A Two-active Site One-Polypeptide Enzyme: The Isomaltase from Sea Lion Small Intestinal Brush-Border Membrane

ITS POSSIBLE PHYLOGENETIC RELATIONSHIP WITH SUCRASE-ISOMALTASE

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The enzyme responsible for all of the isomaltase activity and much of the maltase activity in the small intestine of the Californian sea lion (Zalophus californianus) was isolated by detergent solubilization of the brush-border membrane, followed by immunoadsorption chromatography using antibodies directed against rabbit sucrase-isomaltase. In 0.1% Triton X-100, sea lion isomaltase occurs as a monomer of Mr = 245,000 and is composed of a single polypeptide chain. As judged from the stoichiometry of the covalent binding of the affinity label, conduritol-B-epoxide, this polypeptide chain carries two enzymatically active sites; they are apparently identical and do not show either positive or negative cooperativity.

In addition to cross-reacting immunologically with rabbit sucrase-isomaltase, sea lion isomaltase has similar overall enzymatic properties, with the exception of not hydrolyzing sucrose.

The Alaskan fur seal (Callorhinus ursinus) has a two-active site isomaltase; however, in contrast to the sea lion, this animal is endowed with a small but significant sucrase activity.

Along with (fully active) pro-sucrase-isomaltase, sea lion isomaltase is one of the very few examples of enzymes with more than one active site on a single polypeptide chain acting "in parallel" (rather than "in series"). Furthermore, this enzyme triggers some interesting questions on the phylogenetical pedigree of small intestinal sucrase-isomaltase.

The sucrase-isomaltase complex, which occurs in the small intestinal brush-border membrane of most mammals (reviewed in Ref. 1), is composed of two subunits (a maltase-suarcase and an isomaltase-sucrase, respectively) (1–5). It is synthesized and inserted in the membrane as a single gigantic polypeptide chain acting "in parallel" (rather than "in series").

Materials and Methods

Isolation of Sea Lion Isomaltase—Macroscopically normal small intestines were obtained from a newborn sea lion, everted, and rinsed with saline. Brush-border membranes were prepared by the Ca²⁺ precipitation method (12) as modified in Ref. 13, frozen, and kept at −20 °C.

Isomaltase activity was solubilized with Triton X-100 essentially as described for rabbit sucrase-isomaltase (14), but in the presence of phenylmethylsulfonyl fluoride. Brush-border membranes (29.6 mg of protein/ml), after thawing, were suspended in 10 mM sodium phosphate buffer, pH 7.0, 100 mM NaCl, 0.02% NaN₃, 0.05% Triton X-100, containing 7.5 μM phenylmethylsulfonyl fluoride at 4 °C. After stirring for 2 h at 4 °C, the solution was centrifuged at 120,000 × g for 40 min. For final purification, the Triton extract was applied to a column of Sepharose 4B to which antibodies against rabbit sucrase-isomaltase raised in guinea pigs (15) had been coupled using the cyanogen bromide method (16). The column was then re-equilibrated with the starting buffer, pH 7.0, 100 mM NaCl, 0.02% NaN₃, 0.1% Triton. After application of the sample, the column was washed with the same buffer as the capacity of the column was rather low (0.7–0.8 unit or 0.5–0.6 mg), only a fraction of isomaltase activity was retained. The isomaltase adsorbed to the column was eluted with buffer of low ionic strength (1 mM sodium phosphate, pH 7.0, 0.1% Triton), as described for the purification by immunoadsorption of other brush-border enzymes (17–19). The column was then re-equilibrated with the starting buffer; the isomaltase which had not been retained in the previous step was reapplied and the column was again washed and eluted as before. The procedure was repeated a total of eight times.

