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CHARACTERIZATION OF MICROSOMAL GLYCOSYL-TRANSFERASES OF RAT PANCREAS AND INFLUENCE OF DIETS

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SUMMARY : Four rat pancreatic microsomal glycosyl-transferases (fucosyl-, galactosyl-, mannosyl- and N-acetylglucosaminyl-transferases) are studied and characterized for their optimal conditions and their relation with interfering reactions (glycosyl-nucleotide pyrophosphatases, osidases and proteinases). Dietary treatments of the rats induce modifications : for all the transferase activities, the highest levels are found in a high-starch diet and the lowest one in a high-fat diet. The activities found in the standard diet are at the level of the high-starch or of the high-fat diet depending on the enzyme studied. The observed modifications are not explained by alterations in physico-chemical parameters of the enzymes or by intervention of glycosyl-nucleotide pyrophosphatases, osidases or proteolytic enzymes. The modifications observed for the mannosyl-transferase are predominantly found in a lipid fraction extracted by chloroform-methanol (2/1).

INTRODUCTION

Though secretory proteins of the exocrine pancreas are not usually glycoproteins, attention has focused these last years upon the function of glycosyl-transferases (1-8) and the characterization of membrane glycoproteins of zymogen granules, possibly involved in the secretory process (9,10). On the other hand, the exocrine pancreatic secretion is dependent on the composition of the diet (11-19). Since previous works of our laboratory have shown that rat intestinal glycosyl-transferase activities can also be modified by diets (20-21), it seemed interesting to study the influence of the same diets on glycosyl-transferases activities in an other organ, not directly in contact with the nutriments, but also related to digestion. The present paper characterizes four rat pancreatic glycosyl-transferase activities (fucosyl-, galactosyl-, mannosyl- and N-acetylglucosaminyl-transferase) and their modifications by diets.

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MATERIALS AND METHODS

Animals and diets, cellular fractionation

The animals used in the experiments and the dietary treatments : standard diet used as a control, conditions of supplementation to obtain high-starch and high-fat diets, have been already described (20,21). Two supplemented diets have been studied : diet supplemented with starch, 30 % by weight and diet supplemented by a mixture of corn and rapeseed oil (in a 1/1 ratio), 30 % by weight during two weeks.

Animals were sacrificed by decapitation. The duodeno-pancreatic area was spread, the pancreas was completely excised after elimination of venous trunks and adipose tissue. The organs were homogenized in ice-cold 50 mM Tris-maleate buffer (pH 6.2), 0.3 M sucrose, soybean trypsin inhibitor (0.25 mg/ml) (10 ml/g of wet tissue) in a Potter-Elvejhem homogenizer (10 strokes/min.). The homogenate is centrifugated according to Ronzio (1). The last centrifugation (105,000 g, 90 min.) led to sedimentation of microsomes which, homogenized in a 50 mM Tris-maleate buffer, pH 5.5, whithout neither soybean trypsin inhibitor nor sucrose, are used for the enzymatic determinations.

Determination of enzymatic activities

Four glycosyl-transferase activities are determined : galactosyl-transferase (E.C. 2.4.1.38) with and without exogenous acceptor (chemically desialyzed and degalactosylated fetuin as described by Ko and Raghupathy (22); this treatment eliminates minimum 80 percent of sialic acid and galactose) ; fucosyl-transferase (E.C. 2.4.1.68) with and without desialyzed fetuin (22) as exogenous acceptor (20 µM in incubation mixture), N-acetyl-glucosaminyl-transferase (E.C. 2.4.1.94) and mannosyl-transferase (E.C. 2.4.1.83). Their determination are made in 200 µl of microsomal suspension by study of the incorporation of [^{14}C]-sugar on endogenous and exogenous acceptors using 20 nCi by essay of XDP-[^{14}C]-sugar as substrate (respectively : UDP-[^{14}C]-galactose, 298 Ci/mole ; GDP-[^{14}C]-fucose, 189 Ci/mole ; UDP-[^{14}C]-N-acetylglucosamine, 306 Ci/mole and GDP-[^{14}C]-mannose, 269 Ci/mole, NEN Chemicals). Incorporation was determined in zero order kinetics and optimal conditions for temperature (23° C), pH (5.5) and manganese concentration (as reported in table I). These optimal conditions are not modified by the various diets. In all the enzymatic determinations the reaction was stopped by addition of trichloracetic acid 20 % (w/v).

