

Rat Intestinal β -Carotene Dioxygenase Activity Is Located Primarily in the Cytosol of Mature Jejunal Enterocytes^{1,2}

CHRISTELLE DUSZKA, PASCAL GROLIER,* EL-MOSTAFA AZIM,[†]
MARIE-CÉCILE ALEXANDRE-GOUABAU, PATRICK BOREL
AND VÉRONIQUE AZAIS-BRAESCO

*INRA, Unité des Maladies Métaboliques, 63122 Saint Genès Champanelle, France and

[†]INSERM U71, 63005 Clermont-Ferrand, France

ABSTRACT The purposes of this study were to determine the location of β -carotene dioxygenase (EC 1.13.11.21) activity within the rat gastrointestinal tract, within the villus and within enterocytes, and to identify the metabolites produced in each intestinal fraction. In Wistar female rats, maximal activity was detected in the cytosol (74–93% of the total cellular activity) of mature functional enterocytes harvested from the jejunum (67% of the intestinal activity). The specific activity, expressed in pmol of retinoids/(h · mg protein) rose from 49 ± 3 in the stem cells to 199 ± 12 in the mature functional cells ($P < 0.05$). Thus the intestinal β -carotene cleavage activity might be regulated during the enterocyte maturation process. By using HPLC with diode array and radioactive detectors, retinal, and in the presence of NAD^+ , retinoic acid, were identified as the only metabolites produced. No β -12', 10', and 8'-apo-carotenals were detected, even when various enzyme sources were tested. These results suggest that the major, if not the sole, pathway for the formation of vitamin A from β -carotene in the rat intestine is central cleavage. *J. Nutr.* 126: 2550–2556, 1996.

INDEXING KEY WORDS:

- β -carotene • vitamin A activity
- enterocytes • rats

The β -carotene dioxygenase (β CDIOX)³ enzyme (EC 1.13.11.21) catalyzes conversion of β -carotene to vitamin A in small intestine mucosa of humans and of a variety of animals including ferrets, rabbits, hamsters and rats [Napoli and Race 1988, Van Vliet et al. 1992, Wang et al. 1991]. This activity is also present in liver, lungs and kidneys, but at lower levels [Wang et al.

1991]. Two major biological pathways have been proposed for the β -carotene cleavage reaction: 1) a specific oxidation of the β -carotene central double bond, yielding retinal as the only product [Devery and Milborrow 1994, Goodman and Huang 1965, Olson and Hayaishi 1965, Van Vliet et al. 1992 and 1996] and 2) excentric cleavage of any double bond of β -carotene, yielding several β -apo-carotenals, which could be shortened to retinal [Ganguly and Sastry 1985, Glover 1960, Wang et al. 1991]. Retinal could then be reduced to retinol or oxidized to retinoic acid. Dmitrovski (1991) has suggested that β -carotene may be cleaved both centrally and peripherally by different enzymes. The pathway of β -carotene metabolism may also be dependent on the intestinal sections and on the cellular or subcellular fractions that are used.

In vivo distribution of β -carotene conversion activity along the intestinal tract was previously estimated on the basis of the formation of vitamin A esters in rat intestinal mucosa, after a meal of carotene or after radiolabeled β -carotene was injected into ligated intestinal sections [Olson 1961, Thompson et al. 1950]. The first third of the small intestine was found to be the major site of retinyl ester formation. However, neither

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³ Abbreviations used: β CDIOX, β -carotene dioxygenase; CRBP II, intestinal cellular retinol binding protein type II; HP, phosphate buffer for mucosa homogenates; LRAT, lecithin retinol acyltransferase; NMR, nuclear magnetic resonance; PMSF, phenylmethylsulfonyl fluoride.

retinal, retinoic acid nor apo-carotenals were determined in those experiments. Although most investigators agree that β CDIOX activity is found mainly in the cytosol, the biological sources of enzyme in various in vitro cell-free assays have been mainly cytosolic, post-mitochondrial (9000–20,000 g) or low speed (800 g) supernatant fractions of intestinal mucosa (Glover 1960, Napoli and Race 1988, Van Vliet et al. 1992, Wang et al. 1991). Intestinal cellular retinol binding protein, which can bind retinol and retinal, may be highly associated with β CDIOX in enterocyte cytosol (Sklan 1983) and may accumulate specifically in mature enterocytes of chicks and rats (Crow and Ong 1985, Goda et al. 1993). No information about the distribution of β CDIOX activity along the villus axis has been available.