The isomaltase fractions were concentrated by ultrafiltration (Amicon, PM 10), excess Triton was removed with Bio-Beads SM-2 (Bio-Rad) using 1 g of moist beads for 70 mg of Triton (20), and dialyzed against starting buffer. The preparation finally obtained was essentially homogeneous as judged by SDS-PAGE. Sucrase-isomaltase activity and much of the maltase activity in the small intestine of the Californian sea lion (Zalophus californianus) was isolated by detergent solubilization of the brush-border membrane, followed by immunoadsorption chromatography using antibodies directed against rabbit sucrase-isomaltase. In 0.1% Triton X-100, sea lion isomaltase occurs as a monomer of Mr = 245,000 and is composed of a single polypeptide chain. As judged from the stoichiometry of the covalent binding of the affinity label, conduritol-B-epoxide, this polypeptide chain carries two enzymatically active sites; they are apparently identical and do not show either positive or negative cooperativity.

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It has been proposed (3–5) that this single chain precursor might have arisen by (partial) gene duplication of an ancestor gene coding for a single-site enzyme, splitting both maltase and isomaltase. That is, in the phylogeny of pro-sucrase-isomaltase a similarly gigantic polypeptide chain would occur carrying two identical (rather than different, as in pro-sucrase-isomaltase) active sites, each splitting both maltose and isomaltose.

The Californian sea lion (Zalophus californianus) has no detectable intestinal sucrase activity (11). Although this mammal is obviously not in the direct phylogenetic line which leads to the other mammals who have both sucrase and isomaltase activities, we have investigated the isomaltase activity of the small intestinal brush-borders of a pup of this species and found it to be associated with a large molecular weight polypeptide carrying two apparently identical active sites, each splitting maltose and isomaltose (a "double isomaltase").
maltase complex was isolated from rabbit small intestinal brush border membrane after solubilization with Triton X-100 (14) or controlled papain digestion (21).

Polyacrylamide Gel Electrophoresis in SDS.—This was accomplished in tubes or on slabs using a discontinuous sulfate-borate system modified from Ref. 22, and consisting of a polyacrylamide stacking gel (3.6 × 2.6) buffered in 0.055 M Tris/HCl, pH 6.14, 0.1% SDS, and a separation gel (6.2 × 2.6) buffered in 0.74 M Tris/HCl, pH 9.18, 0.1% SDS. Cathode (upper) buffer was 0.044 M boric acid, 0.065 M Tris, pH 8.5, 0.1% SDS, anode (lower) buffer was 0.43 Tris/HCl, pH 9.28 (8).

Protein samples were denatured by boiling for 5 min in 0.05 M Tris/HCl, pH 6.1, 4% SDS, 10% mercaptoethanol, 25% glycerol. This treatment fully dissociates the subunits of sucrase-isomaltase and of aminopeptidase, produces no protein aggregates when applied to unfraccionated brush-border membranes, and yields a single, high molecular weight band for "pro-sucrase-isomaltase" from hog (7) and rat (9). Denaturation of the samples prior to SDS-PAGE in 6 M guanidinium HCl using dithioerythritol as the reducing agent followed by alkylation with iodoacetamide, yielded identical results. Reference proteins were hog (7) and rat (9) pro-sucrase-isomaltase (M_r = 260,000) and the isomaltase (M_r = 150,000) and sucrase (M_r = 130,000) subunits of rabbit sucrase-isomaltase complex (M_r = 275,000) (24).

Gel Filtration.—The apparent molecular weight of the native enzyme was estimated by gel filtration using a Bio-Gel A-1.5m (Bio-Rad) column (2.0 × 40 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.0, 100 mM NaCl, 0.02% NaN_3, 0.1% Triton. The marker proteins were Triton-solubilized and papain-solubilized sucrase-isomaltase complex from rabbit small intestine (M_r = 275,000 and 235,000, respectively (24)), aldolase from rabbit muscle (M_r = 158,000 (25)), and hemoglobin (human, M_r = 65,000 (26)).

