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REVIEW

Enzymes Phosphorylating Lipids and Polysaccharides

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Abstract—Phosphorylation plays an important role in regulation of living functions of organisms; phosphorylation may significantly alter chemical properties of proteins, lipids, and carbohydrates. Canonical kinases catalyze transfer of terminal phosphate group from ATP (or other NTPs) to specific nucleophilic groups of proteins, lipids, and polysaccharides. Recently, unique kinases, catalytically active antibodies (abzymes) phosphorylating proteins, lipids, and polysaccharides have also been discovered. This review highlights biological functions and enzymatic characteristics of canonical kinases and abzymes phosphorylating lipids and polysaccharides.

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Lipid phosphorylation *in vivo* was originally found in 1953. Stimulation of liver cells by acetylcholine was accompanied by incorporation of [^{32}P]phosphate into one of the minor lipids of pancreatic cells. Subsequent studies revealed that the phosphorylated lipid was phosphatidyl inositol [1]. Now there is evidence that phosphorylation of phosphatidylinositol, phosphatidylinositol phosphates (phosphoinositides), and also another glycerolipid, diacylglycerol, occurs during intracellular signal transduction and represents one of its key moments.

The other known examples of lipid phosphorylation include dolichol phosphorylation during N-glycosylation of proteins in endoplasmic reticulum [2] and ceramide phosphorylation in endoplasmic reticulum and synaptosomal vesicles [3, 4]. Kinases phosphorylating phospho- and sphingolipids are the most studied mammalian lipid kinases. Some kinases phosphorylate not only lipids but also proteins and polysaccharides. There are literature data on monosaccharide kinases (first of all glycokinases) [5, 6]. Little is known about specific kinases of oligo- and polysaccharides. However, recently experimental evidence for specific phosphorylation of lipids and oligo- and polysaccharides by human IgG and sIgA appeared. In this review, we summarize current knowledge on known kinases of lipids and oligo- and polysaccharides.

Phosphatidylinositol-3 kinases (PtdIns-3-kinases). PtdIns-3-kinases are some of the most important regulatory proteins, which are localized at the "crossroad" of various signaling pathways and involved in control of key cell functions. PtdIns-3-kinases exhibit dual enzymatic activity (lipid and protein kinase). They can activate signaling proteins, including oncoproteins, and this feature determines the principal importance of lipid kinases for regulation of such important cell processes as growth, survival, aging and malignant transformation [7]. Figure 1 shows the structural formula of phosphatidylinositol and phosphorylation sites in its molecule. PtdIns-3-kinases phosphorylate the D-3 hydroxyl group of the inositol moiety of phosphoinositide(s). Seven catalytic and five regulatory subunits of PtdIns-3-kinase have been found in mammalian cells. Originally, PtdIns-3-kinase activity was found in mammalian cells and later in other eukaryotic cells. Use of a specific inhibitor of PtdIns-3-kinase, wortmannin, revealed the presence of PtdIns-3-kinase activity in mammary gland secretory cells [8].

There are four major classes constituting the PtdIns-3-kinase superfamily; they differ in amino acid sequences of the catalytic domain [9]. Twelve members of this superfamily have been subdivided into two large groups. The first group consists of lipid kinases exhibiting lipid and

Abbreviations: Ab) antibody; AID) autoimmune diseases; PtdIn) phosphatidyl inositol; PtdIns-3-kinases) phosphatidyl inositol-3-kinases; PtdIns-4-kinases) phosphatidyl inositol-4-kinases; PtdIns-(4,5)P_2) phosphatidyl inositol-4,5-bisphosphate; PtdIns-(3,4)P_2) phosphatidyl inositol-3,4-bisphosphate; PtdIns-4P-5-kinase) phosphatidyl inositol-4-phosphate-5-kinases; PtdIns-4-P) phosphatidyl inositol-4-phosphate; S-1-P) sphingosine-1-phosphate; SLE) systemic lupus erythematosus; TLC) thin layer chromatography.

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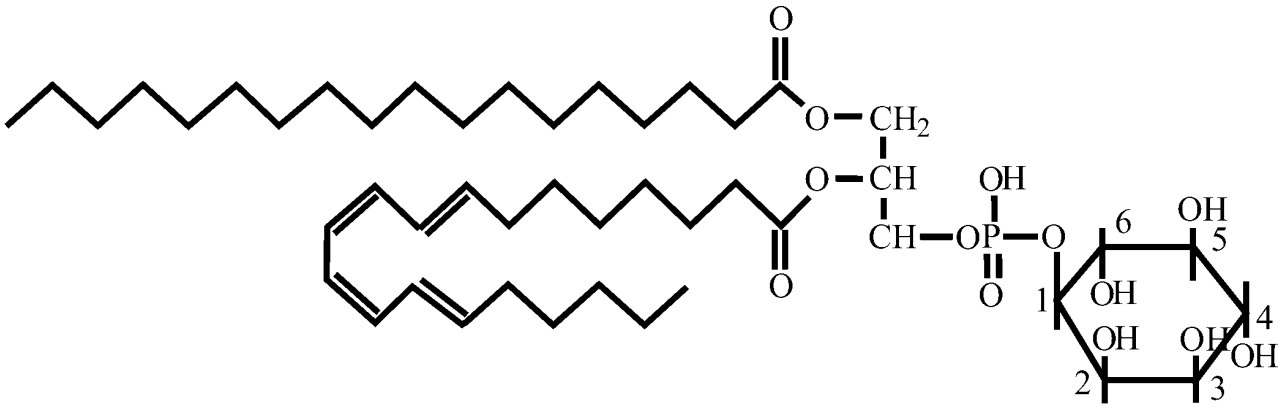

Fig. 1. Structure of phosphatidylinositol (PtdIns). Numbers indicate positions of carbon atoms of the inositol moiety. PtdIns-3-kinases phosphorylate phosphoinositides at the D-3 hydroxyl group of the inositol moiety with formation of PtdIns-3-P; PtdIns-4-kinases and PtdIns-4P-5-kinases phosphorylate phosphoinositides at the D-4 and D-5 hydroxyl groups of the inositol moiety with formation of PtdIns-4-P and PtdIns-(4,5)P_2, respectively.

protein kinase activities; these are PtdIns-3-kinases class I-III. The second group includes PtdIns-3-kinases class IV; these enzymes exhibit only protein kinase activity.

PtdIns-3-kinase is a cytoplasmic protein that consists of two subunits: p85 (regulatory) and p110 (catalytic) [10]. Figure 2 schematically shows the structure of the p85/p110 heterodimer. Interaction of the regulatory p85 subunit is responsible for stimulation of PtdIns-3-kinase activity. The catalytic p110 subunit is positioned at C-end, which is highly conservative and homologous in all PtdIns-3- and PtdIns-4-kinases and shares similarity with catalytic domains of protein kinases. This subunit simultaneously exhibits serine/threonine protein kinase and phosphoinositide kinase activities [11]. It is suggested that interaction of the regulatory p85-subunit with receptor tyrosine kinases and the catalytic p110 subunit is responsible for p110 transportation to cell membranes, where complex formation between the enzyme and a phospholipid substrate occurs [7, 12].

