the same buffer A *(23)*, to remove Abs against polysaccharides, which could possibly co-purify in the preceding column run. The nonbound fraction of sIgA (90% of the total Abs applied on the column) was used for the following purification and analysis.

The amount of sIgA, IgG, and IgM Abs in the bound and nonbound fractions of different adsorbents, including protein A-Sepharose, was evaluated using the Ouchterlony double-immunodiffusion method against anti-IgA, anti-IgG, and anti-IgM Abs.

Affinity Chromatography

Affinity chromatography of Sepharose 4B-purified sIgA on ATP-Sepharose was performed on a column (12 x 55 mm) equilibrated with buffer A, which was washed with 25 vol of buffer A to elute nonbound proteins *(23)*. Adsorbed proteins were eluted in buffer A containing different concentrations of NaCl (0.05, 0.15, and 3.0 *M* NaCl) or 3.0 *M* $MgCl_2$. The eluted fractions were dialyzed against buffer B (10 m*M* Tris-HCl, pH 7.5, and 3.0 m*M* $MgCl_2$), loaded onto a casein-Sepharose column (1 mL) equilibrated with buffer B, washed with buffer B, and eluted using a gradient of 0–1.5 *M* NaCl (in buffer B).

Affinity chromatography of Ab subunits on ATP-Sepharose was performed after sIgA dissociation into single subunits (1 mL, 0.5 mg/mL) for 1 h at 25°C in 0.2 *M* dithiothreitol (DTT), 10 m*M* Tris-HCl, pH 6.8, and 1.0 m*M* $MgCl_2$, as previously described for creatine kinase *(26)* and for sIgA *(23)*. Urea was then added to 6.0 *M*, and, after 15 min of additional incubation, the mixture was applied to an ATP-Sepharose column (5 mL) equilibrated with buffer A containing 4.0 *M* urea and 1.0 m*M* DTT. Elution was in buffer A containing 1.0 m*M* DTT or 1.0 m*M* DTT and 3.0 *M* NaCl. The light (L) and heavy (H) chains of sIgA were identified by SDS-PAGE *(27)* and by immunoblotting *(28)*, as described below (*see* SDS-PAGE and Western Blotting).

Gel Filtration of Antibodies

Gel filtration of sIgA on a TSK HW-55 column (0.8 × 180 mm) was performed after incubation (0.4–1.0 mg/mL, 0.2 mL) for 1 h at 30°C in the following conditions: (1) 0.1 glycine buffer, pH 2.4, 0.2 *M* NaCl; (2) 5 *M* sodium thiocyanate, 50 m*M* Tris-HCl, pH 7.0, 0.2 *M* NaCl; (3) 0.1 *M* NaOH, 0.2 *M* NaCl. The column was equilibrated with the same buffer, except for condition (3), in which case, 0.1 *M* phosphate buffer, pH 10.5, was used (the incu-

bation and gel filtration of sIgA using 0.1 *M* NaOH led to partial fragmentation of the Abs). Fractions were collected and dialyzed against 50 m*M* Tris-HCl buffer, pH 7.0, and their protein kinase activity was measured.

Preparation of Fab Fragment

The homogeneous Fab fragment of sIgA was prepared by papain digestion *(29)* of sIgA purified on casein-Sepharose and sequential chromatography on protein A-Sepharose, DEAE-cellulose, and anti-IgA-Sepharose, as described in Chromatographic Purification of sIgA. The yield was about 20%.

SDS-PAGE and Western Blotting

Nonreducing SDS-PAGE was done in 3–25% gradient gels containing 0.1% SDS, 8 *M* urea, and no 2-mercaptoethanol. Reducing 12% gels contained 0.1% 2-mercaptoethanol and no urea. Samples were boiled for 5 min in 50 m*M* Tris-HCl buffer, 2% SDS, supplemented with 8 *M* urea or 1% 2-mercaptoethanol for nonreducing and reducing gels, respectively.

Two-dimensional electrophoresis of human sIgA possessing protein kinase activity was performed as described in ref. *30*, and polypeptides were revealed by silver-staining *(27)* and by Western blotting on a nitrocellulose membrane *(28)*.