Affinity Labeling of Sea Lion Isomaltase.—Affinity labeling with [3H]conduritol-B-epoxide (27) was done under the same conditions as those worked out by Quaroni et al. (28) to label sucrase-isomaltase. Enzyme Assays.—The glucose, liberated at 37 °C in 33 mM sodium maleate buffer, pH 6.8, from the respective substrates, palatinose (Fluka, Switzerland), isomaltose (BDH, Great Britain,) maltose (Merck, Federal Republic of Germany), and amylose (grade I, Sigma), was determined using a glucose dehydrogenase kit (29) (Merck). Protein—Protein was determined according to a modified Lowry procedure (30) using bovine serum albumin as standard.

RESULTS

Isomaltase from the Small Intestine of Californian Sea Lion (Z. californianus)

Isolation Procedure, Apparent Molecular Weight, and Subunit Composition.—The partial purification of brush-border membranes from small intestine of sea lion and the solubilization of isomaltase using Triton X-100 were accomplished essentially as previously described (14) in connection with the isolation of the sucrase-isomaltase complex from rabbit small intestine.

Sea lion isomaltase is eluted with the void volume in Sephadex G-200 chromatography (in contrast, rabbit sucrase-isomaltase (31) and its isomaltase subunit (32) are retarded by a specific interaction with the dextran matrix). The soluble material was subjected to immunochromatography on Sepharose 4B to which antibodies against rabbit sucrase-isomaltase had been covalently bound. Due to the very low capacity of the immunosorbent (0.7–0.8 unit, or 0.5–0.6 mg) this step had to be repeated a number of times (e.g. 8 times in the preparation of Table I). The overall yield was approximately 35%; the final, essentially homogeneous preparation (as judged by SDS-PAGE) (Fig. 1) had a palatinase activity (at saturating substrate concentration) of 1.5 units·mg⁻¹ protein (or of 1.3 units·mg⁻¹ at 33 mM palatinose) (Table I).

Even after denaturation under the most drastic conditions (see "Materials and Methods"), sea lion isomaltase still yielded in SDS-PAGE a single polypeptide band with an apparent molecular weight of 245,000, 130,000 (275,000 and 120,000) and sucrase 12.9 µg; sea lion isomaltase, 11.3 µg; 4, rabbit sucrase-isomaltase complex, 14.2 µg.

TABLE I

| Fraction | Protein Specific Activity | Activity |
|----------|--------------------------|----------|
|          | % units/mg                | Recover    |          |
| Membrane fraction after Triton X-100 treatment | 100 | 0.061 | 100 |
| After centrifugation (120,000 × g, 40 min) | | | |
| Supernatant | 77.0 | 0.068 | 86.0 |
| Pellet | 37.2 | 0.0023 | 13.1 |
| After concentration (110,000 × g, 16 h) | | | |
| Concentrate | 50.8 | 0.101 | 87.0 |
| Supernatant | 12.8 | 0.0 | 0.0 |
| After Sephadex G-200 chromatography | 8.1 | 0.419 | 55.5 |
| After immunosorbent chromatography (8 runs) | 1.7 | 1.3 | 34.7 |

* Measured as palatinase (isomaltulase) activity. Substrate concentration is 33 mM, in 33 mM sodium maleate buffer, pH 6.8, 37 °C.

** Figures are the results of two runs using buffers of high and low ionic strengths. In each case, isomaltase activity eluted in the void volume (see text).

*Fig. 1. Polyacrylamide gel electrophoresis in SDS of sea lion isomaltase. 1, pig pro-sucrase-isomaltase (7), 4.8 µg; 2, rat pro-sucrase-isomaltase (9), 12.9 µg; 3, sea lion isomaltase, 11.3 µg; 4, rabbit sucrase-isomaltase complex, 14.2 µg.
part and a much larger hydrophilic "body."

