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Intestinal Alkaline Phosphatase of the Fish *Cyprinus carpio:* Regional Distribution and Membrane Association

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ABSTRACT The distribution of alkaline phosphatase along the carp intestine and also its association to the enterocyte membrane have been studied in order to correlate them with the morphological features and functional specialization of the different intestine portions. The intestine was sectioned in seven segments and from each segment a butanol extract of intestinal alkaline phosphatase (IAP) was prepared. The enzyme activity, when expressed as a function of the mucosa or protein content of each segment showed a clear proximo-distal gradient. In addition, IAP was recovered in the precipitate after centrifugation of the homogenate, along the intestine, indicating that it is membrane associated protein. Also, when brush border membranes (BBM) were isolated, IAP was enriched 10-fold. Butanol extracted IAP from all segments exhibited three bands of activity when separated in PAGE-Triton X-100. The slowest migrating band showed a hydrophobic character as it was retained in a phenyl-Sepharose CL-4B column, whereas the other bands represent hydrophilic IAP found in the flow through of the column. Butanol extracted IAP from BBM rendered only one band with hydrophobic character in PAGE-Triton X-100, which could be converted to hydrophilic forms when incubated with phosphatidyl-inositol phospholipase C. These results clearly demonstrate, that in carp as well as in all other species studied, IAP is anchored to the membrane via a glycosyl-phosphatidylinositol. The high IAP content found in the first segment is consistent with the function of dephosphorylation of nutritional compounds proposed in higher vertebrates. The involvement of IAP in other physiological roles such as lipid absorption or protein internalization cannot be ruled out. *J. Exp. Zool. 279:347–355, 1997.*
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Alkaline phosphatase (ALP: EC 3.1.3.1) is present in the membrane of almost all animal cells and has long been studied in many organisms. The enzyme is a member of a growing family of membrane-bound proteins anchored to the outer leaflet of the lipid bilayer via a glycosylphosphatidylinositol (GPI) moiety (Low, '89; Micanovic et al., '88; Amthauer et al., '92). Although alkaline phosphatase has been extensively studied and its clinical usefulness clearly established, its physiological role(s) is still a matter of debate (Moos, '92; Makiya and Stigbrand, '92). Nevertheless, its wide distribution indicates that these enzymes are involved in fundamental biochemical processes. The strong activity found in different tissues like intestine, kidney, mammary gland, and placenta, suggests that ALPs are connected with absorption and transport mechanisms (McComb et al., '79).

Concerning the different aspects related to intestinal alkaline phosphatase (IAP), most of the present knowledge arises from studies done in higher vertebrates. From these studied, some light on the possible physiological functions of this enzyme has emerged. It has been proposed, for example, that in the intestine, ALP is implicated in the absorption of calcium and phosphate (Eguchi, '95). Also, IAP may have a particular function in quenching possible biological signals that enter the organism as nutritional compounds, i.e., phospholipids, sugar phosphates, phosphoproteins, and all phosphorylated intermediates (Hoffmann-Blume et al., '91). Moss ('92) suggested that dephosphorylation of nutritional compounds by IAP

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would be necessary to make them permeable to the plasma membrane. In addition, it has been demonstrated in the trout, that ascorbate phosphoesters are hydrolyzed by IAP. This constitutes an essential step for the utilization of the vitamin C supplied in the diet (Matusiewicz and Dabrowski, '95).

On the other hand, the amount of total IAP activity in the carp closely correlates with the period of maximal feeding of the fish (Penttinen and Holopainen, '92). Recent studies by Halbhuber et al. ('94) have shown that in the calf intestine an important amount of IAP is released attached to vesicles, called chymosomes, that also contain other hydrolases. It has been proposed that the function of these chymosomes is to increase the capacity of extracellular digestion. The amount of IAP released to the lumen is higher in the proximal half of the intestine were total activity of the enzyme is higher than in the distal portion (Hoffmann-Blume et al., '91). Moreover, in rat and human, a surfactant-like particle rich in ALP is not only released to the lumen but also the blood (Deng et al., '92; Domar et al., '93; Alpers et al., '95). The release to the blood is specially enhanced after fat feeding (Eliakim et al, '91; Deng et al., '92).