The transfer of proteins after their separation in SDS-polyacrylamide gels to a nitrocellulose membrane was performed by a procedure described previously *(28)*. The membrane was incubated with specific anti-L- or anti-H-chain Abs conjugated to alkaline phosphatase for 2 h at 37°C, washed 5× with 50 m*M* sodium phosphate buffer, pH 7.5, 0.2 *M* NaCl, and proteins and Abs were detected by amido black and 1-naphthyl phosphate and Fast Blue BB, respectively.

Protein Kinase Activity

Conditions for phosphorylation of casein were optimized by varying the concentrations of all reagents *(23)*. The reaction mixtures (10–100 μL) contained 50 m*M* Tris-HCl, pH 6.8, 1 m*M* $MgCl_2$, 0.1 m*M* EDTA, 50–100 m*M* NaCl, 8 mg/mL casein, and various concentrations of γ-[^{32}P]ATP (optimal concentration 0.1 m*M*).

To analyze the protein kinase activity of sIgA, two approaches were used: ^{32}P-label accumulation in the acid-insoluble fraction, and SDS-PAGE followed by autoradiography *(22,23)*.

For screening the fractions during the purification of sIgA, 5 μL of each fraction was incubated in 20 μL standard reaction mixture containing 10 n*M* γ-[^{32}P]ATP (1×10^5 Mbq/mmol) for 5–20 min at 37°C, and 5–10-μL

aliquots of reaction mixture were applied to Whatman 3MM paper disks presoaked in 5% trichloroacetic acid (TCA). The disks were washed 8× in 5% TCA and once in acetone, and radioactivity was measured by Cherenkov counting.

Specific protein kinase activity of purified Abs was analyzed by ^{32}P-label accumulation in the acid-insoluble fractions, and by SDS-PAGE and autoradiography *(23)*, using the standard reaction mixture containing 0.01–0.03 mg/mL of sIgA and 0.1 m*M* γ-[^{32}P]ATP (1 x 10^2 Mbq/mmol).

The substrate specificity of sIgA was analyzed using optimal concentrations of standard components (*see* above), 10 n*M* γ-[^{32}P]ATP, 10 n*M* γ-[^{32}P]NTP or 10 n*M* γ-[^{32}P]dNTP of the same specific activity as γ-[^{32}P]ATP (1 x 1^{05} Mbq/mmol) and 0.5 mg/mL bovine casein.

Affinity Labeling of sIgA

Many kinases can be affinity-modified by a 2′,3′-dialdehyde derivative of ATP produced by periodate oxidation (oxATP) *(23,26,31)*. Affinity modification of sIgA with α-[^{32}P]oxATP, prepared by oxidation of α-[^{32}P]ATP with $NaIO_4$ according to Nevinsky et al. *(31)*, was carried out in a reaction mixture (0.1 mL) containing 0.5 mg/mL catalytic sIgA, 5 m*M* imidazole-acetate buffer (pH 7.6), 1 m*M* $MgCl_2$, and 10 μ*M* α-[^{32}P]oxATP (500 cpm/nmol). After incubation for 1 h at 30°C, 1 μL of 0.1 m*M* sodium borohydride was added to reduce Schiff's bases, and the mixture was further incubated for 1 h. Incorporation of [^{32}P]oxATP into sIgA subunits was analyzed by SDS-PAGE and visualized by autoradiography.

Analysis of Milk Protein Phosphorylation

Phosphorylation of milk proteins was performed at 37°C for 30 min in the standard reaction mixture (60 μL, *see* Protein Kinase Activity) containing standard components, 10 n*M* γ-[^{32}P]ATP and 50 μL fresh milk *(32)*. In control experiments, phosphorylation of milk proteins was examined in the presence of homogeneous IgG (0.2 mg/mL) purified by affinity chromatography of maternal sera (seven mothers served as the donors of milk and sera). Affinity chromatography of the serum proteins on immobilized catalytic sIgA-Sepharose was done as in the case of anti-IgA-Sepharose chromatography. The catalytic sIgA was prepared using BrCN-activated Sepharose according to the protocol of Pharmacia (1 mg sIgA/mL Sepharose).