**Hydrophobic Region(s) in Sea Lion Isomaltase**—As this enzyme could not be detached from the brush-border membrane by aqueous solutions unless they contained a detergent, it is, by definition, an integral membrane protein, and was expected to possess one or more hydrophobic regions. This was tested by Helenius' and Simons' (23) procedure of charge shift electrophoresis (Table II). Clearly, sea lion isomaltase, much as Triton-solubilized rabbit sucrase-isomaltase, has the highest electrophoretic mobility in the presence of the anionic detergent and the lowest in the presence of the cationic detergent. In contrast, the electrophoretic mobility of papain-solubilized rabbit sucrase-isomaltase, whose hydrophobic "anchor" had been split off by the proteolytic treatment (2), was not detectably affected by the charge of the detergent present. We conclude that sea lion isomaltase, much as Triton-solubilized rabbit sucrase-isomaltase, has at least one hydrophobic domain.

**Catalytic Properties**—Sea lion isomaltase had a pH optimum in the range of 5-6.4 and was fairly stable in the pH range 5.3-7.8 (Fig. 3). It had maltase, isomaltase, and palatinase activities in ratios approximately 1:0.92:0.31 (at saturating substrate concentrations and pH 6.8). The $K_m$ values were 2.4, 3.5, and 3.5 mM, respectively. The Eadie-Hofstee plots were linear with Hill coefficients between 0.97 and 1.03 (Table III). In order to test whether these substrates were split by the same or by independent active site(s), mixed substrate incubations were carried out (Fig. 4). Clearly, the amount of glucose liberated from mixed substrate incubations corresponded closely to that calculated from a mutual fully competitive inhibition between the substrates, using as $K_i$ their respective $K_m$ values as measured in separate experiments. Thus, maltose, palatinose, and isomaltose were split at the same site(s). Table IV reports for comparison the activity

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**Table II**

Relative electrophoretic mobilities of sea lion isomaltase and rabbit sucrase-isomaltase complex (detergent- and papain-solubilized).

| Enzyme                        | Triton X-100 and exolycholate | Triton X-100 alone | Triton X-100 and cetyltrimethylammonium bromide |
|-------------------------------|-------------------------------|--------------------|-----------------------------------------------|
| Rabbit sucrase-isomaltase     | 100                           | 100                | 100                                           |
| (papain-solubilized) (reference) | 100                           | 100                | 100                                           |
| Rabbit sucrase-isomaltase     | 100                           | 64                 | 41                                            |
| (Triton-solubilized)          |                               |                    |                                               |
| Sea lion isomaltase           | 64                            | 38                 | 23                                            |

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**Table III**

Catalytic properties of sea lion isomaltase

| Substrate   | pH | $K_m$ (mM) | $V_{max}$ (μmol mg⁻¹ min⁻¹) | enzyme split |
|-------------|----|------------|-----------------------------|--------------|
| Palatinose  | 6.8| 3.5        | 1.5                         |
| Maltose     | 6.8| 2.4        | 4.9                         |
| Isomaltose  | 6.8| 2.5        | 4.0                         |
|             | 7.6| 3.8        | 5.9                         |
|             | 7.6| 4.3        | 2.4                         |

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*Derived from an Eadie-Hofstee plot assuming Michaelian kinetics (correlation factor for linear regression $r = 0.973$).

*Derived from a Hill plot; Hill coefficient is 0.786.

* Determined using a plot $v$ versus $v^2$.
substrates were split at different sites. Thus, kinetics provided activity on isomaltose and none on palatinose (37, Eadie-Hofstee plots with any of the three substrates gave no curve calculated for a mutual fully competitive inhibition between the substrates, using as \( K_m \) their respective values measured in separate experiments. data determined experimentally.