Studies on fish IAP have been essentially focused on the comparative characterization of the enzyme, especially regarding its enzymatic activity and thermal properties. Much of the work has been done with crude enzyme preparations from rainbow trout, eel, carp, and catfish (Gelman et a., '89; Sorimachi et al., '83; Whitmore and Goldberg, '72a,b; Yora and Sakagishi, '86). Only recently, the purification and characterization of ALP from the pyloric caeca of Atlantic cod has been reported (Ásgeirsson et al., '95).

In this article, we describe the distribution of alkaline phosphatase in the carp intestine and also its association to the enterocyte membrane. These results, in addition to the general morphological features and functional specialization of the different portions of the carp intestine, already described, will be helpful in the further elucidation of the processes in which IAP is involved.

MATERIALS AND METHODS

Materials

Carp (*Cyprinus carpio*) weighing 800–1,200 g were caught in the Cayumapu river. The fishes were acclimated in a tank with river water at 20°C and a photoperiod of 14 h light and 10 h dark for at least 3 weeks before they were sacrificed. Animals were fed to satiation twice every day.

Determination of intestinal alkaline phosphatase distribution in the carp intestine

Alkaline phosphatase was partially purified from carp intestine essentially as described by Whitmore and Goldberg ('72a). The carp intestine is folded into seven segments connected by six loops (Fig. 1). The segments were obtained by sectioning of the intestine as shown in Figure 1. Each segment was washed by flushing 10 ml of ice cold saline (NaCl 0.9% w/v) and then opened by cutting longitudinally. The surface mucus was removed by blotting with absorbent paper. The mucosa from each segment was stripped off using the edge of a glass slide and homogenized with two volumes of cold distilled water for 2 min with a Polytron unit (Janke & Kunkel, Cincinnati, OH). The homogenate was extracted with one-half vol-

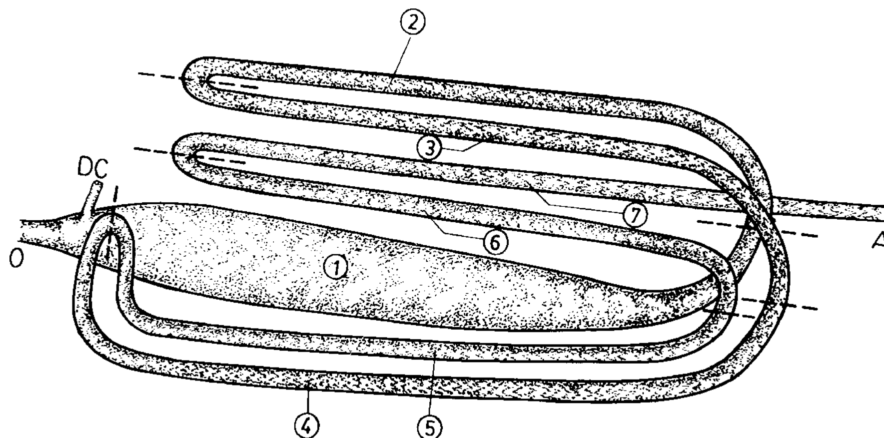

Fig. 1. Schematic drawing of the anatomy of the carp intestine. Dashed lines show where the gut was sectioned to separate the different segments numbered (1 to 7) from oesophagus (O) to anus (A). DC, Ductus choledochus.

ume of n-butanol at room temperature. The butanol solution was stirred for 30 min at room temperature and then centrifuged at 4°C and 9,000*g* for 20 min in a Sorvall RC-5 centrifuge using a SS-34 rotor. The butanol was discarded and the aqueous phase was filtered through glass wool and the alkaline phosphatase activity determined.