The purposes of the present study are to determine the distribution of β CDIOX activity in various parts of the rat intestine, to evaluate β CDIOX activity in cellular and subcellular fractions and to examine the metabolites produced.

MATERIALS AND METHODS

Materials. All nonlabeled carotenoid and retinoid compounds were generously provided by Hoffmann-La Roche (Basel, Switzerland). The [7, 7'- $^{14}\text{C}_2$]-all-*trans*- β -carotene (18.5 MBq/mmol) was synthesized from labeled sodium cyanide (Azim et al. 1996) and was checked for its purity by nuclear magnetic resonance (NMR) spectroscopy and HPLC analyses. β -Carotene was purified (>98%) immediately before use by chromatography on a 5% water-weakened alumina column. Chemicals and protease inhibitors were obtained from Sigma (l'Isle d'Abeau, France).

Animals and diet. Female weanling Wistar rats ($n = 6$ /experiment, INRA Jouy-en-Josas, France) were allowed free access to water and pellets of a nonpurified diet (diet A03, Pietrement, Sainte Colombe, France) for 6 wk. The maintenance diet⁴ for rats contained 230 g/kg crude protein, 50 g/kg fat, 30 g/kg crude cellulose, 90 g/kg ash, 70 g/kg moisture and 530 g carbohydrate. No β -carotene was detected in the diet. All procedures were in accord with the Institute's guide for the care and use of laboratory animals.

Enzyme preparations. Food was withheld overnight, then rats (160–200 g) were anesthetized with ether and killed by exsanguination. The intestine was

immediately removed and flushed with ice-cold phosphate buffer (100 mmol/L, pH 7.4). Stomach, duodenum (4 cm), cecum and six intestinal segments of 15 cm were excised, cut lengthwise and washed in cold phosphate buffer. The intestinal mucosa was scraped off with a glass slide at 0°C and homogenized in a Polytron homogenizer with 3 volumes of 100 mmol/L phosphate buffer (HP buffer), pH 7.7, containing 4 mmol/L MgCl_2 , 15 mmol/L NAD^+ , 1 mmol/L dithiothreitol, 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 10 $\mu\text{mol/L}$ bestatine and 2×10^8 units/L aprotine. After centrifugation for 25 min, the fresh 9000 g-supernatant was used for the enzymatic assay.

In a separate experiment, four sequential fractions of mucosa cells were obtained according to a gradient descending from the villus tip to crypt areas (Alessandri et al. 1993). Six rats, which had been food deprived overnight, were killed and the small intestine was immediately excised and flushed free of intestinal contents with 9 g/L cold NaCl. Thirty-centimeter intestinal segments (proximal jejunum) were everted and mounted onto special stainless-steel rods. Tooled rods (0.3×40 cm) with a blunted tip were used to roll up the jejunum and to keep it in position until complete scouring. Each extremity of the everted intestinal segment was tightly knotted to the rod, and each rod was vertically immersed into individual 35-cm glass tubes full of a phosphate buffer, pH 7.4, containing 3.2 mmol/L KCl, 137 mmol/L NaCl, 1.5 mmol/L KH_2PO_4 , 8.2 mmol/L Na_2HPO_4 , 1.5 mmol/L EDTA, 1 mmol/L dithiothreitol, 1 g/L bovine serum albumin (fatty acid free). The tubes were incubated at 37°C in a deep bathing vat equipped with a mobile platform (30 cycles/min). Four cell fractions were isolated by successive incubations of the tubes (7, 6, 17 and 15 min) followed by longitudinal vibrations of the rods. At the end of each incubation, the nonimmersed tip of the rod was clipped to the mobile head of a vibration apparatus, which produced low amplitude and short-lived vibrations with a frequency of 25 Hz/7 s. Cells were collected in the tube and washed by centrifugation after the last incubation. Cells harvested from the simultaneous incubation and vibration of two individual small intestines were pooled and treated as one unique cell fraction. Thus, three villus tip, upper-third, mid-villus and crypt area cell fractions were obtained from six rats. Pelleted cells were suspended in 2 mL of the HP buffer and homogenized using a Polytron homogenizer and repeated injections through the needle (0.9×25 mm) of a glass syringe. After centrifugation, the 9000-g-supernatant solution was used for β CDIOX, alkaline phosphatase (EC 3.1.3.1; Alessandri et al. 1991) and sucrase (EC 3.2.1.26; Dahlqvist 1970) activities, and protein content (Lowry et al. 1951).

