# Correspondence

## Gastric uptake of nicotinic acid by bilitranslocase

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Bilitranslocase is a 38 kDa membrane protein [1] localised at the vascular surface of the liver cells. The isolated protein is endowed with transport function of sulphobromophthalein (SBP), as directly demonstrated in reconstituted systems. Thus, its proposed physiological role is to mediate the traffic of SBP and related organic anions across the sinusoidal domain of the hepatocyte plasma membrane. On account of the known competition between SBP and bilirubin for the first step of liver uptake observed in vivo, bilirubin had been pointed out as the most obvious physiological substrate for bilitranslocase. A strong indication in this sense has come with the finding, within bilitranslocase primary structure, of the sequence EDSQGQHLSSF [2], which has the peculiarity of being the central portion of a motif highly conserved in α-phycocyanins, biliproteins found in cyanobacteria. Whereas in α-phycocyanins this motif is involved in binding the prosthetic group phycocyanobilin [3], a compound structurally related to bilirubin, in bilitranslocase it has been shown to act as the bilirubin-binding site [2]. Indeed, the affinity of the carrier for the pigment is high enough to ensure bilirubin extraction from the blood stream ($K_d$ of the complex = 2 nM) [2].

Antibodies raised against the sequence in question have been employed in a tissue distribution study in the rat [4] aimed at finding the expression of proteins with the bilirubin-binding sequence in that organism. By these means and somewhat unexpectedly, the sequence has been found in the stomach mucosa, associated with a protein displaying an electrophoretic mobility (38 kDa) identical to that of liver bilitranslocase. Immunohistochemical analysis has shown that this protein is associated with different epithelial cell types of the gastric mucosa.

A critical question therefore concerns the kind of evolutionary pressure that has resulted in the expression, at this level, of a membrane protein endowed with high-affinity bilirubin-binding properties. Obviously, bilirubin in the food is not a likely candidate for such an evolutionary function. In contrast, such a function could be envisaged for nicotinic acid, in view of the fact that bilitranslocase binds this vitamin with high affinity ($K_d$ of the complex = 11 nM) [3]. The possibility could therefore be considered that the physiological role of the sequence EDSQGQHLSSF is related to nicotinic acid absorption at the gastric level.

To test the hypothesis that, indeed, the protein present in epithelial cells of the gastric mucosa could be an electrogenic SBP carrier, like in the liver, the post-mitochondrial fraction from gastric epithelia was tested for electrogenic SBP transport. This substrate was indeed found to be taken up electrogenically by the preparation, with the same kinetics observed with analogous liver preparations ($K_M$ = about 5 μM). Anti-sequence antibodies caused inhibition of electrogenic SBP uptake and, in addition, this effect could be lessened in a concentration-dependent manner by the presence of nicotinic acid. The data obtained fitted the Scrutton and Utter equation, making it possible to calculate the dissociation constants of the carrier–ligand complex. The value ($K_d = 10.23 \pm 0.32$ nM) was, again, virtually identical to that obtained with liver preparations [3]. Interestingly, when the vitamer nicotinamide was tested in the same kind of inactivation analysis, no effect was found, reinforcing the early conclusion that the negative charge on a substrate is a critical molecular requirement for the interaction with bilitranslocase.

All these data make it possible to conclude that the 38 kDa, antibody-reacting protein found in the gastric mucosa is a carrier protein displaying the transport kinetic properties of bilitranslocase. Moreover, it contains its bilirubin-binding sequence, which is the site of high-affinity nicotinic acid binding, too.

Since the epithelial cells of the gastric mucosa are equipped with a SBP transport protein, it was expected that SBP could be absorbed by the stomach. Data obtained in in vivo experiments involving rats showed that SBP was absorbed rapidly and extensively. In particular, the bimodal kinetics of SBP disappearance suggested the involvement of two mechanisms of absorption. A fast, exponential step, typical of carrier-mediated processes, was more evident at low substrate concentrations, whereas a slow, linear one, describing passive diffusion, superimposed at the higher concentrations tested. The decay constants obtained from the fast steps were compatible with a mechanism operating with a substrate affinity in the low micromolar concentration range.

The most direct approach to the study of the involvement of bilitranslocase in nicotinic acid uptake by the gastric mucosa would make use of the anti-sequence antibody. However, under the physiological conditions of the gastric lumen, the antigen–antibody reaction cannot occur, mainly because of pH-dependent denaturation and the proteolytic action of pepsin. An experimental model employing the isolated stomach was therefore set up, satisfying the following requirements: (a) intact gastric mucosa; (b) favourable conditions for the binding of the anti-sequence antibody to its target; and (c) minimal humoral and cellular effects following the immunological reaction.