Determination of particulate alkaline phosphatase form

Intestinal mucosa from the different segments was obtained as described previously except that it was suspended (10%, w/v) in 250 mM sucrose, 10 mM Tris-HCl pH 7.4, 0.2 mM $MgCl_2$, 0.2 µg/ml bestatin, 0.2 µg/ml leupeptin, 0.2 µgl/ml pepstatin A, 0.2 µg/ml aprotinin, 0.2 mM PMSF, and homogenized with 10 strokes of a Potter-Elvehjem tissue grinder. The homogenate was centrifuged at 4°C and 105,000 *g* for 1 h in a Sorvall OTD Combi ultracentrifuge using a T-1270 rotor. The supernatant was separated and the pellet resuspended with the aid of a hand-held glass-Teflon homogenizer in one-half of the original volume of homogenization buffer. Alkaline phosphatase activity was determined in the supernatant and in the resuspended pellet.

Brush border and basolateral membrane preparation

Brush border membrane (BBM) and basolateral membrane (BLM) were isolated from the mucosa obtained from the whole intestine by the method described by Lee and Cossins ('90).

Determination of alkaline phosphatase activity

Alkaline phosphatase activity was routinely measured at 30°C using *p*-nitrophenyl phosphate as a substrate (initial concentration 2.7 mM) in 1.0 M diethanolamine buffer pH 9.8, containing 2 mM $MgCl_2$. Reaction was initiated by addition of the sample and the enzymatic activity followed spectrophotometrically (Shimadzu UV-260) at 405 nm during 6 min. One unit of enzymatic activity was defined as the amount of enzyme that hydrolyzed 1 µmol of substrate in 1 min under the conditions of the reaction ($\varepsilon = 18.800\ M^{-1}\ cm^{-1}$).

Determination of Na^+/K^+ ATPase activity

This enzyme was used as a marker for basolateral membranes. The activity of the ouabain-sensitive Na^+/K^+ ATPase was assayed essentially as described by Lee and Cossins ('90). Membrane aliquots were incubated for 5 min at 30°C in a reaction buffer containing 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 0.5 mM Na-deoxycholate, 4.5 mM ATP and 50 mM Tris-HCl pH 7.4, either in the presence or absence of ouabain (1 mM final concentration). After the reaction was stopped the inorganic phosphate was determined by the Fiske-and Subbarow ('25) method. Ouabain-sensitive activity was calculated from the difference between the activities obtained with and without ouabain. One unit of ouabain-sensitive Na^+/K^+ ATPase was defined as the amount of enzyme that liberated 1 µmole of inorganic phosphate in 1 min under the conditions of the reaction.

Protein determination

The protein content was determined by the bicinchoninic acid method (Smith et al., '85) using bovine serum albumin as standard.

Electrophoresis

Nondenaturing polyacrylamide gel electrophoresis in the presence of 0.5% Triton X-100 was performed as described by Hooper ('93) except that the running buffer used was 90 mM Tris, 90 mM boric acid pH 8.4, containing 0.5% Triton X-100. The separating gel (100 × 80 × 0.75 mm) was 6% T:2.6% C containing 0.375 M Tris-HCl pH 8.8 and 0.5% Triton X-100, and the stacking gel was 3% T:2.6% C containing 0.125 M Tris-HCl pH 6.8 and 0.5% Triton X-100. Samples were preincubated with an equal volume of sample buffer (250 mM Tris-HCl pH 6.8, 1% Triton X-100, 20% glycerol and 0.5% bromophenol blue) for 10 min at room temperature before application to the gel. Electrophoresis was carried out at room temperature and at a constant current of 10 mA for 3 h. After electrophoresis, the gel was equilibrated at room temperature for 10 min in buffer 0.1 M Tris-HCl pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$. IAP activity was detected by incubating the gel at room temperature for 20 min in the equilibrium buffer containing 0.16 mg/ml 5-bromo-4-chloro-3-indolyl phosphate and 0.33 mg/ml nitroblue tetrazoluim. Finally the enzymatic reaction was stopped by washing the gel with 20 mM Tris-HCl pH 8.0, 5 mM EDTA (Hooper '93).

phenyl-Sepharose chromatography

Hydrophobic IAP was separated from nonhydrophobic IAP by phenyl-Sepharose CL-4B chromatography as described by Bublitz et al. ('93). Alkaline phosphatase partially purified by butanol extraction as described previously was loaded on a phenyl-Sepharose CL-4B column equilibrated

with 10mM triethanolamine-HCl pH 8.5, containing 1 mM $MgCl_2$. After washing with the equilibration buffer, the retained alkaline phosphatase was eluted with the same buffer containing 0.5% Triton X-100.