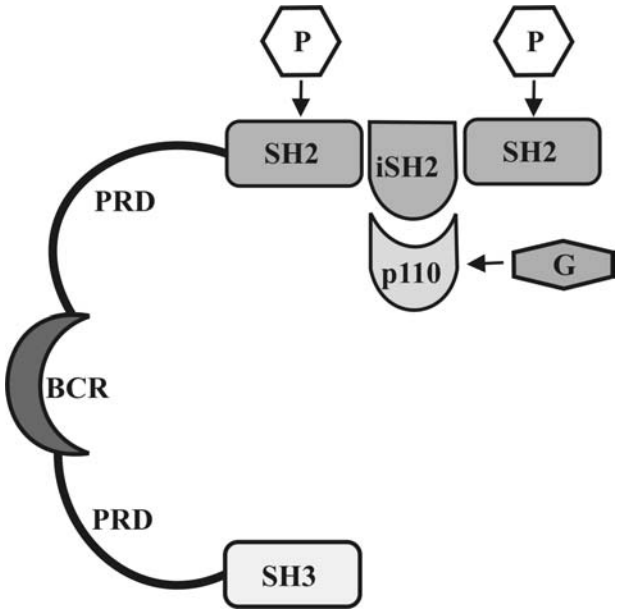

Fig. 2. Schematic structure of p85/p110 heterodimer. The regulatory p85 subunit contains (starting at the N-end) SH3-domain, two proline rich-domains (PRD) separated by BCR homologous domain, and two SH2 domains separated by iSH2 (inter SH2) site involved in binding to catalytic p110 subunit. Arrows indicate positions of heterodimer binding with the main PI3K activators: phosphotyrosine proteins (P) and small G-proteins (G).

In vitro p85 subunit binds to the polypeptide sequence phosphoryl-Tyr-X-X-Met; the presence of phosphotyrosine residue is essential for binding [13]. *In vivo* p85 subunit binds with activated membrane receptors (e.g. platelet derived growth factor receptor, insulin receptor), which contain similar amino acid sequences. Binding of p85 subunit with a receptor and p110 subunit results in formation of the active PtdIns-3-kinase complex. Experiments *in vitro* have demonstrated that the p110 subunit can also exhibit catalytic properties, but it is unable to bind to phosphorylated receptors. Thus, appearance of PtdIns-3-kinase activity during cell stimulation apparently involves transfer of the enzyme complex to plasma membrane where its substrate is located.

According to a known classification [14], this type of PtdIns-3-kinases belongs to class I. This is the most studied class (compared with other representatives of the superfamily of PtdIns-3-kinase). Experiments *in vitro* have demonstrated that enzymes of this class phosphorylate PtdIns, PtdIns-4P, and PtdIns-(4,5)P_2 followed by formation of PtdIns-3P, PtdIns-(3,4)P_2, and PtdIns-(3,4,5)P_3, respectively. Class I PtdIns-3-kinases are subdivided into subclasses IA and IB by activation mechanism [9, 14, 15].

Heterodimeric proteins of subclass type IA comprise catalytic (110 kD) and regulatory (55-85 kD) subunits. The human genome encodes three types of catalytic (p110α, p110β, and p110δ) and regulatory (p85α, p85β, p55γ) subunits of enzymes of this enzyme [9]. Subclass IA enzymes can be activated by tyrosine kinases and by GTP-dependent forms of Ras-proteins, which are involved in stimulation of cell division by growth factors [14].

Subclass IB has only one member, containing catalytic (p110γ) and regulatory (101 kD) subunits. In contrast to p85 subunit, the p101 subunit contains signaling modules. This enzyme is activated by G-proteins (Gβγ) and Ras-proteins [14, 16].

Class II enzymes are large proteins of molecular mass of 170-220 kD. *In vitro* they catalyze phosphorylation of PtdIns and PtdIns-4-P, but not of PtdIns-(4,5)P_2. However, certain evidence exists that PtdIns-(4,5)P_2 is phosphorylated in the presence of phosphatidylserine; this suggests importance of lipid environment for manifestation of the enzyme activity [17]. It is possible that in cells the enzymes of this class also phosphorylate PtdIns-(4,5)P_2. Three types of PtdIns-3-kinases are known within class II. These include PtdIns-3-kinases C2α and PtdIns-3-kinases C2β; they are widely distributed in mammalian tissues, whereas PtdIns-3-kinases C2γ have been found only in liver, mammary gland, and prostate. The class II enzymes (as well as class I enzymes) are involved in numerous receptor mediated signaling such as integrin and insulin pathways and also in epidermal growth factor signaling pathways [9].

The class III PtdIns-3-kinase can phosphorylate only PtdIns [18]. This class includes the PtdIns-3-kinase possessing additional specific functions of protein transporter; it shares similarity in structure and functions with vps34p (vacuolar protein sorting), the yeast analog of PtdIns-3-kinase [19]. Vps34p is involved in distribution of proteins in yeast vacuoles; the same role is also suggested for mammalian PtdIns-3-kinase class III.

The next class of PtdIns-3-kinases, known as class IV, includes proteins exhibiting only protein kinase activity. Nevertheless, they are reasonably included into the class of lipid kinases for several reasons: i) these enzymes are sensitive to widely used inhibitors of PtdIns-3-kinase (wortmannin and LY 294002); ii) the structure of their catalytic domain shares similarity with PtdIns-3-kinases of other classes; iii) like other PtdIns-3-kinases, they play a crucial role in pathogenesis and the development of various diseases such as autoimmune diseases (AID) and tumor processes including breast cancer [9].

PtdIns-3-kinases are important regulatory proteins positioned at the "crossroad"; they control such key processes as cell division (signaling initiated by growth factors) and apoptosis (prevention of its development). PtdIns-3-kinases are especially important in tumor cell transformation. They not only exhibit intrinsic oncogenic activity, but also form complexes with some viral and cell oncoproteins (src, ras, rac, alb, T-antigen); realization of their transforming potential requires obligate presence of PtdIns-3-kinases in the cell. The anti-apoptotic activity of PtdIns-3-kinases is also related to the increased tolerance of tumor cells to chemo- and radiotherapy [15, 16].

PtdIns-4-kinases. These enzymes phosphorylate the hydroxyl group at D-4 of the inositol moiety. Two types of these enzymes are known (type 2 and type 3); they are distinguished by sensitivity to inhibition by adenosine and nonionic detergents. These enzymes phosphorylate only PtdIns with formation of PtdIns-4-P, the major precursor for synthesis of other PtdIns: PtdIns-(4,5)P_2, PtdIns-(3,4)P_2, and PtdIns-(3,4,5)P_3 [20].

The PtdIns-4-kinases type 2 (55 kD) have been isolated from human erythrocyte plasma membrane, pig liver, and cow uterus; these enzymes might be present in all animal tissues. These enzymes exist in two forms, PtdIns-4-kinase 2α and PtdIns-4-kinase 2β. These isoforms differ in the structure of catalytic domain, which is similar to the structures of domains of type 3 PtdIns-3-kinase and PtdIns-4-kinase, respectively. PtdIns-4-kinases 2α are tightly associated with membrane structures of Golgi apparatus and other intracellular compartments. PtdIns-4-kinases 2β are preferential cytosolic proteins that are bound to membranes, activated by small GTPases, and involved in regulation of various intracellular proteins [20].

Mammalian type 3 PtdIns-4-kinases are also subdivided into two isoforms, PtdIns-4-kinase 3α (220 kD) found in plasma membrane and endoplasmic reticulum and PtdIns-4-kinase 3β (110 kD) found in Golgi apparatus. These enzymes are required for regulation of protein transportation from Golgi apparatus to plasma membrane [20, 21]. Studies of PtdIns-4-kinase structure revealed that these enzymes contain highly conservative catalytic domain at the C-end similar to that of the catalytic domain of PtdIns-3-kinases and protein kinases. For example, some amino acid residues essential for catalysis of the kinase reactions are invariant in lipid and protein kinases [21]. Moreover, some PtdIns-3-kinases also exhibit Ser/Thr-protein kinase activity [19]. Thus, lipid and protein kinases may share common origin and have acquired the observed differences during divergent evolution.

