Cyclic AMP dependent protein kinase isoenzyme in gastric cancer

H. OHKURA*, N, HATTORI* and H. KITAOKA**

Clinical Laboratory Division, Internal Medicine and Surgery**, National Cancer Center Hospital, Tokyo, Japan.*

SUMMARY

The isoenzyme of adenosine 3′, 5′-monophosphate (c-AMP) dependent protein kinase and c-AMP binding activity were studied on rat and human gastric mucosa by analytical disc-electrophoresis.

Findings on rat specimen revealed tissue specificity of this enzyme. It was noted that the electromobility was changed by c-AMP or gastrin treatment, and by developmental changes from fetus to adult. On human materials obtained during stomach operation, tissue specific isoenzyme patterns and difference between isoenzyme patterns of normal, regenerative, metaplastic and atrophic gland area were found. Cancer tissue shows a variation in the biological and biochemical behavior of this enzyme, some of which are difficult to distinguish from that of benign tissue, while some are not. The former retains a good responsiveness to gastrin, but the latter lost the responsiveness.

INTRODUCTION

It is well known that many hormones promote their biological activity in specific target cells through the universal amplifying transducer composed of adenyl cyclase, c-AMP and protein kinase[1~3]. Some authors[2] have reproted a similarity of c-AMP dependent protein kinase derived from various origins, regarding their biological and physico-chemical properties.

Kitamura *et al.*[5] set forth a simple and highly sensitive method to analyze this enzyme system electrophoretically, and reported the separation of more than three tissue specific protein kinase isoenzymes. This technique is highly convenient to observe the hormonal response of this enzyme through the electromobility difference between the bound form and the free enzyme[6,7].

The intent of our present study is to clarify the developmental change of this isoenzyme on the glandular stomach in connection with malignant tumors of the stomach.

MATERIALS and METHODS

S. D. strain fetus, newborn, young adult rats were sacrificed and the fundic gland mucous membrane was curated. Human materials are obtained from patients with stomach cancer or other benign diseases in the stomach, who were operated on at National Cancer Center Hospital. Specimens from male and female patients between 37 to 77 years of age were used. A part of tissue is curated as in rats, and the other part is studied histologically.

One hundred miligramms of the scraped tissues were homogenized with a chilled glass homogenizer, in five fold 20% sucrose (w/v). The $105{,}000 \times g$ supernatant is applied on a 7.5% acrylamide gel disc-electrophoresis (Analytical Disc. Joko-Sangyo), with a 3 mA current until the dye front migrates 5.5 cm from the origin. The gel is sliced promptly into 30 to 33 pieces and each slice is extracted by 250 microliter of 50 mM Tris-HCl (pH 7.4), with 10 mM of 2-mercaptoethanol for 18 hrs. at 4°C.

With fifty microliters of the extracts, protein kinase activity is assayed through incorporation of radioactive phosphorous from ^{32}P-γ-ATP to calf thymus histone[6] (type II, Sigma).

The binding activity to c-AMP is assayed through incorporation of ^{3}H-c-AMP at 0°C for 30 minutes. The effect of c-AMP is tested by incubation of the supernatant with cold c-AMP (10^{-4} M, at 0°C for 30 min.) prior to electrophoresis. By gastrin, 10^{-5} M of pentagastrin (Peptavlon, I.C.I.) is incubated with the scraped tissue slices in physiological saline at room temperature for 30 minutes.

RESULTS

Electrophoretical zymogram of protein kinase during the animal growth is shown in rat glandular stomach (Fig. 1, dotted line).

On animals 6 days after birth, a prominent peak of kinase was found in slice number 6 (r/f ratio$=0.20$). Fetal mucosa (18 to 20 days of pregnancy) gives almost the same single kinase as that of the six day animal. In a young adult, 105 days old, the highest activity is seen in slice number 15 ($r/f=0.50$). These two peaks coexist during childhood, with a gradual shift of their relative potencies from the fetal type to that of adult one (18 and 35 day sample).