Treatment of IAP with phosphatidylinositol-phospholipase C (PI-PLC)

Intestinal alkaline phosphatase extracted with butanol from BBM was used in these experiments. Aliquots containing 50 mU of IAP were incubated at 25°C for 2 h in 20 µl of reaction mixture containing 100 mM Tris-HCl pH 7.6, 0.2% Triton X-100, either in the presence or absence of 3 U of PI-PLC. After incubation, samples were subjected immediately to electrophoresis as described earlier.

RESULTS

Figure 1 shows a schematic view of the anatomy of the carp intestine. The length of the intestine is approximately two times the body (1.98 ± 0.11) and it is folded into seven segments of almost equal length (Table 1). To evaluate variations of IAP along the intestine, the mucosa of each segment was stripped off and a crude enzyme extract was prepared as described. The amount of alkaline phosphatase expressed as total units in each segment is summarized in Table 1 and Figure 2A. Most of the IAP activity was found in the first segment accounting for 66% of the total units determined in the intestine. The amount of IAP is drastically reduced in all the other segments, especially in the two more distal. Since the amount of mucosa varies between the different segments and hence also the protein content (Table 1), the amount of IAP was expressed as a function of the amount of mucosa and of the protein content in each segment (Fig. 2B and C, respectively). The amount of IAP, expressed in these form, shows a gradual decrease from segment 1 to 7. These results clearly show that IAP exhibits a craneo-caudal gradient in the carp intestine.

To study if alkaline phosphatase is soluble and/or membrane associated in the intestine and if the proportion of both possible forms varies along it, the mucosa homogenate of the different segments was centrifuged at 105,000 *g* for 1 h and the enzyme activity was determined in the supernatant and in the particulate fractions. As shown in Table 1, almost all the IAP is in the particulate fraction indicating that it is membrane associated. A slight decrease in the amount of IAP was observed in the particulate fraction of the two more distal segments in comparison with the other segments. In order to clearly establish the membrane association of IAP, brush border membrane (BBM) and basolateral membrane (BLM) were prepared from the mucosa obtained from the whole intestine. The IAP was enriched 9.6 times in the BBM fraction with respect to the homogenate and there was 10 times more enzyme in the BBM than in the BLM fraction (Table 2). The Na^+/K^+ ATPase was determined as an enzyme marker for BLM. This enzyme was enriched 5.2 times with respect the homogenate. These results demonstrated that IAP in the carp intestine is mainly associated with the BBM of the enterocyte.

The alkaline phosphatase prepared from the different segments were analyzed in PAGE-Triton X-100 as described. Figure 3 insert, lane 1, shows a representative pattern obtained for the different segments of the intestine and different carps. Three bands of IAP activity were detected, although their intensity varied between the enzyme preparation of the different segments.

In order to assess which of the different migrating IAP forms as hydrophobic, the enzyme preparation was subjected to chromatography in phenyl-Sepharose. Two peaks of activity were observed (Fig. 3). The first peak corresponds to the flow through of the column and when analyzed by PAGE-Triton X-100, it shows two activity bands that comigrate with the fast and middle band of the input to the column (Fig. 3, insert,

TABLE 1. IAP distribution along the carp intestine[1]