In another experiment, subcellular fractions from six small intestines were prepared in the HP buffer by sequential centrifugations (Rigtrup and Ong 1992) and each fraction was tested for purity and β CDIOX activ-

⁴ Mineral and vitamin composition of the diet (g/kg diet): calcium, 16; phosphorus, 10; sodium, 3.5; potassium, 5.3; chlorine, 4.5; magnesium, 2.2; (mg/kg): manganese, 81; zinc, 45; copper, 187.7; cobalt, 1.32; iodine, 0.98; iron, 119; selenium, 0.04; retinyl palmitate, 27.7; *dl*- α -tocopherol, 26; cholecalciferol, 0.125; menadione, 0.47; thiamine, 20.9; riboflavin, 2.5; panthotenate, 11; pyridoxine, 11; cyanocobalamin, 0.05; nicotinic acid, 63; folic acid, 3.31; ascorbic acid, 120; choline, 2380.

ity. Preliminary studies have shown that the purity of these subcellular fractions was >75% (Mercier et al. 1994). Enzyme markers used for specific organelles are: succinate dehydrogenase (EC 1.3.99.1), mitochondria (12,000-g pellet); glucose-6-phosphatase (EC 3.1.3.9), microsomes (105,000-g pellet); glutathione-S-transferase (EC 2.5.1.18), cytosol (105,000-g supernatant); and DNA content, nuclei (1000-g pellet). Activities were measured as described previously (Mercier et al. 1994).

β -Carotene dioxygenase (EC 1.13.11.21) activity assay. All assays were conducted in triplicate using a method modified from Van Vliet et al. (1992). Enzyme homogenates (6 mg of protein) were incubated for 1 h, in a shaking bath at 37°C and in the dark, in the presence of 2.8 μ mol/L all-*trans*- β -carotene in acetone, 3.4 mmol/L SDS, 15 mmol/L NAD⁺, 10 mmol/L glutathione, 12 mmol/L taurocholic acid, 0.4 g/L phosphatidylcholine, 0.25 g/L *dl*- α -tocopherol and 100 mmol/L phosphate in a final volume of 2 mL (pH 7.7). Radiolabeled substrate was used in tissue and subcellular distribution studies to identify more precisely the metabolites produced. Controls were performed in the absence of either protein or β -carotene.

The reaction was stopped by the addition of 1 mL ethanol. All-*trans*-9-(4-methoxy-2, 3, 6-trimethyl phenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoic acid and echinenone were added as internal standards, and samples were extracted twice with hexane. After the organic layer was collected, the aqueous phase was acidified with 50 μ L of 4 mol/L HCl and reextracted with 2 mL ethyl ether/water (1:1, v/v). The organic layers were combined and dried under nitrogen. The residue was then suspended in 120 μ L of a mixture of methanol and dichloromethane (3:1, v/v). An aliquot (80 μ L) of this was injected into a Kontron AG HPLC system (Zurich, Switzerland). All organic solvents (Carlo Erba, Chaussee du Vexin, France) contained 25 mg/L butylated hydroxytoluene as antioxidant.

Retinoids were eluted on two 4.6 $\times$ 250 mm Vydac TP54 reverse columns in series (Vydac, Hesperia, CA) by acetonitrile/100 mmol/L ammonium acetate (70:30, v/v, pH 7.5) as the mobile phase (1.8 mL/min). Carotenoids were separated on a 4.6 $\times$ 250 mm Zorbax reverse column (Shandon, Runcorn, UK) with acetonitrile/dichloromethane/methanol (70:20:10, v/v/v) as the mobile phase (1.8 mL/min). A multiwavelength spectrophotometer detector was used with settings at 350 and 380 nm for retinoids and 450 nm for carotenoids. An additional photodiode array detector was used for measurement of absorption spectra. A radioactive detector was coupled in series with the other detectors when [¹⁴C] β -carotene was used as the substrate.