![Graph](image)

**TABLE IV**

Catalytic properties of sea lion isomaltase and sucrase-isomaltase complex from rabbit

| Enzyme                     | \( K_m \) (mM) | Specific activity (units mg\(^{-1}\)) \( \times 10^3 \) | Palatinose | Sucrose | Palatinose | Sucrose |
|----------------------------|---------------|--------------------------------------------------------|------------|---------|------------|---------|
| Sea lion isomaltase        | 3.5           | 1.3                                                   | 0          | 11.4    | 0          | 11.4    |
| Rabbit isomaltase (Ref. 32)| 4.3           | 8.5                                                   | 1.3        | 11.4    |
| in the native sucrase-isomaltase complex |                   |                                                        |            |         |

* Measured as palatinase or sucrase activity in 33 mM sodium maleate buffer, pH 6.8, 37 °C.

ratios and the \( K_m \) values of the isomaltose moiety from the rabbit sucrase-isomaltase complex (32). Clearly, the overall catalytic parameters of the two enzymes are very similar. As expected (11), sea lion isomaltase does not hydrolyze sucrose to any detectable extent.

Is sea lion isomaltase a glucoamylase or perhaps an \( \alpha \)-limit dextrinase? The question is justified since small intestinal glucoamylase splits, in addition to the \( \alpha \)-1,4-glucopyranoside bonds in maltose and starch, \( \alpha \)-1,6-glucopyranoside bonds also (38-39), albeit slowly, and because \( \alpha \)-limit dextrinase also splits \( \alpha \)-1,4 and \( \alpha \)-1,6 glucopyranosyl bonds (38, 39). Table V shows that sea lion isomaltase has marginal glucoamylase activity only, and that this activity is comparable to that of rabbit sucrase-isomaltase complex. Also, sea lion isomaltase splits equally well maltose and isomaltose, and fairly well palatinose, whereas \( \alpha \)-limit dextrinase has only marginal activity on isomaltose and none on palatinose (37, 38). We conclude that sea lion isomaltase is neither a glucoamylase, nor an \( \alpha \)-limit dextrinase.

**Number of Active Sites/Polypeptide Chain**—How many active sites does sea lion single polypeptide chain carry? The Eadie-Hofstee plots with any of the three substrates gave no indication of either positive or negative cooperativity, or of more than one \( K_m \) value for each of the substrates, or that the substrates were split at different sites. Thus, kinetics provided no indication of whether the single polypeptide chain of the enzyme carried one or more active site(s). We thus reacted the isomaltase with \([^{3}H] \)conduritol-B-epoxide, an active site directed inhibitor of both subunits in rabbit intestinal sucrase-isomaltase (28). Table VI shows that the stoichiometry of label per mole of polypeptide chain of 245,000 was 2.1 at 100% nactivation. In rabbit sucrase-isomaltase, the stoichiometry of 1 mol of \([^{3}H] \)conduritol-B-epoxide bound per active site was not changed even after prolonged incubation with the inhibitor (28).

**A Few Observations on the Isomaltase (Sucrase) from the Small Intestine of the Alaskan Fur Seal (C. ursinus)**

In addition to the small intestinal isomaltase of Californian sea lion, we studied the corresponding enzyme from a pup and a subadult Alaskan fur seal. The enzyme was solubilized and purified as described above for that from Californian sea lion small intestine. The preparation showed one major band in SDS-PAGE with an apparent \( M_r \), of 245 kDa corresponding to the isomaltase band of the sea lion. After reacting the preparation with \([^{3}H] \)conduritol-B-epoxide, this very band was the one showing the highest specific radioactivity. As it proved difficult to determine the amount of protein in this band precisely, the specific radioactivity in Table VI was referred to the protein determined (Lowry) in the sample prior to SDS-PAGE and is, therefore, a conservative estimate (>1.69 mol mol\(^{-1}\)). In contrast to the sea lion, the isomaltase from the fur seal did have measurable, albeit very small, sucrase activity. Due to the minimal amounts available, it was not possible to establish whether sucrose and palatinose (and isomaltose) were split by the same or by different sites.

**DISCUSSION**

Sea lion isomaltase is clearly related to the small intestinal sucrase-isomaltase complex which occurs in most other mammals investigated. In fact, (i) it cross-reacts immunologically with (rabbit) sucrase-isomaltase (the isolation procedure is based on this very property). (ii) Sea lion isomaltase and rabbit sucrase-isomaltase have similar substrate specificities (Table IV) with the single exception of sucrose, which is not split by sea lion isomaltase. (iii) Sea lion isomaltase has the same number of active sites (two) in this enzyme. (iv) The protein (28) was not changed even after prolonged incubation with the inhibitor (28).