However, for the mannosyl-transferase, the reaction was also stopped by the addition of 20 volumes of a chloroform-methanol (2/1 by vol.) mixture (23). The organic phase (called 2/1 extract in table III), washed by a chloroform-methanol-water (3/48/47) mixture, is containing a [^{14}C]-

TABLE I
OPTIMAL CONDITIONS FOR DETERMINATION OF GLYCOSYL-TRANSFERASE ACTIVITIES

Glycosyl-transferase	Optimal range		
	Temperature (°C)	pH	Mn^{++} (M)
Mannosyl-transferase	21-30	5.5	10^{-2}
Galactosyl-transferase	18-23	5.5	5.10^{-2}
Fucosyl-transferase	18-23	5.2-5.5	5.10^{-3}
N-acetylglucosaminyl-transferase	21-23	5.5-7.0	5.10^{-3}

mannolipid. The protein layer at the interface between organic and aqueous phases was collected and extracted by a chloroform-methanol-water (10/10/3 by vol.) mixture (24).

The action of glycosyl-nucleotide pyrophosphatases (E.C. 3.6.1.21) was estimated by chromatography of the incubation mixture (25) and adenosine triphosphate (ATP) (2 mM final concentration) was added for the determination of fucosyl-transferase activity (7), since adenosine monophosphate (AMP), which is a good inhibitor of intestinal pyrophosphatases inhibits the pancreatic transferase. Neutral and acidic proteinases were determined by the use of [^{3}H]-acetyl-hemoglobin (26).

Statistical treatment of the results

For each enzymatic activity, linear regression curves (activity versus time) were determined to estimate the linearity of the reaction (see fig. 1). Then, the values were expressed in pmole.min^{-1}.mg^{-1} protein. The means ± SD for 4 to 12 similar experiments depending on the studied enzyme were calculated and compared using the Student's t test.