Statistical analysis. Values are given as the means $\pm$ SEM and, where appropriate, significance of differences between mean values was determined by one-way ANOVA and multiple range comparison by Fisher's least significant difference procedures (StatView II, Abacus

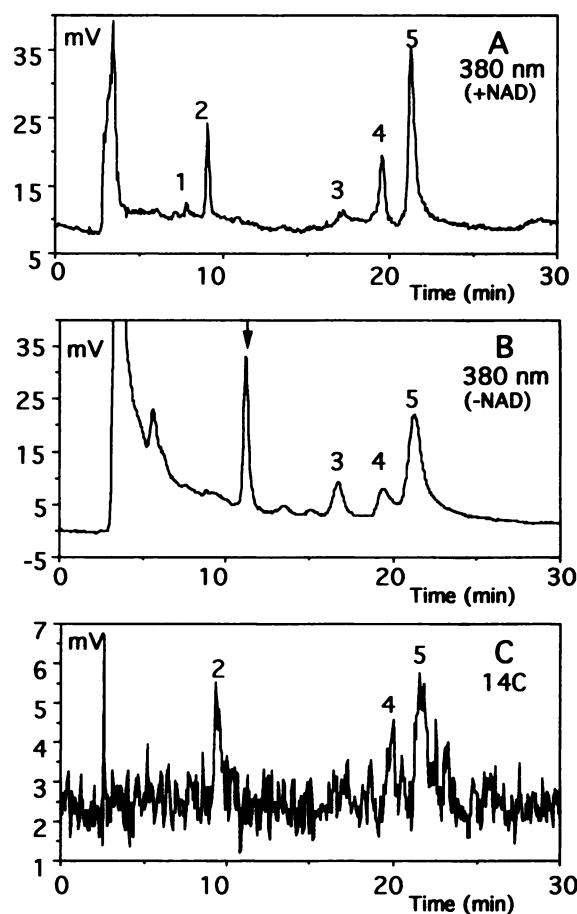


FIGURE 1 HPLC chromatograms of an extract of rat intestine after incubation with 2.8 μ mol/L [^{7,7'-¹⁴C₂}] β -carotene (18.5 MBq/mmol) for 60 min. Sample was extracted twice with hexane and once with ethyl ether after acidification of the aqueous phase. Metabolites were eluted on two 4.6 $\times$ 250 mm Vydac TP54 in series by acetonitrile/100 mmol/L ammonium acetate (70:30, v/v, pH 7.5) as the mobile phase. *Panel A* shows detection at 380 nm when NAD⁺ was added in the incubation mixture. *Panel B* shows detection at 380 nm when NAD⁺ was omitted. The peak marked with an arrow is the internal standard for retinoids. *Panel C* shows radioactive detection. Detected peaks coelute with reference compounds of 1) 13-*cis*-retinoic acid, 2) retinoic acid, 3) retinol, 4) 13-*cis*-retinal and 5) retinal.

Concepts, Berkeley, CA). When heterogeneous variances were identified, variables were log-transformed to achieve homoscedasticity before statistical evaluation. Values of $P < 0.05$ were considered significant.

RESULTS

Authentic standards (20 pmol) were incubated for 1 h at 37°C and the extraction recovery yields were as follows: β -apo-carotenals, 83–95%; retinoic acid 87%; retinol, 81%; retinal 88%. **Figure 1** shows representative HPLC profiles of [¹⁴C] β -carotene metabolites for the hexane-ether extract. Five major peaks which coeluted with the reference standards were detected at

TABLE 1

Retinal and β -apo-carotenal concentrations after a 60-min incubation of β -carotene with or without rat intestinal homogenates¹

Treatment	Retinal	β -apo-10'-carotenal
	pmol/2 mL incubation mixture	
Zero-time control	ND (<3.4)	ND (<3)
60-min time control ²	5.7 $\pm$ 0.4 ^a	4.8 $\pm$ 0.5
Incubation with homogenates from ³		
Duodenum	426 $\pm$ 94 ^{cd}	6.5 $\pm$ 1.1
Jejunum	766 $\pm$ 174 ^d	5.1 $\pm$ 0.3
Ileum	363 $\pm$ 61 ^c	4.4 $\pm$ 0.6
Colon	131 $\pm$ 24 ^b	5.0 $\pm$ 0.3

¹Values are means $\pm$ SEM. Values for retinal were log-transformed before statistical evaluation. Within a column, values not sharing a common letter are significantly different ($P < 0.05$). ND = not detected.