Segment	Length (cm)	Mucosa (g)	Mucosa protein (mg/segment)	IAP (U/segment)	IAP (U/g mucosa)	Particulate %
1	11.8 ± 1.9	2.91 ± 0.58	386.9 ± 23.1	119.75 ± 51.69	40.9 ± 12.6	96.4 ± 1.3
2	10.1 ± 1.9	0.74 ± 0.17	95.0 ± 6.2	20.70 ± 4.91	28.4 ± 5.7	96.9 ± 3.1
3	8.8 ± 1.2	0.44 ± 0.11	47.3 ± 4.9	8.95 ± 1.23	21.3 ± 6.3	95.5 ± 4.6
4	11.9 ± 2.1	0.55 ± 0.19	70.9 ± 6.7	16.28 ± 4.95	33.5 ± 19.4	94.9 ± 6.1
5	10.5 ± 1.4	0.61 ± 0.20	62.2 ± 2.9	9.28 ± 1.68	16.2 ± 3.5	94.2 ± 3.3
6	9.5 ± 1.4	0.66 ± 0.25	52.0 ± 4.2	3.70 ± 1.17	7.0 ± 4.6	83.9 ± 9.7
7	11.1 ± 1.5	0.56 ± 0.19	44.1 ± 2.9	1.70 ± 0.69	3.2 ± 1.1	83.9 ± 8.4

[1]Data are shown as mean ± SD; n = 4. Size of the fishes between 36–40 cm.

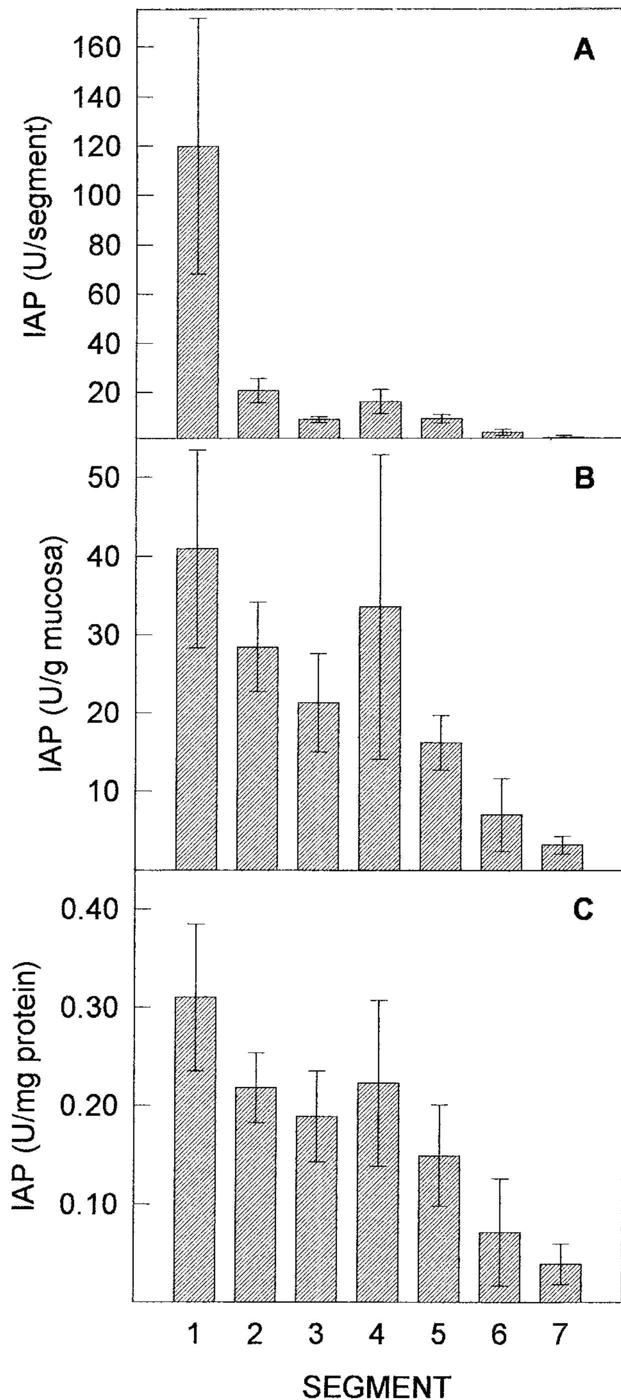

Fig. 2. Distribution of intestinal alkaline phosphatase in the different intestine segments. **A:** Total IAP activity in each segment. **B:** Amount of IAP activity in each segment expressed as a function of the amount of mucosa. **C:** Amount of IAP activity in each segment expressed as a function of the protein content.