Under mitogenic stimulation, PtdIns-4-kinases are associated with other proteins in cells. Cell stimulation with epidermal growth factor causes formation of complex containing receptor tyrosine kinase, PtdIns-4-kinase, PtdIns-5(P)-kinases, phosphoinositide transfer protein, and phospholipase Cγ [21]. Regulation PtdIns-4-kinase activity still requires detailed study.

PtdIns-4P-5-kinases. These enzymes catalyze the reaction of PtdIns-4-P phosphorylation at the D-5 hydroxyl group of inositol forming PtdIns-(4,5)P_2. Several isoforms have been isolated from various tissues.

These include the type 1 enzymes (68 kD) and type 2 (53 kD) isolated from human erythrocytes [22]. The type 1 enzymes are mainly located in plasma membrane, whereas the type 2 enzymes are located in cytoplasm. The type 2 enzymes are activated by phosphatidylserine and sensitive to inhibition by PtdIns-(4,5)P_2; they cannot phosphorylate cell membrane PtdIns-4-P. Inhibition studies employing antibodies revealed that erythrocyte PtdIns-4P-5-kinase activity is mainly determined by the type 1 enzymes.

The family of type 1 PtdIns-4P-5-kinases consists of three isoforms: PtdIns-4P-5-kinase α, PtdIns-4P-5-kinase β, and PtdIns-4P-5-kinase γ. The latter enzyme (PtdIns-4P-5-kinase γ) exists as two spicing variants migrating during SDS-PAGE in the regions of 87 and 90 kD [23]. The amino acid sequence of this isoform shares 80% identity with the sequences of α- and β-isoforms. Among these three isoforms, the β-isoform is characterized by higher V_{max} values than PtdIns-4-kinase and the γ-isoform exhibits higher affinity to phosphatidic acid. Analysis of deletion mutants of all three isoforms has shown that about 300 residues are minimally required for kinase activity. Overexpression of both isoforms (β and γ) induces growth of short actin bundles and reduces tension of actin bundles in the cell [23].

Sphingolipid kinases. Besides the phosphoinositide cycle, there is a sphingomyelin cycle, which also contributes to formation of biologically active lipid mediators. Sphingolipids, products of the sphingomyelin cycle, represent one of the most structurally and functionally diverse class of lipids. They influence structural features of biological membranes and lipoproteins; sphingolipids act as membrane anchors for some proteins, and they are also involved in cell recognition, control of cell growth and differentiation, reception of hormones, toxins, growth factors, and cytokines; sphingolipids also exhibit immunomodulating properties [24]. Sphingolipids are traditionally considered as structural components of cell membranes. Their metabolism results in formation of ceramide, sphingosine, and sphingosine-1-phosphate (S-1-P) involved in regulation of various cell processes.

Ceramide kinase was originally identified in brain tissue [25]. Ceramide phosphorylation by ceramide kinase results in formation of ceramide-1-phosphate [26]. Ceramide hydrolysis catalyzed by ceramidases results in release of sphingosine, which undergoes phosphorylation by sphingosine kinase to S-1-P, an intracellular messenger involved in signaling of growth factors (epidermal growth factor, platelet derived growth factor, and nerve growth factor). In some cells, S-1-P acts as a Ca^{2+}-mobilizing agent [27]. Ceramide kinases regulate cAMP level. Sphingosine kinases are activated by protein kinase C, which prevents ceramide-mediated apoptosis. It has been suggested [27] that the antiapoptotic effect of protein kinase C is mediated by S-1-P. Thus, ceramide metabolite (S-1-P) plays an important role in cell proliferation and survival. Moreover, S-1-P protects cells against apoptosis induced during accumulation of ceramide. Thus, sphingosine kinases play important roles in cell growth, differentiation, and death by regulating S-1-P level [28, 29]. Both enzymes are membrane-associated proteins. Their properties have been described in several studies [28, 30-33] (Table 1).

Table 1. Comparison of characteristics of ceramide- and sphingosine kinases

Characteristics	Ceramide kinase	Sphingosine kinase
Localization	brain synaptic vesicles [3]	lung, spleen, brain, kidney, lymph nodes [25]
pH optimum	6.5 [30]	no data
Substrate	ceramide, dihydroceramide [25]	D-erythro-sphingosine [25]
K_m	for ceramide, 187 μM; for ATP, 32 μM [30]	for D-erythro-sphingosine, 3.4-15.6 μM [31]; for ATP, 93 μM [32]
Metal-dependence	activated by Ca^{2+} and Mg^{2+} [25]	activated by Ca^{2+} [33]

The biological role of various phosphorylated oligo- and polysaccharides is well described in the literature. Monosaccharide kinases are not considered in this review. We failed to find any data on kinases specifically phosphorylating oligo- and polysaccharides.

Certain experimental evidence exists [34, 35] that besides lipid kinases there are some catalytically active antibodies (abzymes) in human milk of healthy parturient women; these abzymes may phosphorylate not only lipids, but also polysaccharides. Below we consider structure, functions, and substrate specificity of the unique abzymes exhibiting lipid and polysaccharide activities and possible pathways of formation of such abzymes in the human body.

Catalytically active antibodies. Mechanisms of immune response are related with production of immunoglobulins; these are characterized by high affinity specific binding of all natural and artificial molecules (antigens) [36]. Antibodies recognize various antigens, and this explains their extreme diversity reaching 10^8-10^{10} of various Ig variants. Five classes of Ig have been found in higher mammals: IgG, IgM, IgA, IgD, and IgE. They differ in biological role, sizes of molecules, amino acid composition, and type and glycosylation level. Theoretically, the human immune system can produce up to 10^6 variants of various antibodies against the same antigen. However, in reality the number of antibodies against the same antigen is significantly lower.

The main function of antibodies was traditionally associated with neutralization of various antigens by their binding. In 1948, L. Pauling noted high similarity between complementary antigen–antibody interaction and enzyme interaction with transition state of substrate. It was suggested that the possibility of antibody generation against such transition states of substrates would result in development of antibodies exhibiting catalytic activity [37]. In 1969, Jencks suggested the possibility of preparation of abzymes as antibodies against stable analogs of transition states [38]. This hypothesis was experimentally confirmed by two research groups in 1986: by Tramontano et al. [39] and by Pollack et al. [40]. For generation of catalytically active antibodies they used haptens simulating transition states of substrates in hydrolysis reaction and hybridoma technology. Such antibodies were called abzymes (abbreviation of antibody enzymes). Later it was demonstrated that abzyme formation could follow the scheme of the antiidiotypic network proposed by Jerne [41]. According to the Jerne's theory (Fig. 3), secondary antibody (Ab2), antiidiotypic Ab against the antigen-binding center of IgG recognizing active site of some enzyme, may exhibit similar activity. Indeed, there is evidence that such network of idiotype–antiidiotypes exists in animals and man. In blood serum of experimental animals, a testable titer of Ab4 was detected.

Use of transition state analogs and hybridoma technology resulted in development of catalytic antibodies catalyzing more than 100 various chemical reactions [42]. Recent development of technologies for preparation of inducible antibodies caused rapid development of not only direct methods for generation of abzymes with certain properties, but also improvement of their properties by directed modification of the antigen-binding site of monoclonal antibodies at the level of their genes using site-directed mutagenesis or selective chemical modification. This resulted in generation of abzymes exhibiting higher substrate specificity compared with corresponding enzymes. Some antibodies are (comparable or) even more effective catalysts of chemical reactions than corresponding enzymes catalyzing similar reactions. Moreover, there are abzymes that lack analogs among natural enzymes. Thus, the design of antibodies with certain catalytic specificity may have numerous applications in biotechnology and medicine. One of the promising directions is generation of abzymes catalyzing rapid cleavage of real and potentially hazardous chemicals including various venoms and drugs [42].

