EFFECT OF GLUCAGON ON PHOSPHORYLATION OF SOME RAT LIVER RIBOSOMAL PROTEINS IN VIVO

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1. Introduction

We have previously shown [1] that some ribosomal proteins of rat liver were phosphorylated in vivo, on serine residues and that isolated ribosomes of rat liver could also be phosphorylated in vitro by ATP and a kinase from microsomal supernatant of rat liver. This phosphorylation was estimated by cyclic AMP at physiological doses (half-stimulation at 5×10^{-8} M). It is well known that cyclic AMP is the cellular mediator of glucagon in rat liver [2]. Therefore we have studied the effects of glucagon on the in vivo phosphorylation of ribosomal proteins of rat liver.

We have observed that 30 min after injection of glucagon and $H_3{}^{32}PO_4$, phosphorylation of ribosomal proteins was strongly increased. Autoradiography after electrophoresis of these proteins showed that phosphorylation of one of these proteins was stimulated 2 or 3 fold after injection of glucagon.

2. Methods

Sprague Dawley rats weighing 300–350 g were injected intraperitoneally with 600 μg of glucagon in 1 ml of 0.14 M NaCl. 2 mCi of carrier-free $H_3{}^{32}PO_4$ in 1 ml of 0.14 M NaCl was administered immediately after hormone injection. Control animals received only $H_3{}^{32}PO_4$. Animals were sacrificed by decapitation 30 min later.

Ribosomes were isolated from the pooled livers of 3 rats according to Littlefield et al. [3], purified by resuspension in 0.44 M sucrose, 1 mM Mg^{2+}, precipitated by raising the Mg^{2+} concentration to 5×10^{-2} M and centrifuged. This operation was repeated twice. The ribosomes were further purified by suspending in a buffer containing 1 mM Tris-HCl (pH 7.6) and 1 mM $MgCl_2$ [4] and dialyzed overnight against the same buffer. The suspension was then centrifuged at 20,000 *g* for 15 min. The supernatant contained the purified ribosomes which were reprecipitated by 5×10^{-2} M $MgCl_2$. The spectral ratio of the purified ribosomes A_{260} nm/A_{280} nm was greater than 1.7.

Ribosomal proteins were extracted from ribosomes with 66% acetic acid [5], precipitated by one volume of 10% TCA, washed with 5% TCA, then redissolved in 6 M urea and dialyzed for 18 hr against several changes of this medium.

Electrophoresis was performed in 7.5% mixed agarose acrylamide gels (agarose 0.8%; acrylamide 7.5%) [6] at pH 4.5 in 6 M urea [7]. Autoradiography was done by applying a Kodirex film on the dry gel. Densitometry of gels after coloration of proteins with Coomassie Blue and autoradiography was effected with a "Vernon" densitometer. Controls of radioactivity were also made after cutting the gels and counting the bands.

Determination of protein-bound phosphate radioactivity after extraction of acyl and acid-labile protein-bound phosphate was performed on isolated ribosomal proteins as follows: 100 μg of ribosomal proteins were deposited on Whatman 3 MM filters. After extraction of acid-soluble material with cold 15% TCA followed by two washings with this reagent, nucleic acids, and acyl and acid-labile protein-bound phosphate were extracted with 5% perchloric acid at 95° for 15 min and washed twice with this reagent at 0°. After washing with alcohol–ether (1:1) the disks were dried and d

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placed in vials for counting the radioactivity in a Packard scintillation counter.

3. Results

The comparative incorporation of alkali-labile phosphate, after injection of glucagon, into total ribosomal proteins was determined (table 1). It appears that glucagon strongly increased the radioactivity incorporated. The observed counts were corrected to account for individual variations in the labeling of phosphate pools which was determinated on the acid-soluble fraction of the liver homogenate. Glucagon did not produce significant changes in this labeling; this is in agreement with the observations of Langan [8].

Fig. 1 shows the comparative electrophoresis of ribosomal proteins. In fig. 1a the electrophoretic patterns of the proteins after coloration are given. It can be seen that glucagon did not change the number of bands or their relative intensity. In contrast, the autoradiographic patterns are different (fig. 1b). There is a general increase of the protein radioactivity after glucagon injection. This increase is particularly visible (2 to 3 fold) on band (M) for which the phosphorylation is the strongest in the controls. However, one also observes the appearance of radioactive bands which were detected with difficulty in the controls. In table 2 we have given the increase in the density of the major band M. We have checked that in our conditions the density was proportional to the radioactivity. As in table 1 we have corrected the values to account for the radioactivity of the precursors. It should be noted that we did not observe in our conditions any change of the ribosomal RNA radioactivity. In some experiments theophylline, an inhibitor of cyclic AMP phosphodiesterase, was injected at the same time as glucagon. It appears that increase of radioactivity was not appreciably modified by this addition.