RESULTS AND DISCUSSION

sIgA was purified by affinity chromatography of milk proteins on Sepharose bearing immobilized anti-IgA Abs (Fig. 1A). The sIgA repre-

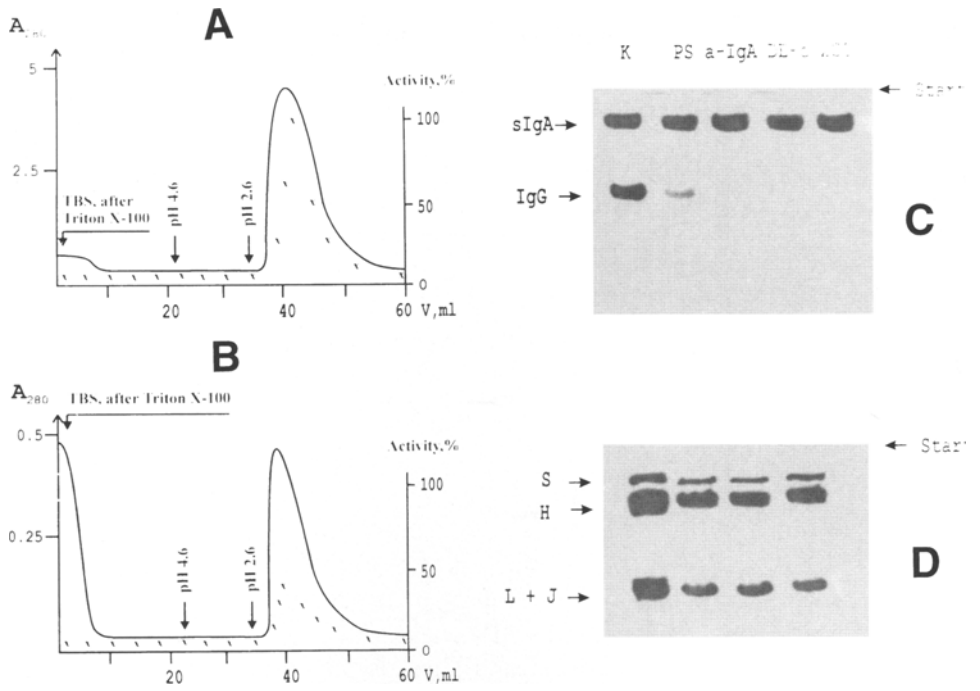

Fig. 1. Purification of human milk sIgA by sequential affinity chromatography on anti-IgA-Sepharose **(A)** and on protein A-Sepharose **(B)** (—), absorption at 280 nm; (x), relative protein kinase activity of Abs assayed by phosphorylation of casein using 5 μL of each fraction in 20 μL reaction mixture (*see* Methods), relative to the activity of the fraction having maximal activity (100%) in (A). Also shown are silver-stained SDS-PAGE analyses of the sIgA fractions in a 3–25% gradient gel in nonreducing conditions **(C)** and a 10% gel in reducing conditions **(D)** (L, light; H, heavy; S, secretory; and J chain of sIgA). Fractions were used after different purification steps of sIgA: PS, protein A-Sepharose; a-IgA, anti-IgA-Sepharose; DE-c, DEAE-cellulose; 2CS, sIgA eluted with NaCl from casein-Sepharose; lane K, control mixture of IgG and sIgA.

sents 95% the total Abs of human milk *(33)*. To eliminate other proteins that could form complexes with sIgA *(24–25)*, the column was washed sequentially with a buffer containing 1% Triton X-100, and then at pH 4.6, before elution of the sIgA in glycine-HCl buffer, pH 2.6 (Fig. 1A). sIgA purified in this manner showed no detectable bands of other proteins on SDS-PAGE (Fig. 1C). The sIgA possessed casein kinase activity (Fig. 2C), which represented 5–40% (depending on the donor; 30 donors) of the kinase activity of the original milk. The kinase activity was not lost by further chromatography on protein A-Sepharose (Fig. 1B), which binds IgA and IgM in addition to IgG *(30)*. Only 4–11% of the total sIgA was bound by this column, and only the bound fraction possessed protein kinase activity.