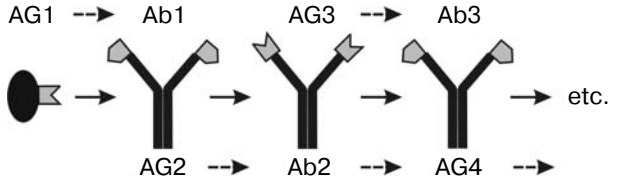

Fig. 3. Scheme illustrating formation of idiotypic antibodies Ab1 (primary) against the active site of an enzyme, acting as the antigen, and subsequent formation of antiidiotypic Ab2 (secondary) and the development of this chain of idiotype–antiidiotypes according to Jerne's theory.

Natural abzymes in autoimmune diseases. Autoimmune and viral diseases are characterized by increased apoptosis, which causes increased blood concentrations of native and partially denatured proteins, DNA, RNA, polysaccharides, lipids, and other cell components and their complexes. IgG hydrolyzing vasoactive peptide was the first example of natural abzymes, which were isolated from blood plasma of patients with bronchial asthma [43]. Subsequent studies revealed the presence of IgG exhibiting DNase [44] and RNase [45] activities in blood plasma of patients with systemic lupus erythematosus (SLE). Now IgG and/or IgM antibodies hydrolyzing DNA, RNA, and polysaccharides have been isolated from blood plasma of patients with various autoimmune diseases: SLE, Hashimoto's thyroiditis, polyarthritis, multiple sclerosis, lymphoproliferative diseases, polyneuritis, malignant tumors, and also viral hepatitis and AIDS [46-51]. There are abzymes hydrolyzing autoantigenic proteins: thyroglobulin (Hashimoto thyroiditis and rheumatoid arthritis) [52, 53], prothrombin (multiple myeloma) [54], protein coagulation factor VIII (hemophilia) [55], basic myelin protein (multiple sclerosis), IgG, IgM, and IgA [56-58].

These abzymes may have different origin. Autoimmune diseases may be accompanied by Ab production against some molecules that can simulate the transition state, and this would definitely cause appearance of abzymes. This pathway of natural abzyme generation has been proposed for appearance of anti-DNA Ab exhibiting DNase activity in SLE patients [51]. Other authors [59] suggest antiidiotypic nature of DNA-hydrolyzing Ab seen in SLE; they are antiidiotype to topoisomerase I (in some cases blood serum of such patients contains Ab against this enzyme). Certain evidence exists that DNA-hydrolyzing Ab are antiidiotype to DNase I [60]. In various autoimmune diseases, abzymes usually represent a complex "cocktail" of primary Ab against substrates mimicking transition state and secondary (antiidiotypic) Ab against active sites of various enzymes [46-51].

Natural abzymes of human milk. According to modern viewpoints, the presence of abzymes in blood is a clear sign of autoimmune processes in the human body [46-51]. So, the existence of natural abzymes in healthy subjects (without any signs of immune status impairments) was considered as impossible situation. Nevertheless, sIgA exhibiting protein kinase activity have initially been isolated from milk of healthy women [61-64]. Later it was demonstrated that small subfractions of polyclonal IgG and sIgA from human milk catalyzed

hydrolysis of DNA, RNA [65-67], ribo- and deoxyribo-NMP, -NDP, and -NTP [68, 69], oligo- and polysaccharides [70], and proteins [71]. Interestingly, all abzymes of human milk usually exhibit higher catalytic activities than known abzymes isolated from blood of patients with autoimmune diseases [46-51].

During pregnancy and in the beginning of lactation, the female organism is characterized by a special immune status; pregnancy initiates temporal appearance of various autoimmune reactions in women [46-51, 72]. However, in contrast to typical patients with autoimmune diseases, disappearance of symptoms typical for the autoimmune diseases in women during the later parturient period is a normal situation; nevertheless, in some cases the "temporal activation of autoimmune processes" is smoothly or sharply (via so-called "autoimmune shock") transformed into typical chronic autoimmune process. According to our viewpoint, rearrangement of the immune system of a pregnant woman involves "triggering" of special immune memory, which accumulates information about hazardous environmental factors surrounding the pregnant woman [46-51, 72]. This information is partially "utilized" by the immune system during pregnancy and mainly right after onset of lactation. Thus, immunization with various antigens within 1-3 months (but nor earlier) before delivery results in appearance of high concentrations of Ab to these antigens in milk [73, 74]. The pathway of antigen penetration into the mammalian organism (injection into blood or oral administration) or its nature (component of food, bacteria or viruses) is not essential.

The time-course of changes of abzyme activity in blood of pregnant and lactating women are important for understanding of a special role of the initial step of lactation for "immune system switch" in pregnant women. There is a tendency to increase in Ab activities hydrolyzing DNA and ATP during the first and/or the third trimesters of pregnancy [69, 72]. In women relative abzyme activities in blood increases by 4-5 times right after onset of lactation. However, abzyme activity in the milk of the parturient women was ~5-600 times higher than in their blood [69, 72]. The level of DNase activity of IgG and IgM from blood of pregnant women with marked pregnancy-induced autoimmune thyroiditis was 4-5 times higher than in healthy women [72]. It should be noted that viral or allergic diseases during pregnancy caused dramatic increase (by several orders of magnitude) of the levels of DNA- and RNA-hydrolyzing abzymes in women's milk [62, 63]. Specific pre-immunization of the female organism during pregnancy may cause "programming" of the immune system for production of not only Ab (which bind antigens) but also specific abzymes playing an important role in protection of newborn babies against hazardous environmental factors [46-51, 72].

Milk abzymes exhibiting kinase activities. The discovery of human milk abzymes exhibiting protein kinase activity was the first demonstration of the presence of catalytically active antibodies in healthy donors lacking any signs of autoimmune pathology [62-64]. sIgA with protein kinase activity was the first example demonstrating the existence of abzymes catalyzing a bi-substrate synthetic reaction (of new bond formation) rather than substrate degradation [62-64].

The authors of [62-67] developed methods for isolation of electrophoretically homogenous preparations of polyclonal IgG and sIgA antibodies from milk of parturient women. SDS-PAGE analysis of ^{32}P incorporation into casein catalyzed by abzymes in the presence of [γ-^{32}P]ATP revealed that the label was incorporated not only into protein, but also into some non-peptide compounds which were not stained with Coomassie and formed high radioactive background of the gel (Fig. 4) [34, 35, 75]. Extraction with chloroform–methanol mixture usually used for lipid extraction from a protein fraction removed the major proportion of non-protein bound radioactivity (Fig. 4). Analysis of extracted products by TLC and the system used for phospholipid separation revealed several ^{32}P-labeled compounds; two compounds (of R_f = 0.83 and 0.66) represented major proportion of the extracted radioactivity (Fig. 4c) [34, 75, 76]. Later it was demonstrated that two compounds denominated as L1 and L2 are ganglioside lipids [75, 76]. Figure 4 shows that after lipid removal, the reaction mixture still contained radiolabeled non-peptide compounds; their electrophoretic mobility was comparable to mobility of proteins with molecular masses 4-12 kD. Casein was removed from this mixture by trichloric acid precipitation and substances remaining in the solution were resistant to proteases, DNases, RNases, but they were sensitive to hydrolysis by some polysaccharide hydrolyzing enzymes [35]. These substances were oligo- and polysaccharides, which were then analyzed TLC (Fig. 4, d and e) and electrophoresis in 20% polyacrylamide gel, respectively.