The binding activity to c-AMP moves quite coincidentaly with protein kinase activity (Fig. 1 solid line). This means that this activity is coupled to protein kinase which forms the bound type of c-AMP dependent protein

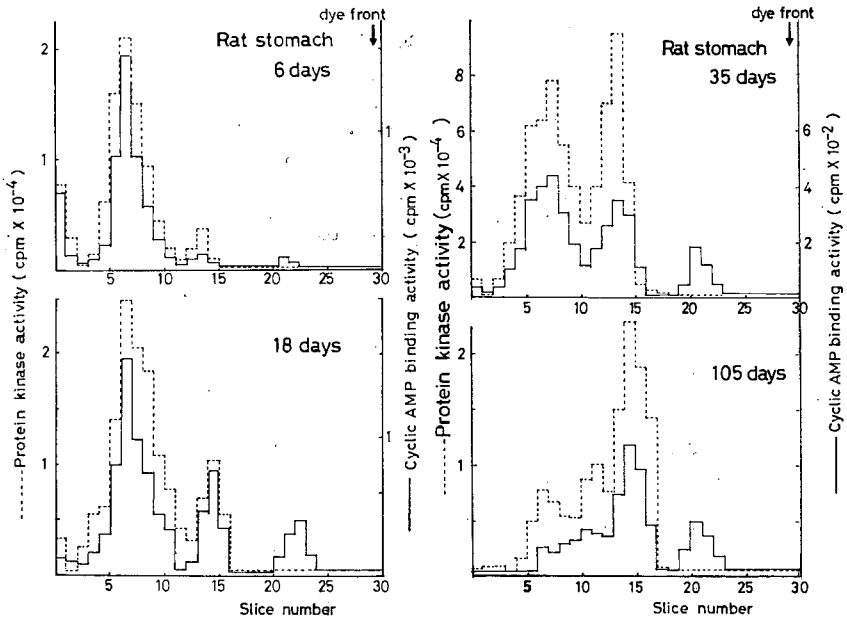

Fig. 1. Isoenzyme changes of cyclic AMP dependent protein kinase in rat fundic gland according to the animal growth.
About one hundred miligrams of the soluble fraction is obtained from the glandular stomach of infant and young adult rats. After electrophoresis in 7.5% acrylamide gel, gel slice extract is assayed for c-AMP binding activity and protein kinase activity. The former is indicated with solid line, the latter, with a dotted line.
The left upper column shows a prominent fetal type peak in a six day baby rat (r/f ratio=0.22). On the right lower column, the prominent peak of a 105 day animal (young adult) is thought to be the adult type kinase (r/f=0.50). A continuous transition is clearly observed through 18 to 35 day patterns.

kinase. These two activities are easily separated through incubation with c-AMP of 01^{-4} M.

Thus the fetal and the adult type of c-AMP dependent protein kinase are thought to compose an isoenzyme group.

Tissue specificity and their hormone dependency are also confirmed but the deta are not shown here (See references 5) and 6)).

In human gastric mucosa, typical "normal" adult protein kinase isoenzyme patterns are shown using histologically "normal" mucosa from an ulcer patient (Fig. 2). There is a difference between rat and human kinase zymograms in the glandular stomach: in the human fundic gland, two increasingly peaks are dominant (Fig. 2 Upper column r/f=0.15 and 0.25)