² β -carotene was incubated in 2 mL of the same medium under the same conditions as that used for studying β -carotene cleavage, $n = 4$.

³ $n = 12$.

380 nm when NAD^+ was added in the incubation mixture (Fig. 1A). The absorption spectra of these peaks, between 280 and 460 nm, corresponded well with those of the respective standards. Radioactive detection showed that retinoic acid and retinal (*cis* and *trans*) are the only metabolites detected under our experimental conditions (Fig. 1C). Neither retinol nor more polar compounds than retinoic acid were observed. We must point out that in most assays, retinal was the major metabolite and that no retinoic acid could be detected when NAD^+ was omitted (Fig. 1B). *Cis*-retinoid isomers found after incubation with enzyme preparations were <15% of total metabolites. When retinal or retinoic acid was used as substrate, similar isomerization rates were observed. A compound that coeluted with β -apo-10'-carotenal was detected in all intestinal fractions (Table 1). Because it was not substantially produced in biological samples and the amount produced did not differ along the intestinal tract, it is probably formed nonenzymatically. No β -apo-carotenals were detected in the cellular and subcellular fractions.

The β -carotene cleavage activity was studied in mucosa from stomach, duodenum, cecum and from six 15-cm segments of intestine. Figure 2 shows that no activity was detected in stomach (A) or cecum (I). In the intestine (C–H), the two metabolites (retinal and retinoic acid) displayed similar distribution curves. β -Carotene could be cleaved in all segments of the intestine. Both retinal and retinoic acid formation increased gradually from duodenum [B; 71 $\pm$ 15 and 35 $\pm$ 14 pmol/(h $\cdot$ cm), respectively] to jejunum (D; 19–34 cm) where maximal values were recorded [142 $\pm$ 51 and

47 $\pm$ 15 pmol/(h $\cdot$ cm), respectively]. The activity was dramatically lower in more distant sections (E–H). In fractions E and F (34–64 cm from stomach) the formation of total metabolites (i.e., retinal + retinoic acid) was significantly lower [72.9 $\pm$ 12.6 and 87.3 $\pm$ 23.0 pmol/(h $\cdot$ cm)] than those measured from section D samples [189.3 $\pm$ 66.1 pmol/(h $\cdot$ cm); $P < 0.05$] but were similar to those from duodenum [105.9 $\pm$ 25.4 pmol/(h $\cdot$ cm)]. A large proportion (67%) of the total activity was concentrated in the first 35 cm of intestine and 33% in the remaining 60 cm. We failed to detect any β CDIOX activity or any β -apo-carotenal production in stomach or in duodenal lumen contents (data not shown).

The four enterocyte fractions harvested from the villus column were associated with the villus tip, the next upper third of the villus, the middle villus and the lower villus region and were shown to contain 16, 14, 30 and 40% of total proteins, respectively, and, expressed in cumulative percentage of proteins from the villus tip to the crypt area, 16, 30, 60 and 100%, respectively. Differentiation marker activities, as well as phosphatase and sucrase activities, were low in stem crypt cells (100% of cumulated protein amounts) and increased gradually to reach maximal values in the upper third of the villus column (30% in cumulative percentage of proteins; Fig. 3A). The distribution gradient of β CDIOX activity along the villus-crypt axis was very closely associated with that of sucrase activity. The specific activity, expressed in pmol of total metabolites/(h $\cdot$ mg protein), rose continuously from 49 $\pm$ 3 in the crypt area (100% in cumulative percentage of proteins) up to 199 $\pm$ 12