The total content of lipids in milk is about 3-5%; lipids are present as fat droplets or milk fat globules surrounded by plasma membrane [77]. This reflects apocrine mode of mammary gland cell secretion. During such secretion, fat droplets are covered by apical membrane fragments of the secreted cells and resultant human milk fat globules (HMFG) are secreted into mammary gland ducts. The nucleus of such HMFGs is mainly formed by triglycerides, the main lipids of milk (98%), which play a major role in energy metabolism. Other components of milk lipids are phospholipids (0.81-1%) and sterols, particularly cholesterol (0.3-0.4%). The lipid fraction also contains desmosterol (a cholesterol-like compound) [77]. Sphingomyelin is the major milk phospholipid; it influences growth and development of tissues of newborn infants by regulating proliferation and differentiation of cells. Milk also contains neutral glycolipids, which belong to a family of gangliosides (glycosphingolipids). Their total content in milk is 11 mg/liter. The major compo-

nents of milk gangliosides are monosialogangliosides 3 (GM3) and disialogangliosides 3 (GD3) and also monosialogangliosides 1 (GM1) and highly polar gangliosides, which play an important role in physiology of newborns [77].

The lipids L1 and L2 phosphorylated by both sIgA and IgG were characterized by exceptionally high R_f values during TLC in various systems used for analysis of phospholipids [75, 76]. It was shown that these lipids as well as previously described milk GM1, GM3, and GD3 [77] may be hydrolyzed by neuraminidase and contain one sialic acid residue [75, 76]. However, in contrast to these gangliosides, L1 and L2 lipids are resistant to oxidation by $NaIO_4$ and, therefore, they lack free *cis*-diol groups. One of these lipids contains four fatty acid residues, the other one having five residues, whereas gangliosides contain just one fatty acid residue [75, 76]. This suggests that L1 and L2 represent previously unknown lipid structures phosphorylated by antibodies.

Oligosaccharides constitute the third fraction of milk (after proteins and lipids). They are subdivided into two large groups. The first group consists of fucosylated oligosaccharides, containing lactose residue in the central part of their molecules and also fucose, galactose, and N-acetylglucosamine. The second group includes acidic or sialated oligosaccharides; besides all the above-mentioned carbohydrates, they contain one or several residues of N-acetylneuraminic acid [78]. Acidic oligosaccharides represent components of glycoproteins and glycolipids, and free oligosaccharides have also been found in physiological body liquids in humans. The diversity of milk oligosaccharides is achieved by differences in genes encoding enzymes responsible for their synthesis in mammary gland cells [79]. During the early lactation period, oligosaccharide concentration may reach 20 g/liter, and then it decreases to 13 g/liter [80].

Protective functions of milk oligosaccharides are determined by features of their structure: they act as analogs of cells receptors in competition for binding of pathogens [81]. Thus, besides a prebiotic function [82], milk oligosaccharides inhibit binding of various pathogens, first of all, enteropathogenic strains of *E. coli* [83, 84]. Acidic oligosaccharides containing N-acetylneuraminic acid residue inhibit hemagglutination induced by *E. coli* toxins. Interestingly, N-glycolylneuraminic acid (which is absent in human milk) lacks this effect [85].

Various glycosidases exhibit narrow substrate specificity and are therefore used for study of polysaccharide structure [86]. For structural analysis, the oligo- and polysaccharides phosphorylated by antibodies were subjected to hydrolysis by 12 glycosidases cleaving various polysaccharides [35, 87]. Electrophoretic analysis of glycosidase hydrolysis of [^{32}P]sIgA and [^{32}P]IgG polysaccharides (Fig. 5) demonstrated the presence of three (in the case of sIgA) and five (in the case of IgG) radioactive bands corresponding to products of polysaccharide phosphorylation. Two (1 and 2 for sIgA and 2 and 4 for IgG) were major polysaccharides, whereas the others (3 for sIgA and 1, 3, and 5 for IgG) represented minor polysaccharides. They were found in all preparations of sIgA and IgG; however, sIgA and IgG labels significantly varied depending on the milk donor. Polysaccharides corresponding to IgG by their electrophoretic mobility and ratio in bands of four major polymers are similar; however, they differ from thee major polysaccharides bound to sIgA [35, 87].

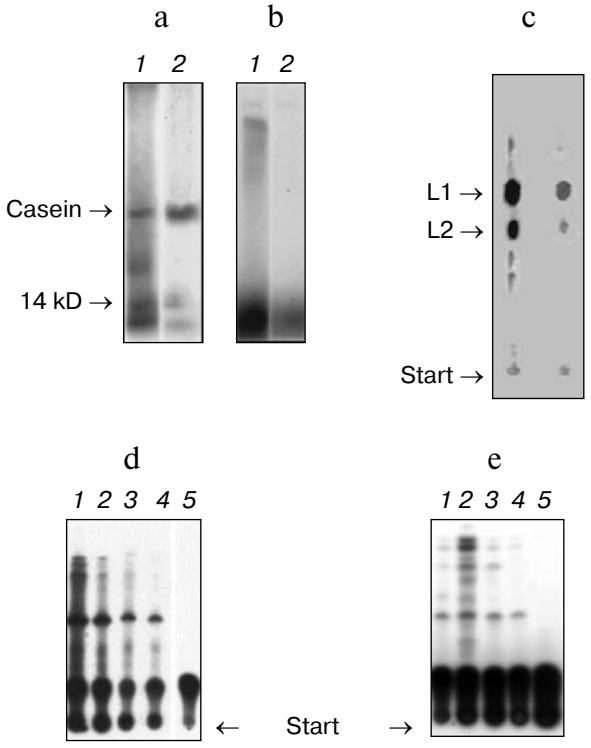

Fig. 4. a, b) SDS-PAGE analysis (12% gel) of sIgA-dependent phosphorylation of various substrates using [γ-^{32}P]ATP in the presence (a) and in the absence of casein (b): lanes *1* and *2* correspond to reaction mixtures before and after their treatment with methanol–chloroform mixture. c) TLC of the extract with methanol–chloroform mixture. Compounds extracted from solutions containing sIgA (*1*) and IgG (*2*) were subjected to chromatography using mixture for separation of phospholipids: chloroform–methanol–water (14 : 6 : 1). d, e) TLC of oligosaccharides. Compounds remaining in the reaction mixture after lipid extraction and protein precipitation (with trichloroacetic acid) were subjected to chromatography using mixture for separation of oligosaccharides: dioxane–ammonium–water (5 : 1 : 4). Lanes *1-4* correspond to four different preparations of sIgA (d) and IgG (e), lane *5* – incubation with ATP but without antibodies.

Effectiveness of hydrolysis of various oligosaccharides bound to sIgA and IgG by various glycosidases was analyzed using TLC. Hydrolysis of sIgA oligosaccharides (Fig. 6) yielded four bands, one of which was the major one (oligosaccharide II). For IgG preparations only two major bands exhibiting similar mobility during TLC were detected (Fig. 6).