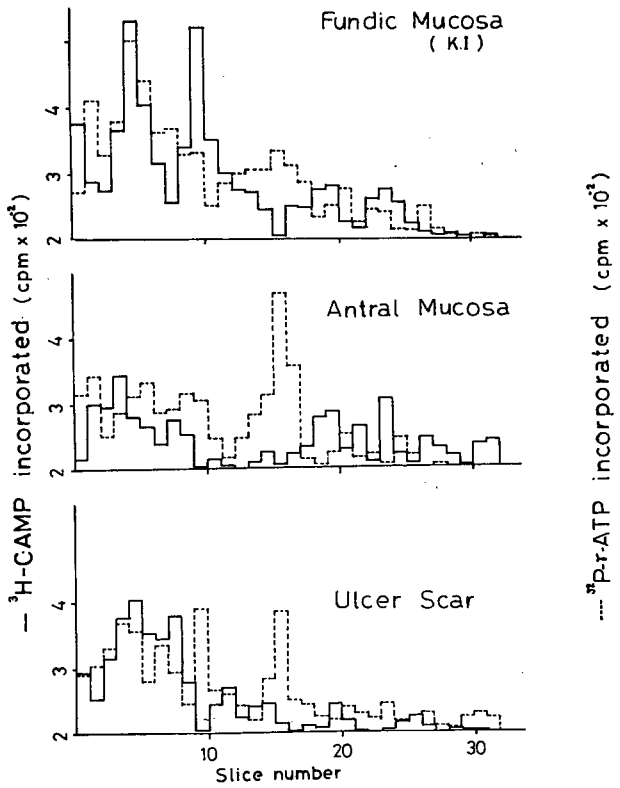

Fig. 2. Protein kinase and cyclic AMP binding activity on a patient with duodenal ulcer.
Subject. Age 37. Male. At the angle of the stomach an ulcer scar was found.
The dotted line shows the kinase activity and the solid line indicates the binding activity to c-AMP. The upper column; normal fundic pattern from histologically normal fundic glands. Middle column; normal antral or pyloric pattern of antrum. The lower column; from a part of ulcer scar composed of young regenerative cells.

which are found in the regenerative tissue of ulcer scar likewise (Fig. 2, Lower column). And these peaks move remarkably when incubated with c-AMP or pentagastrin (Data not shown).

Fig. 3 indicates zymograms obtained from an aged patient with gastric carcinoma. The pyloric glands are highly metaplastic and the tumor is mostly composed of well differentiated muconodullar carcinoma cells (mucocellular cancer cells are also found). The apparent identity of the five protein kinases on the metaplastic antral portion and those in the well dif-

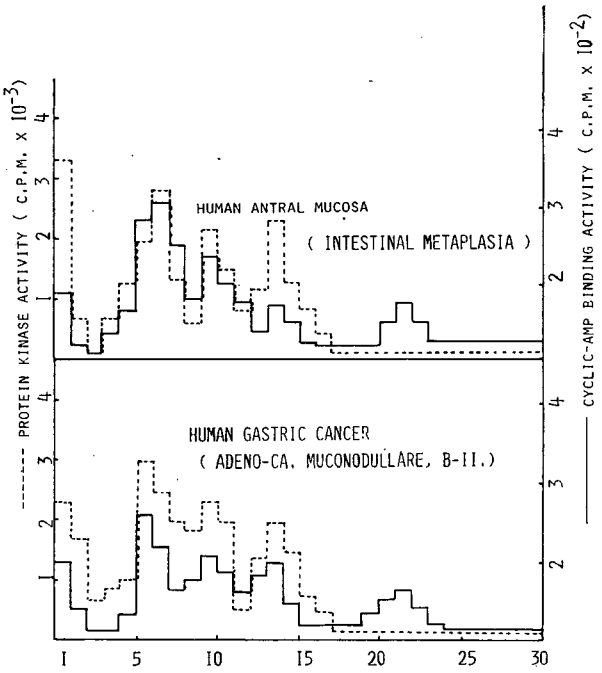

Fig. 3. Protein kinase and cyclic AMP binding activity in a patient with adenocarcinoma of the stomach. Subject male, age 75. Intestinal metaplasia is dominant in the non-cancerous mucous membrane of the stomach.
The upper column; metaplasia with intestinal type cells.
The lower column; well differentiated adenocarcinoma of muconodullar type. Identity of the peaks on two patterns should be noted.

ferentiated cancer tissue should be noted.

Various isoenzyme patterns are obtained from specimens of atrophic gastritis, intestinal metaplasia in the fundus, a part of lympocyte infiltration and malignant tumors including differentiated and undifferentiated cancer cells. It is not clear as to whether there may be a cancer specific fraction of this isoenzyme because of the complexity in overlapping of free and bound type kinase peaks in man. On the other hand, well differentiated adenocarcinoma posesses almost the same isoenzyme pattern as that of the surrounding benign but metaplastic mucosa. And these differentiated tissues respond well to c-AMP and gastrin.

In fresh tissue of any origin when treated with 10^{-4} M of c-AMP, the main peaks of kinase showed a decrease in their activities and more anodic

new peaks appear. In two-thirds of the tissue examined, pentagastrin promoted the same responses as in the case of c-AMP, but its potency is less than c-AMP. The last one-third revealed no activation by gastrin even though they respond well to c-AMP. These groups include undifferentiated carcinomas, so-called schirrus type, profound atrophy and lymphoreticullar hyperplasia.