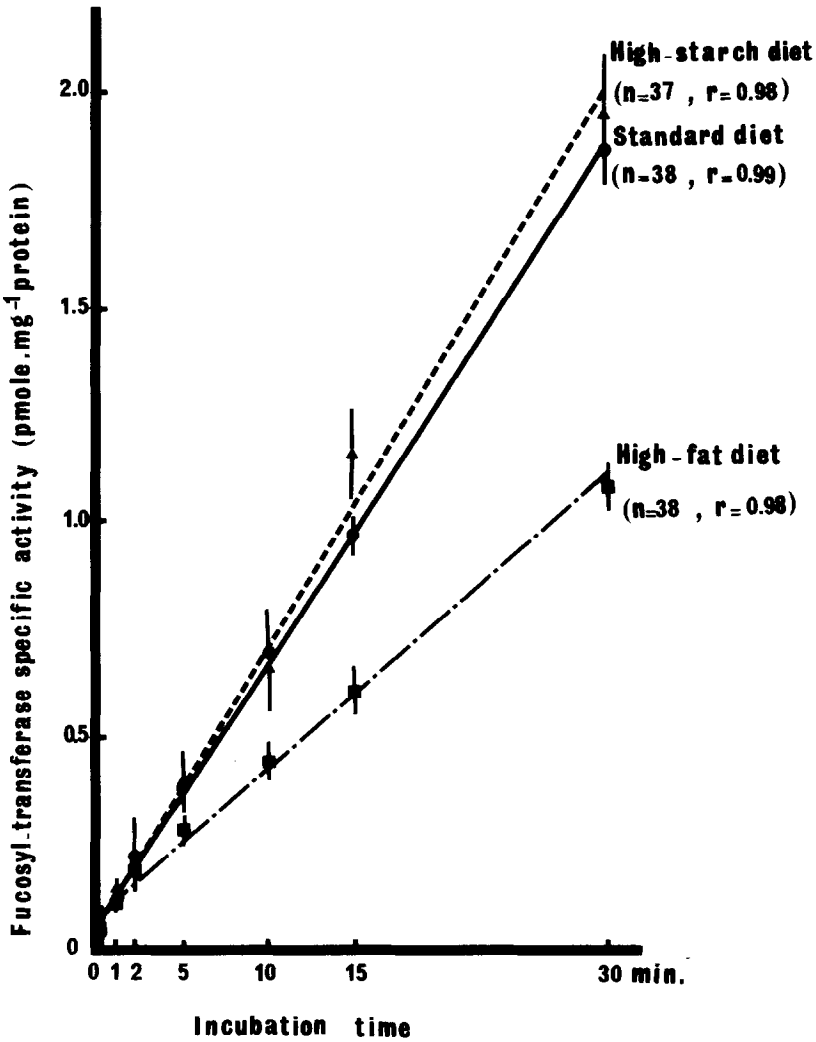

Figure 1. Influence of diets on fucosyl-transferase activity. Kinetics are calculated by linear regression on (n) enzymatic determinations, (r) : coefficient of correlation.

RESULTS

Weight of animals and pancreas, and food intake

Rats fed with an high-fat diet have a lower weight than the others, but differences between the three groups are not significant in the course of the experiment. The daily food intake is also lower with the high-fat diet, though the caloric intake is the same for these animals as that observed for the standard diet (Table II). The daily protein intake varies among the three groups and is in the inverse ratio to the relation energy-protein of the diet. The weight of the pancreas is significantly lower for the animals fed a high-fat diet, with regard to the other groups, the weights of which are quite similar.

Variations of the glycosyl-transferase activities

Figure 1 showns (for example) the regression curves calculated for the fucosyl-transferase activity, measured with exogenous acceptor and ATP. The correlation coefficient (r = 0.98-0.99) indicates an excellent linearity for the reaction from 0 to 30 minutes of incubation and a very good reproducibility, since these curves collect the results obtained in four different experiments. The enzymatic activity is lower in the microsomes of rats fed a high-fat diet than in the microsomes of rats fed a standard or a high-starch diet. Similar curves could be shown for the galactosyl-transferase activity (r = 0.85-0.94, from 0 to 15 min.), the N-acetyl-glucosaminyl-transferase activity (r = 0.88-0.92 from 0 to 15 min.) and for the mannosyl-transferase activity (r = 0.89-0.95 from 0 to 5 min.).

Table III collects the results obtained for these four enzymes and gives the signification of the Student's t test between the three dietary groups ; the behaviours of the enzymes are quite different and can be summarized as follows.

- The comparison of high-starch and standard diets (pointed out by a in the table III) shows a significant difference for the galactosyl- and the mannosyl- transferase activities : the highest activities are found for the high-starch diet. The high-starch versus standard ratio is 1.52 (without exogenous acceptor) and 1.24 (with exogenous acceptor) for galactosyl-transferase and 1.83 for mannosyl-transferase.
- The comparison of high-starch and high-fat diets (b in the table III) gives significant differences for all the enzymes ; the high-starch versus high-fat ratio is : 1.62 for the fucosyl-transferase (with exogenous acceptor) and 1.44 (without exogenous acceptor) ; 1.91 for the galactosyl-transferase (with exogenous acceptor) and 1.85 (whithout exogenous acceptor) ; 1.63 for the N-acetylglucosaminyl-transferase and 1.50 for the mannosyl-transferase.

TABLE II

NUTRITIONAL FACTORS FOR THE THREE DIETARY GROUPS OF RATS

Diet	Dietary intake (g/day)	Weight of pancreas (g)	Energy intake (KJ/day)	Protein intake (g/day)	Ratio Energy/ Protein	Starch intake (g/day)
Standard diet (control)	24.8	0.99±0.09 (n=15)	342	4.2	81	14.6
High-starch diet (30 % by weight)	27.7	0.96±0.09 (n=15)	406	3.3	123	19.7
High-fat diet (30 % by weight)	17.1	0.68±0.09 (n=15) *	357	2.0	178	7.0

Weight of pancreas of high-fat diet groups is significantly different (* $P < 0,001$) from the two other groups.

- The comparison of standard and high-fat diets (*c* in the table III) shows significant differences for the fucosyl- and the galactosyl-transferase activities when measured with the appropriate exogenous acceptor ; the standard versus high-fat ratio is respectively 1.52 and 1.54. When the incubations are performed without exogenous acceptors, the differences are not significant. For the N-acetylglucosaminyl-transferase, the ratio is 1.68.