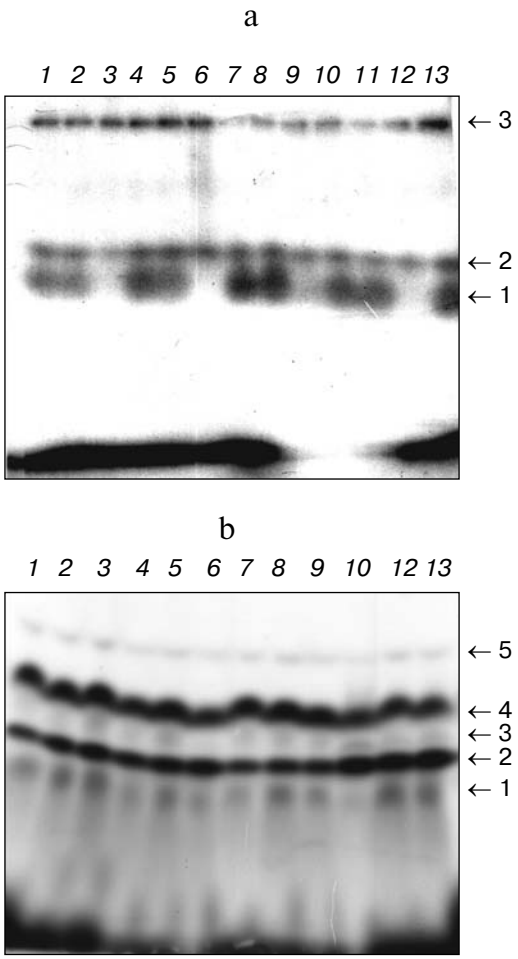

Fig. 5. Electrophoretic analysis (in 20% polyacrylamide gel) of effectiveness of hydrolysis of [^{32}P]polysaccharides phosphorylated by sIgA (a) and IgG (b). The following enzymes were used for hydrolysis of polysaccharides: β-glycosidase (*1*), cellobiohydrolase (*2*), glucoamylase (*3*), galactosidase (*4*), β-1,3-gluconase (*5*), lichenase (*6*), α-mannosidase (*7*), β-gluconase (*8*), α-amylase (*9*), endo-F-enzyme (*10*), chitinase (*11*), and α-L-fucosidase (*12*); *13*) mixture of [^{32}P]polysaccharides incubated without enzymes.

α-Mannosidase was the only enzyme that effectively hydrolyzed polysaccharides bound to sIgA and IgG. Other enzymes (β-1,3-gluconase and endo-F) exhibited weak glycosidase activity with polysaccharides obtained from sIgA and IgG preparations (Fig. 5). Polysaccharides 1 and 3 (sIgA) and almost all IgG polysaccharides (except the second one) were effectively hydrolyzed by glycosidases. The lowest hydrolysis was found only in the case of polysaccharide 2 (sIgA and IgG). Such selectivity was observed in cleavage of polysaccharides isolated from three studied preparations of sIgA and IgG [35, 87].

Maximal hydrolysis of sIgA and IgG oligosaccharides (as well as polysaccharides) was observed in the presence of α-mannosidase [35, 87]. β-Glycosidase and galactosidase demonstrated different effectiveness in hydrolysis of IgG oligosaccharides I and II, but these enzymes were inactive or weakly active in hydrolysis of sIgA oligosaccharides. Another eight glycosidases exhibited weak activity with respect to IgG oligosaccharides. However, some of these enzymes (glucoamylase, exo-1,3-β-gluconase, and α-F-fucosidase) effectively hydrolyzed sIgA oligosaccharides. Thus, these results suggest structural diversity of oligo- and polysaccharides bound and phosphorylated by IgG and sIgA [35, 87].

Interestingly, none of these enzymes hydrolyzed oligo- and polysaccharides to monomers. Moreover, in all cases of hydrolysis there was some amount of the label corresponding to oligo- and polysaccharides [35, 87]. All glycosidases used significantly differed by effectiveness of hydrolysis of IgG and sIgA oligo- and polysaccharides, corresponding to radioactive bands with various electrophoretic and chromatographic mobility. There is evidence [35, 87] that oligo- and polysaccharides bound to IgG and sIgA contain various monosaccharide units linked by various types of glycoside bonds. Moreover, these oligo- and polysaccharides can have complex branched structure, and in discrete bands observed during electrophoresis and TLC, they are obviously heterogeneous in content of various monosaccharides in these compounds corresponding to each band with certain mobility during electrophoresis and TLC [35, 87].

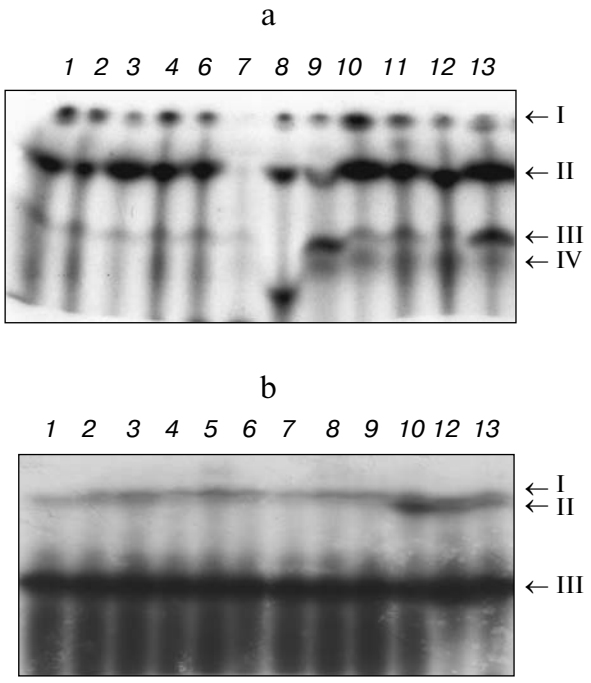

Fig. 6. TLC analysis of effectiveness of hydrolysis of [^{32}P]oligosaccharides phosphorylated by sIgA (a) and IgG (b). The following enzymes were used for hydrolysis of oligosaccharides: β-glycosidase (*1*), cellobiohydrolase (*2*), glucoamylase (*3*), galactosidase (*4*), β-1,3-gluconase (*5*), lichenase (*6*), α-mannosidase (*7*), β-gluconase (*8*), α-amylase (*9*), endo-F-enzyme (*10*), chitinase (*11*), and α-L-fucosidase (*12*); *13*) mixture of [^{32}P]oligosaccharides incubated without enzymes.

Evidence for catalytic activity of antibodies. Human polyclonal Ig represent a very wide range of antibodies with different structures of variable sites responsible for antigen [36]. Moreover, Ig may form complexes with various enzymes, which may be potentially co-purified with antibodies during their purification. So, demonstration of catalytic activity directly associated with a particular antibody represents one of the most complex problems of abzymology [46-51]. Direct association of lipid and polysaccharide kinase activities with IgG and sIgA antibodies [34, 35, 75, 87] was previously shown to meet strict criteria developed earlier [43, 46-51]: electrophoretic homogeneity of antibody preparations (silver staining); total sorption of catalytic activities of homogeneous IgG and sIgA from solution by adding sorbents with immobilized antibodies against human IgG and IgA, and coincidence of peaks of activity and protein density of the antibody during subsequent antibody elution by means of acidic buffer (pH 2.6). Such coincidence of protein absorbance peaks of IgG and sIgA antibodies and the peaks of catalytic activities was demonstrated during gel-filtration of antibodies in an acidic buffer (after antibody preincubation with this buffer before loading onto the sorbent). It was also shown that Fab-fragments of antibodies exhibited catalytic activity. Thermal treatment resulted in disappearance of catalytic activity of antibodies; no labeled lipids or oligo- and polysaccharides were found during their incubation with ^{32}P-labeled substrates in the absence of abzymes.