TABLE 2. Distribution of the marker enzymes in membrane fractions isolated from carp intestinal mucosa[1]

Marker enzyme	Homogenate	BBM	BLM
IAP (U/mg protein)	0.215	2.060	0.209
Na^+K^+ ATPase (U/mg protein)	0.132	0.368	0.687

[1]Values represent the average of two preparations.

lane 2). The hydrophobic form of IAP retained in the column and eluted with the buffer containing Triton X-100, migrates in the gel as the slow form.

When analyzed by PAGE Triton X-100, IAP solubilized by butanol extraction from isolated BBM renders only one band of activity corresponding to the slow migrating hydrophobic enzyme (Fig. 4, lane 1). These results suggested that this enzyme form is anchored to the membrane through the GPI moiety. To clearly demonstrate this, an aliquot of the BBM solubilized IAP was incubated with PI-PLC, which specifically cleaves the GPI anchor. As shown in Figure 4, lane 3, the treated IAP migrated much faster than the controls (lanes 1 and 2), indicating that the hydrophobic moiety was cleaved. Treatment of BBM with PI-PLC showed that 65% of the IAP

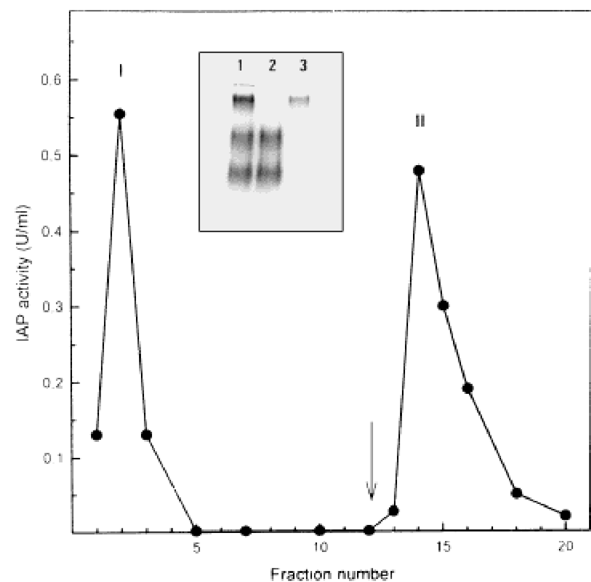

Fig. 3. Elution profile of carp IAP from phenyl-Sepharose CL-4B. Chromatography was performed as described in Materials and Methods. The arrow indicates the beginning of the elution step with the buffer containing 0.5% Triton X-100. **Insert:** Nondenaturing PAGE containing Triton X-100 of IAP. Lane 1: input (7 mU of IAP); lane 2: peak I, IAP not retained in the column (5 mU of IAP); lane 3: peak II, IAP eluted with 0.5% Triton X-100 (3 mU of IAP).

Fig. 4. Effect of PI-PLC treatment on IAP obtained from BBM. Lane 1: IAP extracted with butanol from BBM; Lane 2: the same as 1 but incubated at 25°C for 2 h; lane 3: the same as 1 but incubated with PIPL-C at 25°C for 2 h. Five mU of IAP activity was added to each lane.

associated with the membranes was susceptible to the phospholipase cleavage.

DISCUSSION

Cyprinid fishes, such as carp or goldfish, have one of the simplest types of digestive tract among vertebrates (Yamamoto '66). The carp does not have a true stomach and the intestine is a simple coiled tube having no valves and no caeca (Curry, '39; Al-Hussaini, '49a; Kapoor et al., '75). Taking in consideration morphological differences of the epithelia and physiological functions, four different regions may be distinguished in the carp intestine (Al-Hussaini, '49a,b; Noaillac-Depeyre and Gas, '73; Vernier, '90). The dilated portion down the opening of the ductus choledochus, forms the intestinal bulb or anterior intestine. A small intestine extends from it with a proximal portion (or medium 1) and a distal portion (or medium 2). Finally, a short posterior intestine or rectum ends at the anus. Since the proximal and distal portion accounts for almost 70% of the total intestine length, for the purpose of this study, the gut was sectioned in seven segments as depicted in Figure 1. The segments obtained in this way were almost of equal length and allowed us to clearly show the variation of alkaline phosphatase content along the intestine (Table 1 and Fig. 2). Although most of the IAP activity was found in the first segment, in all the remaining segments the enzyme was also present. This result is in agreement with previous histochemical studies, where IAP was detected along the entire intestine of the carp (Al-Hussaini, '49b; Noaillac-Depeyre and Gas, '73).