The extraction of the mannosyl-transferase incubation mixture by organic solvents shows that almost all the radioactivity is found in the first organic phase (chloroform-methanol (2/1) extraction), while the radioactivity is very low, either in the proteic pellet or in the second organic phase (extraction of the proteic layer by chloroform-methanol-water (10/10/3)). The table III indicates that the modifications shown in the organic phase are similar to that observed in trichloracetic insoluble material

Control of the glycosylation reaction

In order to prove the validity of the observed differences in the enzymatic behaviour with respect to the various diets, several critical points have been investigated.

- The optimal temperature, the optimal pH and the optimal ionic concentration are the same, for each enzyme, whatever the diet may be.
- The nucleoside-diphospho-sugars and their degradation products (by the glycosyl-nucleotide-pyrophosphatases) are separated by chromatography of the supernatant of the incubation mixture at : 15 min., 10 min. and 5 min. for the fucosyl-, galactosyl- and mannosyl- transferases respectively. The rate of glycosyl-nucleotide hydrolysis is similar in the microsomes

TABLE III

INFLUENCE OF DIFFERENT DIETS ON PANCREATIC MICROSOMAL GLYCOSYL-TRANSFERASES

Glycosyl-transferase	Diet		
	Standard diet	High-starch diet	High-fat diet
Fucosyl-transferase without exogenous acceptor	0.030 ± 0.015 (26)	0.036 ± 0.017 (25) *b* *	0.025 ± 0.015 (26) *b* *
with exogenous acceptor	0.064 ± 0.010 (26) *a* ***	0.068 ± 0.011 (26) *b* ***	0.042 ± 0.009 (25) *b* *** *c* ***
Galactosyl-transferase without exogenous acceptor	0.033 ± 0.014 (34) *a* ***	0.050 ± 0.018 (32) *a* *** *b* ***	0.027 ± 0.014 (29) *b* ***
with exogenous acceptor	0.054 ± 0.022 (44) *a* ** *c* ***	0.067 ± 0.021 (44) *a* ** *b* ***	0.035 ± 0.013 (46) *b* *** *c* ***
N-acetylglucosaminyl-transferase	0.032 ± 0.023 (18) *c* **	0.031 ± 0.015 (18) *b* *	0.019 ± 0.008 (17) *b* * *c* **
Mannosyl-transferase trichloracetic precipitate	2.133 ± 0.792 (89) *a* ***	3.898 ± 1.381 (86) *a* *** *b* ***	2.163 ± 0.779 (90) *b* ***
Mannose-containing lipid (2/1 extract)	2.034 ± 0.568 (51) *a* ***	4.044 ± 1.217 (49) *a* *** *b* ***	2.144 ± 0.621 (51) *b* ***

- Glycosyl-transferase activities are given in pmol.min^{-1}.mg^{-1} protein.
- Results are given as means ± SD of (n) determinations of enzymatic activity and compared by Student's *t* test : *a* = comparison of standard diet versus high-starch diet groups ; *b* = comparison of high-starch versus high-fat diet groups ; *c* = comparison of standard versus high-fat diet groups . Results are significantly different : * P < 0.025, ** P < 0.005, *** P < 0.001.

prepared from rats fed with the three diets. This rate remains low and does not alter the linearity of the reaction ; it varies from 4 to 10 % for the fucosyl-transferase (incubated in presence of ATP, as compared with 50 to 75 % breakdown whithout ATP), from 22 to 27 % for the galactosyl-transferase and from 8 to 25 % for the mannosyl-transferase.

- Contrary to the pyrophosphatases, the proteolytic activities of the microsomes vary with the nature of the diet : the acidic proteinases are less active in the microsomes from high-fat diet than in those from the two

other groups, as well in terms of specific activity as in terms of the total activity of the fraction. On the other hand, the neutral proteinases are more active in the microsomes prepared from rats fed a high-starch diet than in those from the two other groups, which are very similar.