Table 1
Effect of glucagon on ribosomal proteins phosphorylation.

Experiment	cpm/mg prot.		Stimulation (%)
	C	G	
A	350	750	115
B	370	710	92

Each experiment was performed with pooled livers of 3 rats after injection of $H_3{}^{32}PO_4$ (C) or $H_3{}^{32}PO_4$ + glucagon (G).

Table 2
Increase of major band (M) labeling after injection of glucagon.

Experiment	Increase of radioactivity (%)
1	180
2	280
3	290
4	210
5	200

In experiments 3, 4 and 5 the animals received 18 mg of theophylline with glucagon and $H_3{}^{32}PO_3$.

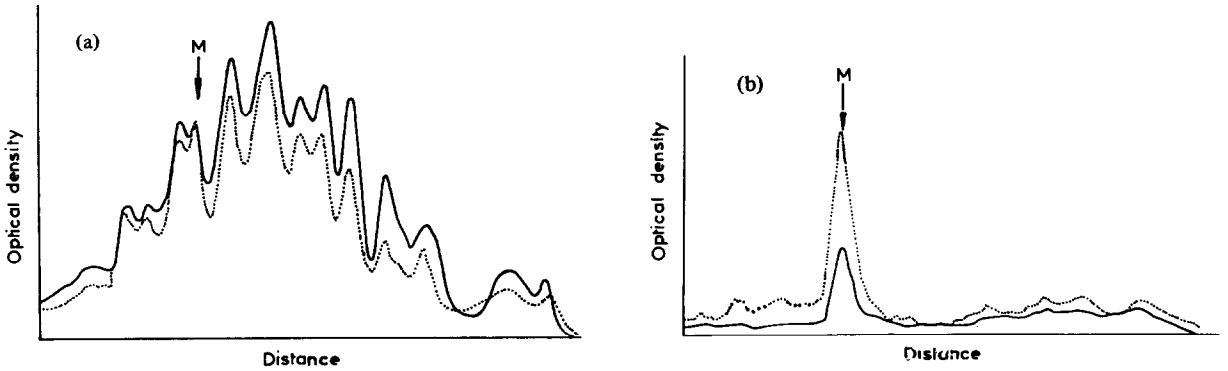

Fig. 1. (a) Densitometry after electrophoresis and coloration of the ribosomal proteins. (exp. no. 5). —— control; - - - - glucagon. (b) Densitometry of the autoradiography of the same electrophoresis as (a). —— control; - - - - - glucagon.

4. Discussion

We have found that ribosomal protein phosphorylation was strongly stimulated 30 min after glucagon injection. With the same doses of glucagon Butcher et al. [9] have observed that the level of cyclic AMP was increased 40 fold. This stimulation is particularly clear on one of the ribosomal proteins, perhaps because it is the most strongly labeled band of the controls, but it appears that the phosphorylation of other proteins is also increased. We have previously shown [1] that a cyclic AMP-dependent kinase from rat liver microsomal supernatant was able to phosphorylate in vitro proteins of isolated ribosomes. This kinase has been purified. It seems to be analog of the cyclic AMP-dependent histone kinase of liver [10] and can phosphorylate, as shown by electrophoresis, several ribosomal proteins (unpublished results). Eil and Wool [11] have also purified a cyclic AMP-dependent kinase which phosphorylated several proteins of rat liver ribosomal subunits.

It is known that glucagon or cyclic AMP can induce the synthesis of some rat liver enzymes like tyrosine transaminase [12] and serine dehydratase [13]. There are indications that cyclic AMP acts at the translational level not only in rat liver but in other mammalian tissues [14, 15]. Our results are in favour of this hypothesis and suggest as we have already proposed [1] that at least one of the actions of cyclic AMP at this level is mediated by the phosphorylation of some ribosomal proteins. This is not exclusive of an action at the transcriptional level (Langan) [8]. Walton et al. [16] have found that a cyclic AMP-dependent kinase from adrenal glands can phosphorylate ribosomal proteins in vitro, and have proposed that this phosphorylation has a regulatory role in the cyclic AMP translational control of adrenal cortical protein synthesis. Kabat [17], who found that in reticulocytes several ribosomal proteins were phosphorylated and related the phosphorylation of two of these proteins with the activity of ribosomes, did not observe an effect of cyclic AMP, but the mechanism of action of this nucleotide in reticulocytes can be different.

The purification of the ribosomal proteins which are phosphorylated in vivo and the study of the turnover of their phosphate groups should allow a better understanding of the mechanism by which they could regulate the synthesis of specific proteins.

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