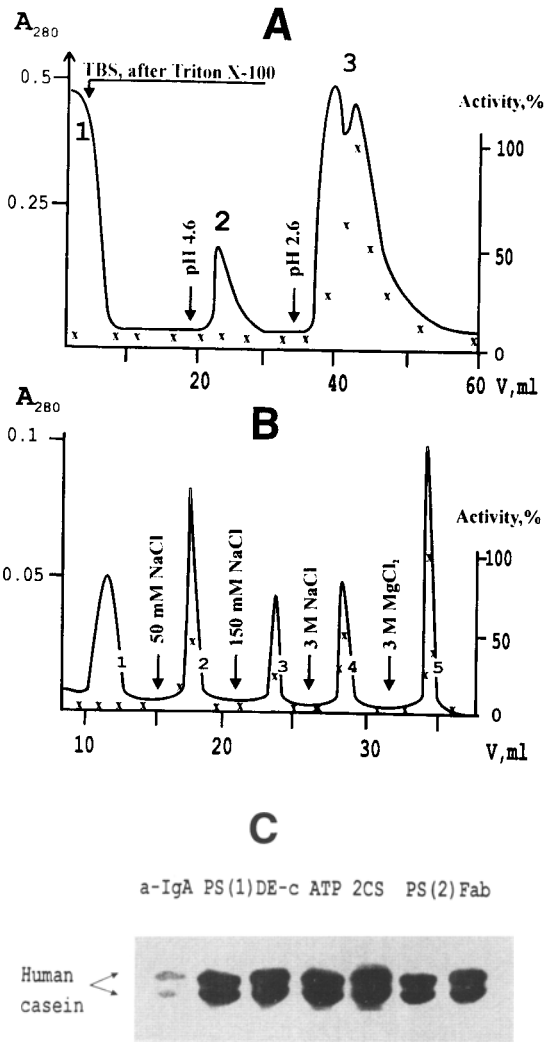

Fig. 2. Purification of human milk sIgA by affinity chromatography on protein A-Sepharose **(A)** and ATP-Sepharose **(B)** (—), absorption at 280 nm; (x), relative protein kinase activity of Abs assayed by phosphorylation of casein, as in Fig. 1. **(C)** An autoradiograph of an SDS-gel showing casein phosphorylation by sIgA fractions from (B) a-IgA, anti-IgA-Sepharose; PS(1), protein A-Sepharose; DE-c, DEAE-cellulose; ATP, peak 5 from ATP-Sepharose (Fig. 2B); 2CS, NaCl-eluted sIgA from casein-Sepharose; PS(2), protein A-Sepharose; Fab, Fab fragment of sIgA.

To confirm the casein kinase activity, the authors purified sIgA by reversing the above sequence of chromatographic steps (Fig. 2A). Protein A-Sepharose bound 4–10% of the sIgA present in milk, as shown by immunoprecipitation. About 5–40% (depending on the donor) of the kinase activity did not bind to protein A-Sepharose on a second cycle of chromatography (data not shown). The eluted protein had protein kinase

activity 10–25× higher (Fig. 2A) than sIgA purified on anti-IgA Sepharose (Fig. 1A; compare also lanes PS and a-IgA of Fig. 2C), suggesting that sIgA with kinase activity bound more strongly to protein A-Sepharose than the catalytically inactive sIgA.

sIgA so recovered was essentially completely bound (97–99%) by anti-IgA-Sepharose. The protein kinase activity of the bound sIgA (90–95%) was significantly higher than that of the unbound IgG fraction (5–10%; data not shown). Chromatography of Abs on the two adsorbents yielded homogeneous sIgA fractions with the same protein kinase activity, regardless of the sequence in which the columns were run. However, chromatography on protein A-Sepharose as the first step had the advantage of separating catalytic sIgA from inactive Abs and immediately yielding sIgA with higher protein kinase activity (Fig. 2C). It should be mentioned that, after two sequential chromatographies in any sequence, the total protein kinase activity of purified sIgA was 2–3× lower than after one chromatography. This suggests a partial inactivation of sIgA kinase activity because of exposure to acidic treatments twice during the sequential purification procedure.

