but a proteolysis product having a strong tendency to precipitate at pH 8.0 [6], gave support to our previous hypothesis of a pathogenic role for the premature zymogen activation of over-stimulated acinar cells in pancreatitis [9,10].

REFERENCES

[1] Rouimi, P., Bonicel, J., Rovery, M. and De Caro, A. (1987) FEBS Lett. 216, 195–199.
[2] De Caro, A., Lohse, J. and Sarles, H. (1979) Biochem. Biophys. Res. Commun. 87, 1176–1182.
[3] De Caro, A., Multigner, L., Lafont, H., Lombardo, D. and Sarles, H. (1984) Biochem. J. 222, 669–677.
[4] Montalto, G., Bonicel, J., Multigner, L., Rovery, M., Sarles, H. and De Caro, A. (1986) Biochem. J. 238, 227–232.
[5] Guy-Crotte, O., Amouric, M. and Figarella, C. (1984) Biochem. Biophys. Res. Commun. 125, 516–523.
[6] Gross, J., Carlson, R.I., Brauer, A.W., Margolies, M.N., Warshaw, A.L. and Wands, J.R. (1985) J. Clin. Invest. 76, 2115–2126.
[7] Giorgi, D., Bernard, J.P., De Caro, A., Multigner, L., Lapointe, R., Sarles, H. and Dagorn, J.C. (1985) Gastroenterology 89, 381–386.
[8] Figarella, C., Amouric, M. and Guy-Crotte, O. (1984) Biochem. Biophys. Res. Commun. 118, 154–161.
[9] Figarella, C. (1980) Gastroenterol. Clin. Biol. 4, 631–635.
[10] Steer, M.L., Meldolesi, J. and Figarella, C. (1984) Dig. Dis. Sci. 29, 934–938.

Volume 227, number 1, 82–84 FEB 05471 January 1988

Answers to Dr. Figarella's comment

P. Rouimi, J. Bonicel and M. Rovery and A. De Caro+

Centre de Biochimie et de Biologie Moléculaire, CNRS, 31, Chemin Joseph-Aiguier, BP 71, 13402 Marseille Cedex 9 and *+Groupe de Biochimie Fondamentale, Unité de Recherches de Pathologie Digestive, U 31, INSERM, 46 Blvd. de la Gaye, 13258 Marseille Cedex 9, France*

Received 25 November 1987

1. P. ROUIMI, J. BONICEL AND M. ROVERY'S ANSWER

First we would like to specify that our research group in the Centre de Biochimie et de Biologie Moléculaire, CNRS, Marseille, had nothing to do with the misunderstandings by several members of the same team which led to its breaking up in 1981.

In 1985, Professor H. Sarles and Dr A. De Caro asked us to collaborate in the determination of the primary structure of the human pancreatic stone protein prepared from calculi (PSP) as well as of its immunoreactive forms from the pancreatic juice (PSP S1–5). Our research group is indeed equipped for protein sequencing and trained in structural determination methods. Having worked for several years on pancreas proteolytic enzyme precursors, their activation, their proteolyses, the proposed research was of great interest to us and fitted into the frame of our research field. Together with Dr A. De Caro we endeavoured a thorough study of the various PSP forms.

In the first report [1] written in collaboration, our contribution dealt with amino acid compositions and N-terminal sequence determinations of the 40 and 65 first amino acids of PSP and PSP S1, respectively. At that time we mentioned the report of Dr Figarella's group [2] since the 15 first amino

Correspondence address: P. Rouimi, Centre Biochemie et de Biologie Moléculaire, CNRS, 31, Chemin Joseph-Aiguier, BP 71, 13402 Marseille Cedex 9, France; or A. De Caro, Groupe de Biochemie Fondamentale, Unité de Recherches de Pathologie Digestive, U 31, INSERM, 46 Blvd. de la Gaye, 13258 Marseille Cedex 9, France

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

acids determined for 'Protein X' were identical to those of PSP and PSP S1. No supplementary experiment on the PSP extracted from calculi was carried out, owing to the very small quantity of protein available. A year later we published the entire sequence of PSP S1 [3].

The immunoreactive proteins prepared by A. De Caro, PSP S1 and PSP S2–5 (14 kDa and 16–19 kDa, respectively, evaluated by SDS-gel electrophoresis) have a common C-terminal group and a different N-terminal group (Ile for PSP S1 and a blocked end group for PSP S2–5). It was therefore obvious that a proteolysis in the N-terminal sequence of PSP S2–5 had transformed the protein into PSP S1 [4]. A peptide of PSP S2–5 peptic hydrolysate demonstrated that the cleaved bond was an Arg-Ile bond. The single N-terminal residue which appeared after a mild tryptic proteolysis of PSP S2–5 was Ile. SDS-gel electrophoresis showed that PSP S2–5 bands had practically disappeared to the advantage of a single band comparable to that of PSP S1 [4]. In contrast, with chymotrypsin a weak proteolysis was obtained and several N-terminal residues appeared [4].