In discussing the properties of sea lion isomaltase, we thus compare them first with those of the sucrase-isomaltase complex. This enzyme is composed of two subunits of apparent molecular weight 130,000 and 150,000, respectively (2). Each subunit is composed of a single polypeptide chain, a glycoprotein (32), and carries a single enzymatically active site i.e. that of the sucrase moiety splits maltose, sucrose, and a number of aryl-\( \alpha \)-glucopyranosides. The active site of the isomaltase subunit splits maltose, isomaltose, palatinose (isomaltulose), a number of 1,6-\( \alpha \)-branched dextrans and some aryl-\( \alpha \)-glucopyranosides (31, 40). The properties of the two subunits in the sucrose-isomaltase complex are very similar, and both the limited sequence known in the active sites (41) and the number of fingerprints from tryptic digestion (32) indicate that they are likely to have a high degree of homology.

The subunit composition (one), and similar size as the fully active (hog, rat) "pro-sucrase-isomaltase" (7, 10).
Sea Lion Small Intestinal Isomaltase

TABLE V
Glucosylase and isomaltase activities of some intestinal α-glucosidases
Sources of amylose were as follows: A, amylose according to Zulkowsky (Merck); B, amylose from potato starch (BDH); C, amylose from potato (type I, Sigma); D, amylose (Koch-Light); E, soluble starch (Baker); F, starch of unspecified source.

| Enzyme                                | Glucosylase activity (μmol glucose liberated·min⁻¹·mg⁻¹ protein) | Isomaltase activity (Vmax, μmol glucosidic bonds split at optimum pH·min⁻¹·mg⁻¹ protein) |
|---------------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------------------------|
| Sea lion isomaltase (present paper)   | A 1.13 B 0.02 C 0.056                                          | ~4.5                                                                             |
| Rabbit sucrase-isomaltase complex (present paper) | A 7.6 B 0.04 C 0.064                                          | ~10                                                                              |
| Rat glucoamylase (from Ref. 36)       | A 28.4                                                          | ND*                                                                              |
| Rat glucoamylase (from Ref. 37)       |                                                                  |                                                                                  |
| Hog glucoamylase (from Ref. 38)       |                                                                  | ~0.65                                                                            |
| Hog α-limit dextrinase (from Ref. 39) |                                                                  | ~1.6                                                                             |

*Glucosylase activity was assayed in the present paper at 37 °C in 50 mM Na maleate buffer, pH 6.0, 2.5 mM EDTA, containing 10 mg of amylose/ml. The conditions in Ref. 36 were the same; those in other references were similar, although not necessarily identical.

In order to explain these three groups of characteristics of the small intestinal sucrase-isomaltase complex (i.e., the analogy, if not homology, between the two subunits, their common or related biological control mechanism(s), and the unusual mode of anchoring to the membrane), the following has been suggested (3–5). (i) Sucrase and isomaltase have arisen phylogenetically by (partial) duplication of an original isomaltase-maltase gene. (ii) This would have led first to a gene coding for a single polypeptide chain carrying two identical domains, each endowed with enzymatic activity i.e., a double isomaltase. Subsequent mutation would have transformed one of these domains from an isomaltase-maltase into a sucrase-maltase with the appearance of a single chain two-active site precursor (pro-sucrase-isomaltase). (iv) This single chain pro-sucrase-isomaltase would be synthesized, glycosylated, and inserted in the membrane of the endoplasmic reticulum and transferred, along with other plasma membrane proteins, to the brush-border membrane. (v) Post-translational proteolytic modification of this single chain (perhaps by one or more pancreatic proteases), would lead to the two subunits of the “ripe” sucrase-isomaltase complex; they would still remain associated via interactions formed during the folding of single chain pro-sucrase-isomaltase.