In further experiments, sIgA was first purified on protein A-Sepharose and then chromatographed on DEAE-cellulose, which separated sIgA from IgG *(20)*, as shown by immunoprecipitation and gel filtration on Toyopearl HW-55 (Fig. 3C). Upon gel filtration through Sepharose 4B, 90% of the sIgA was excluded. The excluded sIgA retained protein kinase activity, showing that this activity could not be ascribed to Abs reacting with Sepharose, because the Abs would remain bound to this column.

The protein kinase activity in sIgA was bound by an affinity column of ATP-Sepharose (Fig. 2B) and was eluted at 0.15–3 *M* NaCl or at 3 *M* $MgCl_2$, conditions which should disrupt even stable immune complexes. In contrast, commercially available bovine protein kinases (Sigma) and milk-protein kinases of nonimmunoglobulin nature (which do not bind protein A-Sepharose; *see* below), were eluted at NaCl concentrations lower than 0.1 *M* (data not shown). All the fractions of sIgA eluted from ATP-Sepharose showed kinase activity when purified from human milk (Fig. 2B, peaks 2–5). Using the sIgA fraction purified from the milk of 35 donors, 70–90% of the sIgA was bound by the ATP-Sepharose, but the ratio of the different peaks depended on the donor. 70–80% of the sIgA bound to ATP-Sepharose was also bound by casein-Sepharose (Fig. 3A), and showed casein kinase activity (Fig. 3A and C).

SDS-PAGE of the sIgA in nonreducing conditions revealed a single protein band (Fig. 1C). Three bands were seen in a reducing gel, in which the L- and J-components co-migrate (Fig. 1D). All four components of the 370-kDa form of sIgA (1 S chain, 72 kDa; 4 H chain, 62 kDa; 4 L chain, 23

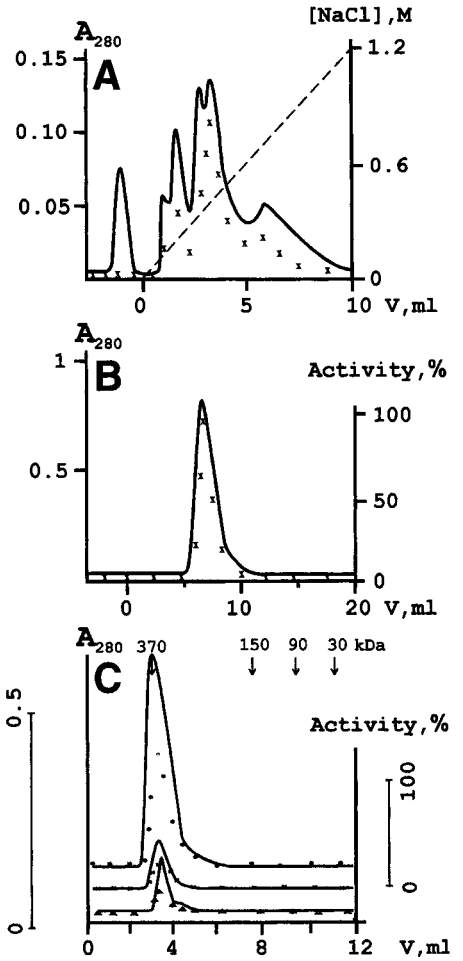

Fig. 3. Purification of human milk sIgA by affinity chromatography on casein-Sepharose **(A).** (—), absorption at 280 nm, (x), relative casein kinase activity of sIgA. Affinity chromatography of purified sIgA on anti-sIgA-Sepharose **(B).** The column was washed with TBS containing Triton X-100 and 3 *M* NaCl, and the protein was eluted with glycine-HCl buffer, pH 2.6. (—), absorption at 280 nm; (x), relative casein kinase activity of sIgA. Gel filtration of sIgA on a TSK HW-55 column **(C)** after exposure to acidic conditions (profile 1), thiocyanate (profile 2), or alkaline conditions (profile 3). (—), Absorption at 280 nm; (o, x, ▽), relative casein kinase activity of sIgA.

kDa, and 1 J chain, 23–26 kDa; *25,34,35*) were identified using two-dimensional electrophoresis (two coordinates: molecular size and isoelectric point; data not shown). The above data strongly suggest that protein kinase activity is an intrinsic property of the sIgA.