DISCUSSION

The results reported here describe significant differences in four glycosyl-transferase activities measured in pancreatic microsomal fractions prepared from rats fed three different diets. For all these enzymes, the microsomal fractions prepared from rats fed a high-starch diet have the highest activity, while the microsomal fractions prepared from rats fed a high-fat diet have the lowest activity. With the standard diet, used as a control, the enzymatic activities are at the level of one of the other diet, depending on the studied enzyme : similar to high-starch preparations for fucosyl- (with exogenous acceptor) and N-acetyl-glucosaminyl-transferases ; similar to high-fat preparations for galactosyl- (without exogenous acceptor) and mannosyl-transferases ; at a middle level for fucosyl- and galactosyl-transferases (without and with exogenous acceptor respectively).

In order to relate these data to the glycosyl-transferases themselves, several parameters, which are known to interfere with the reaction of glycosylation, have been studied. Firstly, we have controled the optimal conditions for the measure of these transferase activities and verified that they are not modified by diets (table I). Secondly, the breakdown products resulting from the action of glycosyl-nucleotide pyrophosphatases and/or from the action of osidases on the glycoconjugates, are found in low amounts in the incubation mixtures, except for the fucosyl-transferase where the use of an inhibitor (ATP) is needed, in good agreement with the works of Völkl (7). The action of these two degradative enzymes is similar in the three diets and does not alter the linearity of the transfer reaction which is always measured at initial velocity. Thirdly, in the pancreas, it was imperative to estimate the residual activity of proteolytic enzymes, which can destroy either the enzyme or the glycoproteinic acceptor. Indeed, soybean trypsin inhibitor is used during fractionation steps, but not in the incubation buffer. Some differences appear in proteolytic activities which cannot explain the observed variations of glycosyl-transferase activities : neutral and acid proteinases are the less active in the fractions prepared from rats fed a high-fat diet as are the glycosyl-transferase activities ; the variations of proteolytic activities are therefore parallel to that of glycosyl-transferases and do not interfere with these last activities.

The last point of the controls deals with the endogenous amounts of acceptor or of glycosyl-nucleotides. When an exogenous glycoproteinic acceptor is available (fucosyl- and galactosyl-transferases), its addition to the incubation mixture enhances the transfer of the radioactive sugar multiplication factor from 1.3 to 2.1 (table III). Besides, the linearity of the reaction remains correct in presence of exogenous acceptor. This fact suggests that the endogenous amounts of available glycosyl-nucleotides, although not known, are sufficient, since the radioactive glycosyl-nucleotides are always added in low amounts (in the range of 0.5 µM final concentration).

On the other hand, the addition of a glycoproteinic acceptor gives further informations for the interpretation of the observed variations. For the galactosyl-transferase, the increase in activity is greater for the preparations obtained from rats fed a standard diet than from rats fed a high-starch diet, which suggests that this last group has a greater amount of endogenous acceptor. This phenomenon is less marked for the fucosyl-transferase. Inversely, the increases for the fucosyl- and galactosyl-transferases activities are significantly lower in the preparations obtained from rats fed a high-fat diet than in those from the two other groups ; in this case, the hypothesis are either a deficience in the actual amount of glycosyl-transferase quantity or a modification of the fatty acids composition of the membranes which could alter the enzymatic mechanism.

Since the four enzymatic activities studied have not the same behaviour with the three different diets, the nutritional interpretation is still unclear. Although high-starch and high-fat diets are known to induce adaptation of some secreted pancreatic enzymes (27), the observed differences of glycosyl-transferase activities cannot be related for all the enzymes to the nutritional factors reported in table II : neither the protein intake, the energy intake nor the starch intake seems to be the factor which can explain all the modifications. In the rat small intestine mucosa, only the fucosyl-transferase activity is modified (decreased) by high-fat diet (20) while high-starch diet does not induce modifications. The mechanism consists of a decrease of the enzyme (21) and an additional regulatory effect of free fatty acids (28). By contrast, in the rat pancreas, the results remain still a phenomenological approach and require more defined studies. The mannosyl-transferase is choosen as a model, because of its high activity and of the possible existence of a polyprenic intermediate (as suggested by extraction with chloroform-methanol (2/1)), which has not been yet described in the rat pancreas ; moreover, this product has been reported to vary with diet in the rat adipose tissue (29), or in rat liver (30,31). We characterize this product and demonstrate that it is the rate limiting factor in an other paper (32).

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