A large patch of the organ, comprising nearly the entire corpus and antrum, was mounted on a two-chamber device, thus delimiting a serosal and a mucosal compartment. Both surfaces were in contact with a buffered saline solution containing glucose. Nicotinic acid was added in the mucosal compartment and its concentration was estimated by reversed-phase HPLC. With time, the vitamin concentration in the mucosal compartment was found to lessen. The vitamin was not detected in the serosal compartment, indicating that, under the prevailing experimental conditions, plain leakage from one compartment to the other did not take place and nicotinic acid was indeed accumulated in the preparation. The effect of anti-bilitranslocase antibodies on nicotinic acid disappearance is shown in Fig. 1. Two curves were obtained by fitting the data to a single-exponential decay equation. As also indicated by the decay constants ratio ($k_{ab}/k_0 = 0.15$), a strong inhibitory effect of the anti-bilitranslocase antibody on nicotinic acid uptake was observed.

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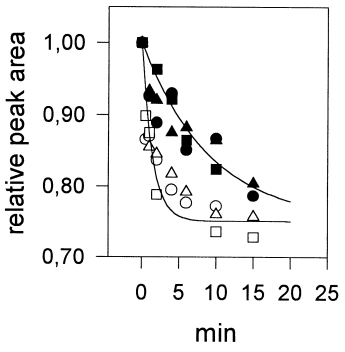


Fig. 1. The effect of the anti-bilitranslocase antibody on nicotinic acid absorption by the isolated rat stomach. Isolated rat stomach patches consisting of the entire surface of the antrum and the corpus were mounted onto a two-chamber device. The serosal compartment consisted of 3 ml of a buffered saline solution (5.55 mM glucose, 115 mM NaCl, 8 mM KCl, 1.25 mM $CaCl_2$, 1.20 mM $MgCl_2$, 2 mM $KH_2PO_4$, 25 mM Tris–HCl pH 7.4). The mucosal surface was pre-incubated at 37°C in 1 ml 0.1 M Tris pH 7.4, 1% (w/v) bovine serum albumin, 5.44 mM glucose without (open symbols) and with (closed symbols) anti-bilitranslocase antibody (96.5 μg IgG). After 10 min, the tube holding the stomach patch was transferred into a vial with 8.5 ml 40 μM nicotinic acid in the buffered saline solution mentioned above. Samples were withdrawn at the indicated times and analysed by HPLC: 20 μl sample was injected into a reversed phase column (Ultrasphere ODS, 5 μm particles, 4.6 mm × 15 cm, Beckman Instruments Inc, San Ramon, CA, USA) and eluted isocratically with 5 mM potassium phosphate buffer pH 6.8/acetonitrile (97:3, v/v). The flow rate was 0.8 ml/min and the elution was monitored at $\lambda = 260$ nm. Under these conditions, at room temperature, nicotinic acid eluted with a retention time around 3 min (typically 2.9). Each type of symbol refers to a single animal. Data were fitted to the single-exponential decay equation. The following decay constants were found: $k_0 = 0.700 \pm 0.08$ $min^{-1}$ ($n = 3$) and $k_{ab} = 0.109 \pm 0.013$ $min^{-1}$ ($n = 3$).

These data show the carrier's accessibility to the antibodies added from the mucosal side, indicating that also in this organ the carrier is expressed at the cell membrane level and, in more detail, that the bilirubin-binding motif is exposed at the outer surface of the cells, as expected for its proposed function.

The most interesting outcome of this work is the demonstration that nicotinic acid can be taken up at the gastric level via a mechanism involving bilitranslocase. The function envisaged for this carrier in the stomach seems to be a real evolutionary advantage, accounting for the maintenance of its expression at the gastric level, since it enables the organism to extract quite minute amounts of nicotinic acid from the food as early as possible during digestion. It may be useful to remember in this context that gastric absorption of nicotinic acid is described both in humans [5] and in other ruminants and bilitranslocase therefore stands as the molecular mechanism fitting this role. Absorption at the level of the small intestine would involve substantially higher concentrations of nicotinic acid, set free during the digestion process. The mechanisms proposed are both passive diffusion driven by post-absorptive metabolism and carrier-mediated transport. The latter consists in two low-affinity carriers, i.e. MCT1, a monocarboxylate–proton co-transporter and AE2, a monocarboxylate–bicarbonate antiporter. Both of these systems display apparent $K_M$ values in the millimolar range.

## References

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