Further control experiments were performed to rule out co-purifying protein kinases. Incubation of sIgA at any stage of purification with immobilized anti-sIgA IgG led to essentially complete binding of Abs to the

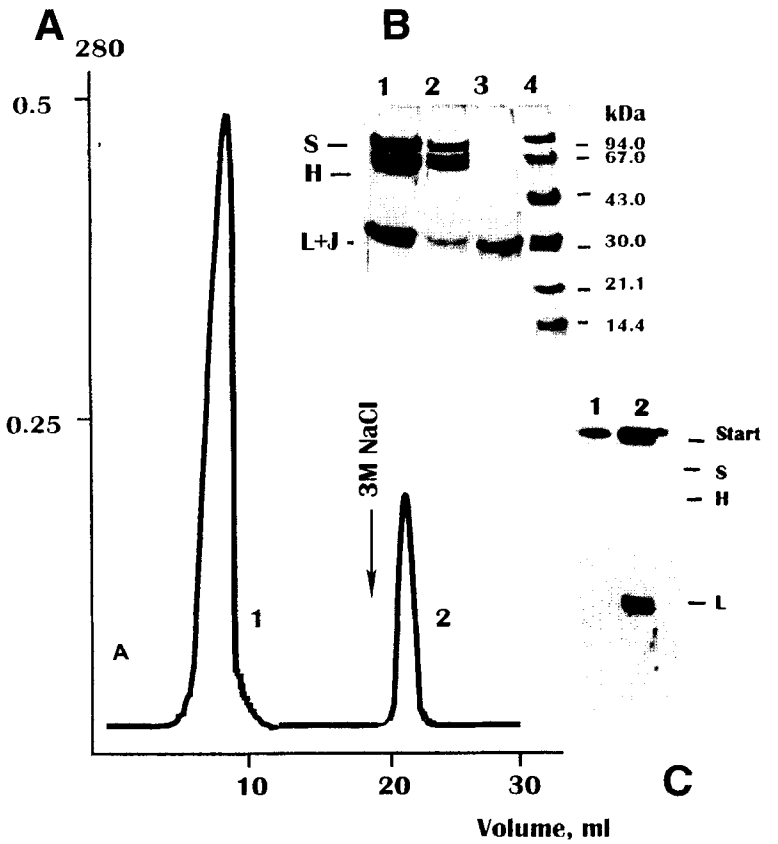

Fig. 4. Chromatography of sIgA subunits generated using DTT and urea on ATP-Sepharose **(A).** Proteins were eluted in buffer containing 1 m*M* DTT (peak 1) or 1 m*M* DTT and 3 *M* NaCl (peak 2). 12% SDS/PAGE in reducing conditions **(B)** of peak fractions from (A). Lane 1, initial nontreated sIgA; lane 2, peak 1; lane 3, peak 2; lane 4, molecular mass markers. Radioautograph of the reducing SDS gel **(C)** showing modification of sIgA subunits by the 2′, 3′-dialdehyde derivative of α-[^{32}P]ATP (lane 1, time zero; lane 2, incubated with the reagent for 1 h).

adsorbent, and to disappearance of protein kinase activity from the solution. By elution of anti-sIgA column with pH 2.6 buffer, the protein kinase activity tracked exactly with the sIgA peak (Fig. 3B), and there were no other peaks of activity.

sIgA incubated in acid, in alkali, or with thiocyanate showed only one protein peak upon gel filtration, which contained about 50–70% of the initial protein kinase activity (Fig. 3C). The kinase activity coincided exactly with the sIgA peak observed by gel filtration. Strong noncovalent protein complexes would be expected to dissociate in these conditions *(10,23).* Moreover, the homogeneous Fab fragment of sIgA showed casein kinase activity (Fig. 2C).