This “one-chain pro-sucrase-isomaltase hypothesis” seems now well established: a single chain enzymatically active pro-sucrase-isomaltase has indeed been isolated from the small intestines of hogs whose pancreases had been disconnected from the duodena three days prior to death (7); a fast fluoscence-labeled, high molecular weight band could be isolated from the rat intestine (6) and from subcutaneous transplants of small intestine from fetal rats (9); and finally, in vitro translation of RNA from rabbit small intestine yielded a high molecular weight band (8), which was identified as pro-sucrase-isomaltase (or pre-pro-sucrase-isomaltase). Both hog and rat pro-sucrase-isomaltases are converted into “normal” sucrase-isomaltase bands by treatment with pancreatic endoproteases. In addition, recent work on glucoamylase (51) has shown that this enzyme also is synthesized as a large single chain polypeptide which is split post-translationally. Thus, points i and ii above seem to be secured. Points i and ii are of course made likely by the very probable homology between sucrase and isomaltase.

The observations of the present paper show that sea lion isomaltase carries two active sites/polypeptide chain. If the

TABLE VI
Stoichiometry of labeling of sea lion isomaltase with [3H]conduritol-B-epoxide
The enzyme (0.62 mg) was incubated for 7 h in 1 ml of 92 mM sodium maleate buffer, with 23.9 μmol of [3H]conduritol-B-epoxide (specific radioactivity 1.25 × 10⁶ cpm/μmol). Excess reagent was removed by exhaustive dialysis (3 days) against 50 mM sodium maleate buffer pH 6.8, followed by vacuum dialysis against distilled water. The concentrate was percolated (13.2 ml/h) through a Bio-Gel P-100 column (2 x 26 cm) equilibrated in 10 mM ammonium carbonate, 0.05% Triton X-100. The fractions eluted with the void volume were pooled, protein concentration was determined by a modified Lowry procedure (30), and the radioactivity of the fractions was counted.

| Enzyme                                | Inactivation | Radioactivity | Ratio of epoxide to enzyme |
|---------------------------------------|--------------|---------------|----------------------------|
| Sea lion isomaltase                   | 100          | 1055          | 2.07                       |
| Alaskan fur seal                      | 100          | 6867*         | >1.69*                     |
| Rabbit sucrase-isomaltase complex     | 100          | 1708*         | 1.96                       |

* Specific radioactivity of [3H]conduritol-B-epoxide 8.80 × 10⁶ cpm/μmol (0.99 μCi/μmol).
* See text.
* Specific radioactivity of [3H]conduritol-B-epoxide, 1.92 × 10⁶ cpm/μmol (0.197 μCi/μmol).

monofactorial genetic disease (47).³

One more point worth mentioning is the unusual mode of anchoring of the sucrase-isomaltase in the brush-border membrane, i.e., the COOH-terminal regions of both subunits and the NH₂-terminal regions of the sucrase subunit are not involved in the anchoring to the membrane fabric and are exposed to the outer, luminal face of the membrane (2). The isomaltase subunit is anchored in the membrane fabric via a highly hydrophobic segment which is located not far from the NH₂ terminus of the polypeptide chain (2, 5); the sucrase subunit has a peripheral position and seems to interact with the membrane fabric via the isomaltase subunit only (2, 48, 49). (For a very recent review on sucrase-isomaltase, see Ref. 50).