We did not deem advisable to cite the article [2] in this matter since the authors had reported that a chymotryptic proteolysis was at the origin of the transformation. Since we knew that the split bond was actually an Arg-Ile bond, it was illogical to assume that the specific cleavage was due to the chymotrypsin. Two hypotheses may be put forward: (i) the cleaved bond in this work [2], was not an Arg-Ile bond but another close bond specifically hydrolyzed by chymotrypsin. It would have been interesting to know the N-terminal residue(s) appearing after this proteolysis. (ii) The chymotrypsin preparation used was contaminated by trypsin. It is regrettable that the authors [2] had not studied the tryptic hydrolysis at the same time.

2. A. DE CARO'S ANSWER

We have studied several molecular forms of PSP. The form present in pancreatic stones, although obviously derived from the secretory form, is peculiar in several aspects: (i) it is associated with pathology; (ii) it has generally remained into calculi for a long time (several years); (iii) its study is difficult given the very small amount found in stones and the small amount of stones available. This is why, after the publication of the partial characterization of PSP [5], we decided to study the secretory forms of this protein. Monoclonal antibodies against PSP were prepared in collaboration with Immunotech, and are now commercially available. Immunoabsorbent columns allowed the fast purification of PSP S, and the further purification of PSP. The amino acid composition of highly purified PSP was different from the first one. This remark has already been noted and published in the same paper [1] as the sequence of the first forty amino acids. The phosphate content of PSP is presently under reinvestigation. The presence of sugar in PSP was claimed on the basis of detection by the Schiff reagent only. A more thorough study is presently being carried out.

Also, we recently described the presence of proteolyzed (unpublished) PSP in certain calculi [6], which could explain the presence of Asx N-terminal, as previously reported.

In [7] we wrote this sentence "These results (obtained with mRNA) support the hypothesis that PSP is a molecular entity, and not a degradation product of trypsinogen 1 or another pancreatic protein". We meant that PSP was a molecular entity and had no similarity with trypsinogen 1 or another already known pancreatic protein.

From the beginning of our work, we disagreed with Dr Figarella's hypothesis that PSP was not a new protein entity, but a degradation fragment of trypsinogen 1 [8]. We have shown that: (i) the samples of trypsin 1 used by Dr Figarella in her work were in fact contaminated by PSP [9]; (ii) using two monoclonals against PSP (D4 and 2E7) no common epitope could be revealed between PSP and pure trypsin [9]; (iii) immunoprecipitation of human pancreatic mRNA translation products with antibodies against PSP and trypsin 1 gave distinct products [7]; (iv) the complete sequence of PSP S1, one of the immunoreactive forms of PSP S, has been determined [3]. It shows no significant homology with the sequences of human trypsin 1 and 2, as derived from the nucleotide sequence of their mRNA [10].

In [2] the authors suggested that PSP could be derived from a precursor which could not be trypsinogen 1, but another protein immunologically related to trypsinogen 1. The above mentioned

elements do not support the possibility of any relationship of PSP S and trypsinogen 1.

REFERENCES

[1] Montalto, G., Bonicel, J., Multigner, L., Rovery, M., Sarles, H. and De Caro, A. (1986) Biochem. J. 238, 227–232.
[2] Guy-Crotte, O., Amouric, M. and Figarella, C. (1984) Biochem. Biophys. Res. Commun. 125, 516–523.
[3] De Caro, A.M., Bonicel, J.J., Rouimi, P., De Caro, J.D., Sarles, H. and Rovery, M. (1987) Eur. J. Biochem. 168, 201–207.
[4] Rouimi, P., Bonicel, J., Rovery, M. and De Caro, A. (1987) FEBS Lett. 216, 195–199.
[5] De Caro, A., Multigner, L., Lafont, H., Lombardo, D. and Sarles, H. (1984) Biochem. J. 222, 669–677.
[6] Multigner, L., Daudon, M., Montalto, G., De Caro, A., Etienne, J.P. and Sarles, H. (1986) N. Engl. J. Med. 314, 248.
[7] Giorgi, D., Bernard, J.P., De Caro, A., Multigner, L., Lapointe, R., Sarles, H. and Dagorn, J.C. (1985) Gastroenterology 89, 381–386.
[8] Figarella, C., Amouric, M. and Guy-Crotte, O. (1984) Biochem. Biophys. Res. Commun. 118, 154–161.
[9] Provansal-Cheylan, M., Lusher, M., De Caro, A., Multigner, L., Montalto, G., Sarles, H. and Delaage, M. (1986) Biochimie 68, 1109–1113.
[10] Emi, M., Nakamura, Y., Ogawa, M., Yamamoto, T., Nishide, T., Mori, T. and Matsubara, K. (1986) Gene 41, 305–310.