Following dissociation of the subunits of sIgA by DTT and urea, the L chain was bound by ATP-Sepharose (Fig. 4A, peak 2). The protein adsorbed by this affinity adsorbent (peak 2) had the electrophoretic mobility of the L chain (Fig. 4B, line 3) and reacted with Abs against L chain (data not shown).

An affinity label for ATP-binding sites, the 2′,3′-dialdehyde derivative of ATP (oxATP) *(23,26,31)*, inactivated the protein kinase activity of sIgA. The L chain was labeled covalently by α-[^{32}P]oxATP with a stoichiometry of 2.5–3.0 mol oxATP bound per mol of sIgA (Fig. 4C). ATP, at a 20-fold excess over α-[^{32}P]oxATP, essentially completely prevented both the inactivation of kinase activity and the covalent binding of oxATP (80–90%). ^{32}P-sIgA labeled with α-[^{32}P]oxATP showed a positive reaction with Abs against sIgA (data not shown). The stoichiometry of the affinity modification shows that oxATP binds covalently to the L-subunit of sIgA, and not to a co-purifying protein kinase.

The optimum reaction conditions and substrate specificity of the sIgA protein kinase differed from those of known protein kinases. Its optimum $MgCl_2$ concentration (1 m*M*) was about 10–20× lower (10–20 m*M; 36*) and its pH optimum (pH 6.8), compared with a pH value of about 8.0 for known kinases *(36)*. Catalytic sIgA did not show autophosphorylation, phosphorylation of histones, or activation by iron (Fe) ions *(23)*, which are typical properties of other protein kinases *(36)*. In contrast to other protein kinases *(36)*, sIgA transferred [^{32}P]phosphate only onto serine residues of casein *(23)*, and utilized as a substrate not only ATP, but also other NTPs and dNTPs with comparable efficiencies (Fig. 5A; ATP [100%], dATP [70–80%], GTP and dGTP [200–300%], UTP and dTTP [30–60%]).

Fractionation of sIgA on ATP-Sepharose (and casein-Sepharose) showed that it is polyclonal in origin and that different fractions have different affinities for ATP (and for casein). The authors also detected a different affinity for ATP of sIgA fractions corresponding to different sIgA peaks (Fig. 2B, peaks 2–5). The K_m values for ATP ranged from 15 μ*M* (peak 2) to 0.1 μ*M* (peak 5).

The specific activity of catalytic sIgA was 4–25 U/mg protein, depending on the donor of the milk (17 donors were tested), which is about 2–10% of the specific activities of known protein kinases *(36)*.

The authors recently compared *(32)* the protein kinase activities of catalytic sIgA and other milk proteins of a nonimmunoglobulin nature, isolated from milk using specific affinity adsorbents after removing sIgA on protein A-Sepharose. All fractions of sIgA (corresponding to peaks 2–5, Fig. 2B) were capable of phosphorylating not only casein, but also several other proteins of the milk, the spectrum of which was dependent on the sIgA fraction used *(32)*.