³ In some pedigrees of this pathological condition, the pattern of sucrase and isomaltase activities left indicates a fairly complicated situation (for a review, see Ref. 56).
sites are identical, as all observations till now indicate, sea lion isomaltase would mimic or perhaps even be identical with the hypothetical double isomaltase suggested in point ii of the "one-chain pro-sucrase-isomaltase hypothesis" (see above). It is premature, however, to regard sea lion isomaltase as a dead end remnant of the double isomaltase in the phylogenetic pedigree of sucrose-isomaltase suggested above. In fact, whether Pinnipedia are monophyletic or biphyletic (52, 55), the eared seals (Otorioida, to which the Californian sea lions, Z. californianus, belongs) arose from the same ancestor as did the dog-bears (Ursidae). Now, one does find sucrase activity in the small intestine both of species which have branched off from the phylogenetic tree prior to the eared seals and in others which have branched off after them.4

The occurrence of a double isomaltase in the small intestine of Californian sea lion can be given, therefore, more than one explanation, e.g. (i) a back mutation from the "one-chain sucrase-isomaltase" reproducing the double isomaltase; (ii) other, more hypothetical, mechanisms changing one of the isomaltases into a sucrase, which need not be discussed in detail; (iii) that sea lion isomaltase may indeed be a dead-end remnant of the double isomaltase in the suggested phylogenetic tree of sucrose-isomaltase. This would imply, however, that the isomaltase → sucrase mutation producing the "one-chain sucrase-isomaltase" from the double isomaltase occurred more than once; i.e. the appearance of small intestinal sucrase activity would be an example of convergent evolution, perhaps related to the advantage of digesting sucrose-containing foodstuffs present in the diet of animals living on land. It is not possible at this moment to distinguish among these, and perhaps other, possibilities.

The one-chain, two-active sites isomaltase from the small intestine of the Alaskan fur seal may have, as mentioned above, traces of sucrase activity. Unfortunately, the minute amounts of the enzyme which we could prepare did not allow us to establish whether this sucrase activity is due to one catalytic site, or to both. It is thus futile at this moment to speculate as to the phylogenetic position of this enzyme.

Whatever the phylogenetic significance of the "isomaltases" in the small intestine of Pinnipedia, the situation in these animals is clearly different from that in ruminants, which seem to lack both sucrose and isomaltase (i.e. isomaltase) activity (e.g. the cow (54)) or have only minute amounts of the latter (perhaps due, however, to the "maltase," e.g. the camel (65)). The isomaltases in these Pinnipedia, and the lack of sucrose-isomaltase in (at least some) ruminants reminds one of the variants described in the various pedigrees of human sucrose-isomaltase malabsorption (reviewed in Ref. 56).

The intestines which we had at our disposal were from young individuals of Z. californianus and of C. ursinus. Attempts at splitting the double isomaltase of the former using the pancreatic endopeptidases (trypsin, chymotrypsin, and elastase, alone or in mixtures), which normally bring about the transformation pro-sucrase-isomaltase → sucrase-isomaltase (6, 7, 9), failed (data not shown). It is nevertheless possible that in the individuals used by us the pancreas or some other digestive organ had not reached full development; the small intestine from adult animals may possess a two-5

The alternative possibility, i.e. that the hypothetical original gene may have coded for sucrose and the duplication may have produced a double sucrase, is very unlikely because isomaltase activity is present in the small intestine of all vertebrates investigated, whereas sucrose does not occur in some species of Pinnipedia, in a few ones of Monotremata and Marsupialia (among mammals) and in some penguins (among birds). For a review, see Ref. 1.

chain, two-active site isomaltase, if the one-chain, two-active site isomaltases described here are processed in the adult by some protease, as is the case of pro-sucrase-isomaltase in most other mammals. These considerations do not invalidate the conclusions above; if anything, a (hypothetical) proteolytic processing of the double isomaltase in the adult may instead have rendered difficult to detect the peculiarity occurring in this species.

Polypeptides carrying more than one enzymatically active site are rare among eukaryotes (for reviews, see Refs. 57-59). In general, the enzymes associated together on the same polypeptide chain catalyze a sequence of reactions belonging to the same metabolic pathway (57-59), and indeed this may be an advantage in eukaryotes (59). To our knowledge, it is quite exceptional for two similar or identical active sites (e.g. acting in parallel rather than in series) to occur on the same polypeptide chain. The only other examples known to us are pro-sucrase-isomaltase (7, 9) and the ATPase of myosin filaments (e.g. Ref. 60).

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