The height of the mucosal ridges is maximal in the proximal region and decreases gradually to the anus (Lee and Cossins, '88; Weinberg, '76). Accordingly, the calculated mucosal surface area decreases sharply in the first three segments and then remains almost constant to the anus (Lee and Cossins, '88). A similar pattern for the mucosa quantity in each segment was observed. Therefore, to obtain a closer approximation to the IAP distribution along the intestine, the amount of mucosa in each segment must be considered. In effect, a proximo-distal gradient of IAP distribution was observed when the enzyme activity was expressed either as a function of the mucosa or protein content in each segment. In higher vertebrates, IAP distribution has been determined in the small intestine, dividing it in a proximal and distal half. It was found that in calf, mouse, adult rat, hen, and rabbit, IAP was significantly lower in the distal portion in comparison with the proximal portion. The opposite happens in 14-day-old rat and goat (Hoffmann-Blume et al., '91).

In all species studied intestinal alkaline phosphatase is normally associated with the brush border membrane linked via glycosyl-phosphatidylinositol (GPI) moiety. Therefore, usually over 90% of IAP is recovered in the precipitate of an intestinal homogenate centrifuged at 105,000 *g* for 1 h. This is the case of the adult rat. In the suckling rat, although IAP is also found in a particulate form in the proximal half of the intestine, in the ileum in contrast, it is mainly recovered in the soluble form (Yedlin et al., '81). Our results show that in the carp, IAP is associated to the membrane along the intestine. Indeed, in all the seven segments, IAP was recovered in the particulate fraction, thus resembling the adult rat. Furthermore, when BBM and BLM were isolated from the intestinal mucosa, IAP was selectively enriched (10 times) in the BBM fraction. These results clearly demonstrate that IAP is associated to the BBM of the enterocyte and are consistent with the histochemical localization reported by Noaillac-Depeyre and Gas ('73).

Several criteria are used to demonstrate the GPI anchor in a protein. Due to the hydrophobic nature of the GPI moiety, the protein is retained on a phenyl-Sepharose column and shows a slow migrating band when it is fractionated on PAGE-Triton X-100 (Bublitz et al., '93; Hooper '93).

However, the most relevant feature of GPI anchors is its ability to be cleaved by specific phospholipases like PI-PLC. This enzyme removes the lipid portion of the GPI, resulting in the lost of the hydrophobic character of the protein. All the above mentioned criteria were fulfilled by the intestinal alkaline phosphatase of the carp. In fact, in the butanol extracted IAP preparation obtained from the different segments, a slow migrating band of the enzyme activity that corresponds to the fraction retained in the phenyl-Sepharose CL-4B column, could be detected (Fig. 3, and insert). In addition, only the hydrophobic form of IAP was observed in a butanol extract from BBM. This form of IAP was converted in a more hydrophilic form after incubation with Pl-PLC (Fig. 4). These data strongly support that in the carp intestine, as well as in all species studied so far, IAP is anchored to the BBM of the enterocyte via GPI. Recently, Ásgeirsson et al. ('95) reported that in the pyloric caeca of *Gadus morhua,* IAP seems to be also a GPI anchored protein, although they could only release 10% of the total IAP activity after 5 h incubation with Pl-PLC from tissue slices. In the carp, we were able to release 65% of the IAP activity associated to BBM. A great variation in the sensitivity (20–90%) to phospholipases, between alkaline phosphatases from different human cell lines and bovine tissues have been observed (Wong and Low, '92; Zekri et al., '89). The resistance to phospholipase has been attributed to acylation of the inositol ring in the GPI moiety (Wong and Low, '92).