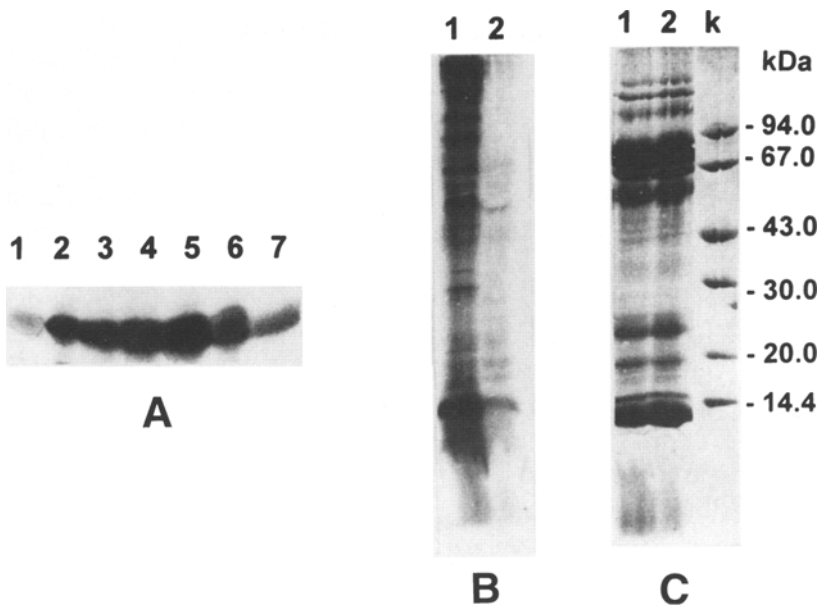

Fig. 5. **(A)** Radioautograph of reducing SDS-PAGE showing human casein phosphorylated by sIgA (casein-Sepharose purified) in the presence of various γ-[^{32}P]NTPs or γ-[^{32}P]dNTPs with the same specific activity (10^5 Mbq/mmol). dTTP (lane 1), dATP (lane 2), ATP (lane 3), GTP (lane 4), dGTP (lane 5), CTP (lane 6), UTP (lane 7). **(B)** Radioautograph of reducing SDS gel showing human milk proteins phosphorylated by protein kinases of unfractionated milk in the absence (lane 1) or presence (lane 2) of 0.2 mg/mL IgG purified from maternal sera by affinity chromatography on catalytic sIgA-Sepharose. **(C)** Silver-stained gel corresponding to (B); k, molecular size markers.

The kinases of a nonimmunoglobulin nature were found to phosphorylate casein at different sites from sIgA. Proteolysis of casein phosphorylated with non-Ab kinases yielded very small labeled polypeptides; digestion of casein phosphorylated by sIgA yielded only two labeled polypeptides of high molecular mass *(32)*.

There are several possible explanations for the catalytic function of natural Abs. The first possibility is based on the recently described induced-fit mechanism for Ag–Ab, complex formation *(38)*. Such a mechanism might lead to a conformational rearrangement in the Ag and introduce electronic strain in the scissile bond. A second possible explanation for the formation of natural abzymes is that anti-idiotypic Abs to antienzyme Abs are potential biocatalysts *(10,14)*. This idea has found experimental support *(14)*, and it presupposes the existence of a first Ab, the interaction of which, with enzymes and with corresponding anti-idiotypic Abs, should lead to inhibition of their catalytic activities.

In order to elucidate the possible presence of anti-idiotypic Abs to catalytic sIgA, the authors analyzed the interaction of sIgA-Sepharose with proteins from maternal sera. A small fraction of serum IgG was observed to bind tightly to immobilized catalytic sIgA and was eluted from the column by acidic buffer, pH 2.6 (data not shown). The electrophoretically homogeneous fraction of IgG purified from maternal sera did not possesses protein kinase activity. The serum IgG did not inhibit the phosphorylation activity of control nonimmunoglobulin nature protein kinases from bovine or from human milk (IgG:protein kinase ratio 1:1.5). However, under the same conditions, the IgG from maternal sera significantly inhibited casein phosphorylation catalyzed by catalytic sIgA (70–80%). Moreover, addition of serum IgG directly to human milk led to a significant decrease of milk protein phosphorylation (Fig. 5B). The inhibitory activity of the IgG correlated with the relative amount of protein kinases of immunoglobulin and nonimmunoglobulin nature in the different milk samples. The results speak in favor of the absence of crossreactivity of catalytic sIgA and normal milk protein kinases. In addition, the presence in human maternal sera of IgG with a high affinity for catalytic sIgA allows us to suppose that the Abs can, in principle, be of an anti-idiotypic nature.

Data reported here provides evidence that protein kinase activity is an intrinsic property of sIgA derived from milk of healthy mothers and not a result of co-purifying protein kinases. The authors could not detect Abs possessing catalytic activities in the sera of nonpregnant women *(37)*, suggesting that healthy mothers may possess an increased immune status concerning catalytic Abs.

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