DISCUSSION

There is little doubt that analytical disc-electrtophoresis is a superior tool for isoenzyme study. But no reports are available regarding the use of gel slice for protein kinase assay. In our unpublished data, protein kinase inhibitors, co-existing in the soluble fration, can be excluded through disc-electrophoresis and therefore we can obtain a highly sensitive enzyme assay system. In our system, a peak fraction of kinase free from c-AMP binding activity indicates a free type (catalytic unit) of this enzyme, or a c-AMP independent enzyme. And a peak of kinase activity combined with that of c-AMP binding property indicates, in most cases, the bound form (regulatory unit-catalytic unit complex) but not in all cases. Especially on man, more than three peaks of kinase are crowded in the cathodic one third of the gel and they are overlapped with the peaks of c-AMP binding activity of both free and bound type.

But c-AMP promotes migrations of those peaks and revealed new peaks of free protein kinase and free binding protein.

Rat stomach has a simple fraction of protein kinase and seems to be a good model to study the carcino-fetal relationship of this enzyme, although we have not examined gastric carcinoma materials in rat. Hence our discussion must be limited to data in clinical materials. As described before, there is no apparent difference between benign cells and well differentiated tumors. But in undifferentiated cancer tissue, clearly different isoenzyme patterns are seen which showed no response to the gastrin.

When a cell responds to c-AMP well but does not respond to the hormone, the possible mechanisms are as follows ; 1) difference in the hormone receptor at the cell surface and the resulting change in the next information into the cell, 2) appearance of unknown protein kinase inhibitor of c-AMP independent type, 3) another kinase system coupled to other nucleotides (c-GMP, etc···) or 4) changes in the substrate specificity of the kinase.

It is interesting that young or undifferentiated cells possess different (usually more cathodic) isozymes compared with those of adult or old well differentiated cells. Thus it may be reasonable to refer to this c-AMP

dependent protein kinase as a new member of the carcinofetal isozyme group.

REFERENCES

1. REIMANN, E. M., BROSTROM, C. O., CORBIN, J. D., KING, C. A. and KREBS, E. G.: Separation of regulatory and catalytic subunits of the cyclic 3′, 5′-adenosine monophosphate-dependent, protein kinase (s) of rabbit skeletal muscle. *Biochem. Biophys. Res. Commun.*, **42**, 187 (1971).
2. KUMON, A., *et al.*: Mode of action of adenosine 3′, 5′-cyclic phosphate on protein kinase from rat liver. *Biochem. Biophys. Res. Commun.*, **41**, 1290 (1970).
3. GILL, G. S. and GARREN, L. D.: Role of the receptor in the mechanism of action of adenosine 3′: 5′-cyclic monophosphate. *Proc. Nat. Acad. Sci. U.S.A.*, **68**, 786 (1971).
4. TAO, M., SALAS, M. L. and LIPMANN, F.: Mechanism of activation by adenosine 3′: 5′-cyclic monophosphate of a protein phosphokinase from rabbit reticulocytes. *Proc. Nat. Acad. Sci. U.S.A.*, **67**, 408 (1970).
5. KITAMURA, T., YAMAMURA, T., OHKURA, H. and SAKODA, M.: Study on specificity of various tissues to (cyclic 3′, 5′)-AMP binding protein and protein kinase. *Rinsho kagaku Symposium* (*Proc. Symp. Chem. Physiol. & Path.*, **11**, 211 (1971). (in Japanese)
6. OHKURA, H., KITAMURA, T. and YAMAMURA, T.: On the role of cyclic AMP in gastric juice secretion (4) c AMP bound protein and protein kinase of gastric mucosa. Igaku no Ayumi **86**, 739 (1973). (in Japanese)
7. OHKURA, H. and YAMAMURA, T.: *Nippon Shokaki-byo Gakkai Zasshi* (Jap. J. Gastroenterol). (in Japanese) (in press).
8. BERNARD, E. A. and WASSERMANN, G. F.: Protein kinase activities in rat epididymis. Effects of maturation and testosterone treatment. *Biochem. Biophys. Res. Commun.*, **49**, 434 (1972).
9. FURIHATA, C., KAWACHI, T. and SUGIMURA, T.: Premature induction of pepsinogen in developing rat gastric mucosa by hormones. *Biochem. Biophys. Res. Commun.*, **47**, 705, (1972).
10. SANO, R., FUKUTOMI, H. and TAKAZAWA, H.: Four points biopsy of chronic gastritis. *I to Cho* (*Stomach and Intestine*), **6**, 227 (1971). (in Japanese)