Three bands of enzyme activity were obtained when butanol extracted mucosal IAP was fractionated in PAGE-Triton X-100. In contrast, the enzyme extracted from BBM exhibits only one band that corresponds to the slow migrating GPI containing IAP. This difference can be explained based on the high content of proteases or phospholipases present in the mucosa homogenate; these enzymes wash out during the BBM isolation. In fact, two groups (Malik and Low, '86; Stinson and Hamilton, '94) have demonstrated that during the butanol extraction, endogenous phospholipase can convert the alkaline phosphatase to a hydrophilic form that lacks the GPI anchor. In addition, Hawrylak and Stinson ('88) reported that proteases can also release alkaline phosphatase from intact plasma membranes. On the other hand, crude IAP preparations from different species, separated using different nondenaturating electrophoretic systems, can be resolved in several bands (Whitmore and Goldberg, '72a; Chang and Moog, '72). One of them could correspond to the GPI-IAP form already discussed and the other bands have been attributed to differences in the states of aggregation and/or net charge (Whitmore and Goldberg, '72; Chang and Moog, '72). This may explain why the flow through IAP fraction in the phenyl Sepharose column can be separated in two bands in PAGE-Triton X-100. The possibility that these IAP forms could represent different isoforms cannot be ruled out. In fact, in the rat intestine two IAP isoform that arise from the expression of two different mRNA has been demonstrated. Both IAP isoforms are membrane-bound by the GPI moiety, although they have different carboxy-terminal sequences (Engle et al., '95). Further studies will be necessary to explain the nature of the difference between the two IAP forms in the carp.

Among the different physiological roles that have been proposed for IAP, the dephosphorylation of nutritional compounds seems to be an important function in the carp intestine, especially considering the high amount of the enzyme found in the first segment. The fact that the activity of digestive enzymes, including IAP, closely correlates with the annual period of maximal feeding of the carp, further supports this hypothesis (Penttinen and Holopainen, '92).

In different teleost fish species the internalization across the intestinal epithelium of a wide variety of intact proteins like: peroxidase (McLean and Ash, '86, '87; Roumbout et al., '85), bovine growth hormone (Le Bail et al., '89), salmon growth hormone (Moriyama et al., '90), human growth hormone (Hertz et al., '91), insulin (Hertz et al., '92; Vera et al., '93), rabbit IgG (Nakamura et al., '90), carp apolipoprotein A-I (Vera y col., '92), and lactoferrin (Sakai et al., '95) has been demonstrated. Several studies performed in adult fishes, have been oriented to dissect the mechanism of the transepithelial transport of proteins. Using morphological approaches, they have demonstrated that this process occurs primarily by a transcellular route and is initiated by endocytosis of the protein in the columnar epithelial cells (Noaillac-Depeyre and Gas, '73; Roumbout et al., '85). In polarized epithelia of higher vertebrates this pathway of internalization is mediated by the binding of the macromolecules to specific sites in the apical membrane (Lamaze and Schmidt, '96). In fish intestine the presence of binding sites has not been demonstrated yet. In placenta, an other tissue active in protein internalization, it has been demonstrated, that placental alkaline phosphatase plays the role of Fc receptor

for the internalization of maternal IgG (Makiya and Stigbrand, ’92). It has also been reported that human IAP is able to bind IgG (Mäder et al., ’94). In addition, Tsonis et al. (’88), found sequence similarities between alkaline phosphatases and several proteins with protein binding capacity suggesting that these enzymes may specifically interact with other proteins. On the other hand, in the posterior intestine of the carp, where macromolecular uptake of proteins occurs, IAP has been localized in brush border membrane, in the tubulo-vesicular network and absorption vesicles (Noaillac-Depeyre and Gas, ’73). Taking together these observations the participation of IAP in the internalization of protein cannot be ruled out. In an attempt to assess this possibility, we are currently studying the binding capacity of IAP to different proteins.

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