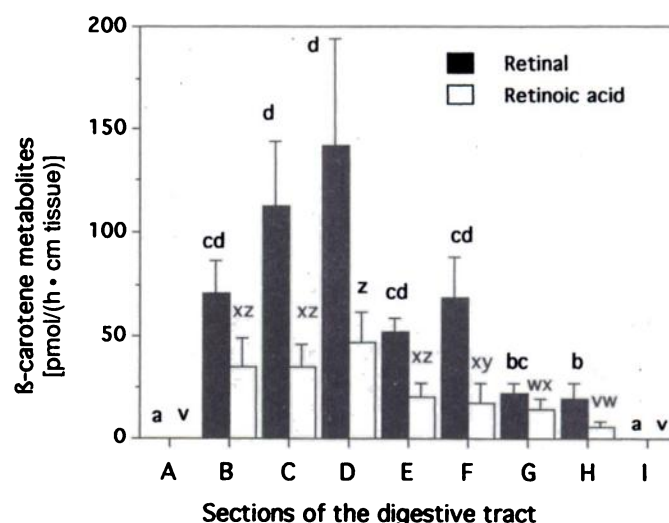


FIGURE 2 β -Carotene dioxygenase activity in various segments of rat digestive tract: (A) stomach, (B) duodenum, (C) 4–19 cm, (D) 19–34 cm, (E) 34–49 cm, (F) 49–64 cm, (G) 64–79 cm and (H) 79–94 cm sections of intestine, and (I) cecum. Bars represent means $\pm$ SEM for six rat intestines. Values were log-transformed and analyzed by ANOVA and by Fisher's least significant difference procedure. For each retinoid, means that do not share a common letter are significantly different ($P < 0.05$).

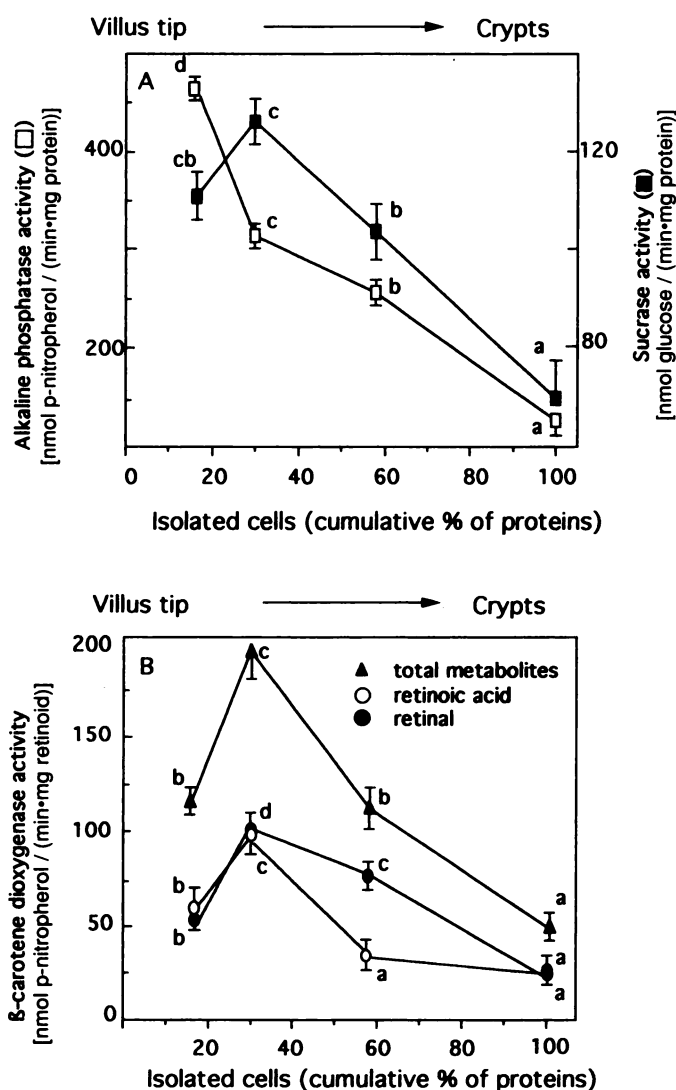


FIGURE 3 Distribution of β -carotene dioxygenase, alkaline phosphatase and sucrase activities along rat jejunal villi. Enterocytes were obtained from six rat proximal intestines, according to a gradient descending from the villus tip to crypt areas by sequential incubations and longitudinal vibrations of everted jejunum segments. Cells harvested from two simultaneous incubations and vibrations were pooled and subsequently treated as one unique cell fraction. Each point represents the mean $\pm$ SEM of measurements on three independent cell fractions. Results are expressed as a function of cumulated protein amounts. The villus tip, upper-third villus, mid-villus and crypt area correspond to 14, 30, 60 and 100% of cumulated protein levels, respectively. *Panel A* shows alkaline phosphatase and sucrase activities, expressed in nmol of *p*-nitrophenol or glucose released/(min $\cdot$ mg protein). *Panel B* shows the β -carotene dioxygenase activity expressed in pmol of retinal, retinoic acid and total retinoids/(h $\cdot$ mg protein). For each metabolite, values not sharing a common letter are significantly different ($P < 0.05$, ANOVA and Fisher's procedures).