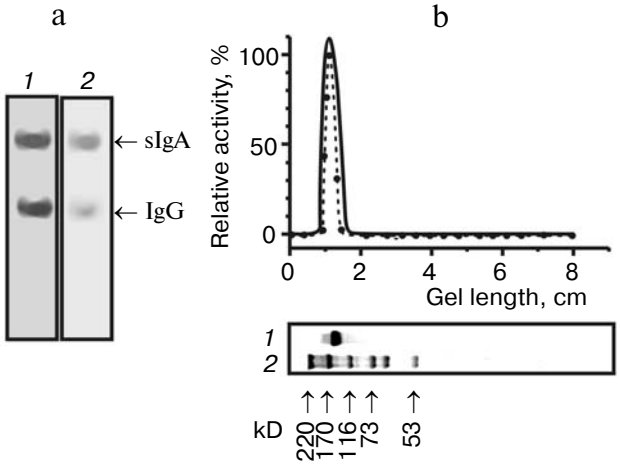

Fig. 7. Analysis of lipid kinase activity of IgG and sIgA antibodies *in situ* (a) and oligosaccharide kinase activity of IgG antibodies by the method of gel fragmentation (b) after SDS-PAGE of abzymes. a) After the electrophoresis gel was washed (to remove SDS) and incubated in the reaction mixture containing 100 nM [γ-^{32}P]ATP (lane *2*); lane *1* corresponds to gel stained with Coomassie R-250. b) After electrophoresis of IgG antibodies, the gel lane was separated into fragments of 2-3 mm. After extraction of proteins from these gel fragments, relative activity of eluates in phosphorylation of exogenous oligosaccharides was determined (•); eluate activity with maximal level of oligosaccharide phosphorylation was defined as 100%; the lower part of this figure shows the position of IgG (lane *1*) and protein markers (lane *2*); after electrophoresis proteins were stained with Coomassie R-250.

It should be noted that there are certain criteria that give almost 100% probability of association of catalytic activity with antibody rather than with possible contaminations. These criteria include demonstration of catalytic activity in the antibody after protein separation by means of SDS-PAGE [46-51]. It has been demonstrated that this is the strictest criterion, and if catalytic activity under study meets this criterion, it will definitely meet other (less strict) criteria [46-51]. Applicability of this criterion to lipid and polysaccharide kinase activities of antibodies was also demonstrated (see Fig. 7 and [35, 75, 87]).

Abzymes are known to exhibit higher affinity to substrates than canonic enzymes, and they can significantly differ from enzymes in substrate specificity. Kinase activities of abzymes meet these criteria as well.

Effectiveness of interaction and substrate specificity of milk abzymes. Minor lipids and oligo- and polysaccharides form rather stable complexes with sIgA and IgG abzymes [34, 35, 75, 87]. They gradually dissociate from complexes with antibodies during sequential chromatographic steps or severe treatments such as gel filtration and affinity chromatography on protein G-Sepharose and protein A-Sepharose using acidic buffers (pH 2.6) and buffers containing nonionic detergent Triton X-100. The complexes of antibodies with ^{32}P-labeled oligo- and polysaccharides were well degraded under conditions of dissociation of tight immune complexes after their incubation in buffer containing 2-3 M $MgCl_2$; the complexes of antibodies with ^{32}P-labeled lipids degraded after incubation in buffers containing 20-30% ethanol or dioxane followed subsequent gel filtration on columns equilibrated with these buffers [35, 75, 87]. During SDS-PAGE in the presence of 0.1% SDS, the complexes with oligo- and polysaccharides degraded completely, whereas less than 1-2% of lipids remained in the complexes with antibodies. However, during electrophoresis the complexes of antibodies with polysaccharides degraded, and ^{32}P-labeled polysaccharides were characterized by electrophoretic mobility similar to mobility of proteins with molecular masses of 4-15 kD (Fig. 4).

It was shown that preincubation of sIgA preparation with six various canonic polysaccharides for 5 h did not cause significant reduction in phosphorylation of endogenous oligosaccharides [35, 87]. However, prolonged incubation for 24 h caused significant inhibition of phosphorylation of sIgA-bound oligosaccharides in the case of β-glucan (which contains 1-3- or 1-6-linked glucose residues) and xylan (β-1,4 polymer, which consists of β-D-xylopyranose chains "decorated" with L-arabinose, D-glucuronic acid, and 4-O-methylglucuronic acid, linked sequentially or separately). Interestingly, two polymers containing fructose residues, inulin (a linear polyfructan, in which fructose residues are linked by β-(2→1) glycoside bond and which contains terminal

sucrose) and arabinogalactan (β-D-(1-3)-galactan, in which the main and side chain contain different number of D-galactose and L-arabinose residues) almost totally displaced oligosaccharides from sIgA. Other canonic polysaccharides caused insignificant effect on sIgA-dependent phosphorylation of oligosaccharides [35].

Similar experiments with IgG preparations revealed that none of the above-mentioned canonical polysaccharides inhibited IgG-dependent phosphorylation of endogenous oligosaccharides even after 24 h preincubation. Moreover, in the cases of mannan and lichenan there was marked and reproducible activation of phosphorylation of tightly bound oligosaccharides. This suggests high stability of IgG complexes with lipids and oligo- and polysaccharides and the existence of significant differences in the structure of oligo- and polysaccharides bound to IgG and sIgA [87].

IgG and sIgA phosphorylate only tightly bound minor lipids. Addition of high concentrations of various exogenous lipids may cause inhibition of phosphorylation of L1 and L2, but label incorporation into exogenous lipids has not been observed [34, 75].

Substrate specificity of IgG and sIgA exhibiting kinase activities with a phosphate group donor also differs from that of canonic kinases. It is known that almost all protein, lipid, and polysaccharide kinases use ATP (and in some cases other nucleoside-5′-triphosphates) as the phosphate group donor [88, 89]. Kinases utilizing any NTP or dNTP as the phosphate donor have not been described in the literature yet. However, small subfractions of polyclonal IgG and sIgA preparations (each of which is less than 5% of total amount of antibodies) can effectively phosphorylate milk proteins, antibody tightly bound lipids and oligo- and polysaccharides in the presence of any of the NTP and dNTP used [34, 35, 63, 75, 87]. Milk sIgA and IgG abzymes with protein kinase activity phosphorylate casein (and also up to 10-15 other milk proteins) by transferring terminal phosphate from (d)NTP with comparable effectiveness: ATP (100%), dATP (70-80%), GTP and dGTP (200-300%), and UTP (30%). Phosphorylation selectively occurred at serine residues [63]. sIgA and IgG antibodies with lipid kinase activity also phosphorylated minor lipids using not only ATP but also other (d)NTP with the following effectiveness: ATP (100%), dATP (40%), GTP and dGTP (3-8%), UTP and dTTP (50-60%) [34, 75]. A similar situation was observed in the case of antibodies with oligo- and polysaccharide kinase activity [31, 83]. Subfractions of sIgA and IgG abzymes catalyzing phosphorylation of proteins, lipids, and polysaccharides may significantly differ in relative activity assayed with various nucleotides [34, 35, 63, 75, 87].

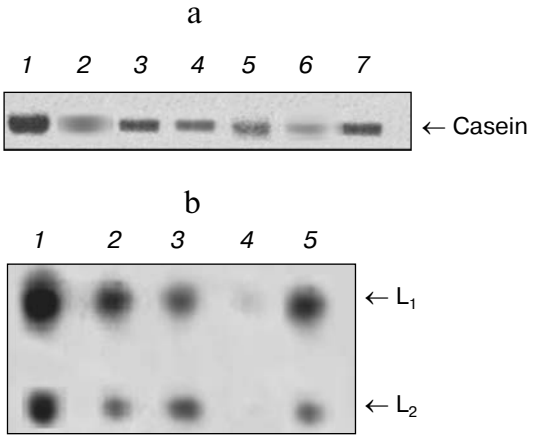

Fig. 8. Comparison of effectiveness of phosphorylation of casein (a) and L1 and L2 lipids (b) using various nucleotides and *ortho*-phosphate as the donors of phosphate group. a: *1*) GTP; *2*) dGTP; *3*) ATP; *4*) dATP; *5*) UTP; *6*) CTP; *7*) *ortho*-phosphate; b: *1*) ATP; *2*) dATP; *3*) TTP; *4*) GTP; *5*) *ortho*-phosphate.