($P < 0.05$) in the mature functional cells section (30% in cumulative percentage of proteins amounts) and then declined to 118 ± 9 in the villus tip (14% in cumulative percentage of proteins; Fig. 3B). In this experiment, retinoic acid and retinal were produced at a similar rate

in all fractions except in the middle-villus cells where retinal was the main metabolite.

Subcellular fractions were prepared from six rat small intestines and β CDIOX activity was measured in each fraction (Table 2). The mean purities of the different preparations were estimated to be $>77\%$, as judged by marker enzyme activities. An exception is the nucleus/debris pool which contained 40% of total succinate-cytochrome c reductase activity (a mitochondrial enzyme). Cytosol was essentially free of microsomal and mitochondrial contamination. β -Carotene cleavage activity was largely associated with cytosol where 93% of retinoic acid [275 pmol/(h $\cdot$ g mucosa)] and 74% of retinal [542 pmol/(h $\cdot$ g mucosa)] formation were detected, respectively. Very low activities [<45 pmol/(h $\cdot$ g mucosa)] were recovered in mitochondria and in microsome preparations. When expressed as pmol retinoid/(h $\cdot$ mg protein), β CDIOX activity was low in the microsomal fraction and was significantly higher in cytosol, ($P < 0.05$).

DISCUSSION

We report here, for the first time, that β CDIOX activity is located primarily in mature functional enterocytes of rat small intestine. The activity was absent in undifferentiated crypt cells, low in mid-villus cells and reached maximal rates in differentiated cells which contained maximal sucrase activity. No β CDIOX activity could be detected in lower muscular layers of intestine (data not presented), suggesting that the activity of β -carotene conversion to vitamin A is limited to the enterocytes. Similarly, intestinal retinol binding protein type II (CRBP II), which can bind retinol and retinal, has been shown to be restricted to mature enterocytes (Crow and Ong 1985, Goda et al. 1993). Thus, β CDIOX activity and the subsequent cellular transport

TABLE 2
 β -Carotene dioxygenase activity in subcellular fractions of rat jejunum¹

	Retinoic acid		Retinal	
	Retinal	Retinoic acid	Retinal	Retinoic acid
	pmol/ (h $\cdot$ g mucosa)		pmol/ (h $\cdot$ mg protein)	
Nucleus				
+ debris	30 $\pm$ 6 ^a	0 ^a	2.7 $\pm$ 0.4 ^a	0 ^a
Mitochondria	65 $\pm$ 16 ^a	13 $\pm$ 6 ^b	6.9 $\pm$ 0.9 ^{bc}	1.3 $\pm$ 0.6 ^a
Microsomes	45 $\pm$ 26 ^a	6 $\pm$ 3 ^{ab}	4.7 $\pm$ 2.4 ^{ab}	0.6 $\pm$ 0.2 ^a
Cytosol	402 $\pm$ 111 ^b	256 $\pm$ 57 ^c	9.1 $\pm$ 1.6 ^c	6.8 $\pm$ 1.7 ^b

¹Each value represents a mean $\pm$ SEM, $n = 6$. Values were log-transformed before statistical evaluation by ANOVA and Fisher's least significant difference procedure. Within a column, values with different superscripts are significantly different ($P < 0.05$).

of retinal could be regulated during the enterocyte maturation process to become highly effective in the cells which efficiently absorb vitamin A and β -carotene. Whether synthesis or turnover of the β CDIOX protein, or cellular regulation of the activity is modified along the villus-crypt axis is still unclear. A similar distribution gradient of CRBP type II and of β CDIOX activity could also support the hypothesis of a close cytosolic association between the two proteins. Sklan (1983) isolated a fluorescent high molecular weight lipid-protein aggregate from the cytosol of chick intestinal mucosa. This aggregate exhibited β -carotene-cleavage activity and contained zinc, copper, retinyl esters and a retinol binding protein of $\sim 15,000$ Da. The author suggested that this intestinal protein represented the intracellular retinol binding protein. However, Devery and Milbrow (1994), who attempted to purify the β -carotene cleavage enzyme, failed to detect any such protein aggregate in guinea pigs.

The distribution study along the stomach-cecum axis confirms that β -carotene conversion activity is restricted to the upper third of the intestine, i.e., duodenum and jejunum (Olson 1961, Thompson et al. 1950). Similar gradients of CRBP II, retinal reductase and lecithin retinol acyltransferase (LRAT) levels down the duodenal-ileal axis were found in rat intestine (Herr et al. 1993). This suggests coordinated regulation of proteins that participate in intestinal β -carotene metabolism and the subsequent metabolism and transport of retinoids formed. However the 33% of total activity contained in distal sections should not be overlooked because bile salts, which improve β -carotene solubilization in intestinal lumen and stimulate β CDIOX activity (Olson 1961), could be efficiently absorbed through the ileal epithelium (Hofmann 1977). Nonetheless, the physiological importance of such β CDIOX activity in the distal intestine remains unclear.

In confirmation of past studies, β CDIOX activity was primarily, if not solely, found in the cytosolic fraction. Mitochondrial, microsomal and nuclear fractions did not contribute substantially to the total β CDIOX activity.

In our three assays, retinoic acid and retinal were the only metabolites of β -carotene detected. We observed low levels of *cis*-retinoid isomers. These isomers may have been chemically formed from their *trans*-isomers in the presence of sulphhydryl groups and the SDS detergent in the incubation mixture, as suggested by Urbach and Rando (1994). Assays were conducted by mixing pure *trans*-retinal and *trans*-retinoic acid with boiled proteins in the incubation buffer. Detection of 10–15% *cis* retinoid isomers after 1 h supported the hypothesis of a retinoid isomerization induced by sulphhydryl groups (data not shown). *Cis*-retinoids may also be enzymatically formed from *cis*- β -carotene. However, in our experimental conditions, all-*trans*- β -carotene was stable over 1-h incubations and little 9-*cis* and 13-*cis*- β -carotene (< 4%) could be detected.

The metabolite profiles were similar when different intestinal tissues, enterocytes or subcellular fractions, including low speed centrifugation supernatants, were used as enzyme sources. When [^{14} C] all-*trans*- β -carotene was added as substrate, β -apo-12'-, -10'-, or -8'-carotenals were not identified. This result cannot be attributed to analytical problems because extraction yields of apo-carotenal standards, in assay mixtures containing bile salts and SDS, were >90% and the HPLC detection limit for these products was <3 pmol. Herr et al. (1993) reported that PMSF inhibited the LRAT activity in rat intestine. In our experiments, addition of protease inhibitors in the mucosa homogenate, and of SDS in the incubation mixture, improved the β CDIOX activity yield (data not shown). This is in agreement with data reported by Devery and Milbrow (1994) and by Dmitrovski (1991). However, we cannot exclude the possibilities that compounds which were added in the assay mixture, e.g., SDS, or in the HP buffer, e.g., protease inhibitors, inhibited specifically β -apo-carotenal formation or that any β -apo-carotenals formed were very rapidly converted to retinal. In the same way, we cannot exclude the possibility that the very low speed centrifugation supernatant (800 g), used by Wang et al. (1991), contains specific components which produce apo-carotenals from β -carotene.

Thus, our results further support the hypothesis that all-*trans*- β -carotene is centrally cleaved in rat intestine mucosa. We further suggest that all-*trans*- β -carotene metabolites formed did not change when different enzyme preparations are used. However, other factors such as species, incubation methods or β -carotene solubilization may influence the cleavage pathway. To summarize, we detected a maximal all-*trans*- β -carotene dioxygenase activity in the cytosol of mature enterocytes from rat jejunum. The only detected metabolites consisted of retinoic acid and retinal, suggesting a central cleavage of β -carotene by the β CDIOX. This profile of products was not modified by the nature of the enzyme preparation used.

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