A unique property of milk sIgA and IgG kinases consists in the fact that in contrast to all known kinases, they can use not only such macroergic compounds as NTPs, but also *ortho*-phosphate (Fig. 8). The of interaction proteins with *ortho*-phosphate resulting in covalent bond formation is an exceptionally rare event. Incubation of alkaline pyrophosphatase with *ortho*-phosphate was accompanied by phosphorylation of this enzyme [89-92]. However, we have not found in the literature any example of enzyme-dependent transfer of *ortho*-phosphate from solution onto any substrate (proteins, lipids, polysaccharides, etc.). However, catalytically active IgG and sIgA from human milk exhibit such unique property. In the case of sIgA and IgG abzymes, the level of phosphorylation of proteins and lipids was comparable with both ATP (100%) and *ortho*-phosphate (60-80%). Antibody-dependent phosphorylation of oligosaccharides was more effective in the presence of *ortho*-phosphate (150-200%) [34, 35, 63, 75, 87].

Direct protein phosphorylation by *ortho*-phosphate is impossible in view of the thermodynamics of aqueous media, because of large energy requirements. For explanation of alkaline phosphatase phosphorylation by *ortho*-phosphate, authors [89-92] employed results of Roentgen structure analysis. According to these results, phosphorylation of this protein occurs in a hydrophobic site of this protein, in which phosphorylation follows thermodynamics of non-aqueous media like vacuum or a gaseous phase. It is possible that certain amino acid residues localized in the hydrophobic environment of active sites of specific subfractions of IgG and sIgA may undergo phosphorylation by *ortho*-phosphate as it occurs in the case of alkaline phosphatase (with formation of a macroergic bond between phosphate and the protein). Subsequent steps possibly involve transfer of the activated phosphate group onto the substrate via a ping-pong mechanism [75].

Catalytic heterogeneity of abzymes with kinase activity. There is evidence that abzymes hydrolyzing DNA,

RNA, ATP, polysaccharides, and proteins are very heterogeneous [46-51]. They may contain abzyme subfractions differing in type of light chain (λ or κ), total charge, dependence on metal ion, thermal stability, pH optimum, substrate specificity, and affinity to substrates [46-51].

The plot of the dependence of accumulation of ^{32}P-labeled lipids and ^{32}P-labeled oligosaccharides on concentration of both [^{32}P]*ortho*-phosphate and [γ-^{32}P]ATP is characterized by the presence of reproducible intermediate plateaus (from 1 to 3). This complex kinetic behavior is characterized by 2-3 means of K_m and V_{max} (k_{cat}) values, which may differ up to 20 times (e.g. Tables 2 and 3). Abzymes with lipid kinase activities were separated into several fractions during chromatography on ATP-Sepharose; these fractions exhibited different affinity for ATP [63, 75, 87]. This suggests that preparations of polyclonal IgG and sIgA abzymes are heterogeneous and may contain at least 2-3 types of monoclonal antibodies, which are characterized by different K_m and V_{max} values for both polysaccharides and lipids.

Since lipids and oligo- and polysaccharides are tightly bound to antibodies, their maximal phosphorylation may evaluate relative amount of Ig (%) bound to these ligands [35, 75, 87]. For evaluation of maximal phosphorylation, abzymes were incubated up to reaching a plateau using saturating concentrations of [^{32}P]*ortho*-phosphate or [γ-^{32}P]ATP. It was shown that in dependence on milk donor, various preparations of IgG and sIgA contained about 2-7% of abzymes with tightly bound minor lipids and oligo- and polysaccharides provided that each antibody molecule contained just one molecule of a bound ligand. If each oligomeric molecule of IgG abzymes or sIgA antibodies can be phosphorylated two or four such bound ligands, the total amount of antibodies does not exceed 1-3.5% of the total, respectively.

For most proteins and enzymes interacting with *ortho*-phosphate, the K_d value for P_i is about 10^{-2}-10^{-3} M and only a small proportion of enzymes exhibits K_d value of ~10^{-4} M [93]. Consequently, affinity of milk abzymes to P_i (Tables 2 and 3) is 1-3 orders of magnitude higher than for canonic proteins and enzymes interacting with this ligand.

The biological role of abzymes exhibiting protein, lipid, and oligo- and polysaccharide kinase activities remains unknown. There is evidence [30, 31, 58-60, 71, 72, 83] for the presence of unique protein, lipid, and polysaccharide kinases in the human body; their catalytic properties and substrate specificity significantly differ from known properties of kinases described in the literature. It is known that during the first 4-6 months, the immune system of newborn babies is still under formation and IgG and sIgA of the mother's milk play an important role in maintenance of passive humoral response. Catalytic activity of milk antibodies may significantly

Table 2. Kinetic parameters (K_m and k_{cat} for *ortho*-phosphate) characterizing antibody-dependent phosphorylation of endogenous tightly bound lipids*

Antibody	$K_m(1)$, μM	$K_m(2)$, μM	$k_{cat}(1)$, min^{-1}	$k_{cat}(2)$, min^{-1}
IgG1	4.0 ± 2.0	14.5 ± 1.0	0.22 ± 0.04	0.38 ± 0.07
sIgA1	5.6 ± 1.8	32.0 ± 3.9	1.62 ± 0.4	2.1 ± 0.4
IgG2	3.1 ± 1.4	20.0 ± 5.0	0.23 ± 0.05	0.46 ± 0.1
sIgA2	2.4 ± 0.8	12.0 ± 2.0	0.13 ± 0.02	0.22 ± 0.04
IgG3	1.9 ± 0.8	42.0 ± 13.0	0.14 ± 0.03	0.5 ± 0.1
sIgA3	1.6 ± 0.1	3.5 ± 1.9	0.034 ± 0.01	0.041 ± 0.01

* Data are taken from [71].

Table 3. Kinetic parameters (K_m and k_{cat} for *ortho*-phosphate and ATP) characterizing IgG-dependent phosphorylation of oligosaccharides tightly bound to these antibodies*

Antibody	Substrate	$K_m(1)$, μM	$K_m(2)$, μM	$k_{cat}(1) \times 10^2$, min^{-1}	$k_{cat}(2) \times 10^2$, min^{-1}
IgG1	P_i	9.0 ± 3.0	43.0 ± 15.0	0.17 ± 0.07	1.0 ± 0.3
	ATP	9.7 ± 2.5	29.0 ± 10.0	0.19 ± 0.07	0.47 ± 0.015
IgG2	P_i	34.0 ± 12.0	71.0 ± 25.0	0.8 ± 0.2	7.5 ± 0.06
	ATP	21.0 ± 8.0	60.0 ± 10.0	0.12 ± 0.03	0.62 ± 0.25

* Data of [83].

extend biological action of Ig, because abzymes may not only bind components of pathogenic viruses or bacteria but also hydrolyze foreign DNA, RNA, polysaccharides, and proteins [46-51].

Phosphorylation plays an important role in regulation of vital functions of the organism; phosphorylation of proteins, lipids, and polysaccharides is accompanied by changes in their biological function. Phosphorylated proteins, lipids, and polysaccharides are important biological effectors, regulators, and mediators involved in almost all important physiological processes in animal and human cells. Considering the active role of IgG and sIgA in the development of passive immunity in children, it is possible that protein, lipid, and polysaccharide kinase activities of these Ig play an important (but still unknown) role in the protective effects of these Ig in regulation of cell processes.

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