THE FULLY-ACTIVE NATURE OF SYNTHETIC AND HYDROLYTIC ACTIVITIES OF GLUCOSE-6-PHOSPHATASE OF INTACT NUCLEAR MEMBRANE*

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SUMMARY: Carbamyl-P:glucose phosphotransferase, mannose-6-P:glucose phosphotransferase, and mannose-6-P and glucose-6-P phosphohydrolase activities of D-glucose-6-P phosphohydrolase (EC 3.1.3.9) have been demonstrated in avian and mammalian liver (and kidney) nuclear membrane. In marked contrast with activities of this enzyme of fragmented endoplasmic reticulum ("microsomes"), those of the intact membrane of isolated nuclei are totally, or nearly-totally, manifest without the need for preliminary activation by detergents or similar treatments. Disruption of nuclei and isolation of nuclear membranes results in the acquisition of detergent-sensitivity of such activities. Physiological implications of these observations are discussed.

Glucose-6-phosphatase (D-glc-6-P[†] phosphohydrolase; EC 3.1.3.9) of liver, kidney, small intestine, and pancreas is now known to possess potent synthetic as well as hydrolytic activities (1). For example, in addition to its traditional hydrolase activity (Reaction 1), the enzyme also catalyzes the transfer of a phosphoryl group from such compounds as PP_i, various nucleoside tri- and diphosphates, phosphoenolpyruvate, carbamyl-P (Reaction 2), and mannose-6-P (Reaction 3) to Glc. The enzyme also effectively hydrolyzes these phosphate compounds in addition to Glc-6-P (Reaction 4, for example).

$$\text{Glc-6-P} + H_2O \rightarrow \text{Glc} + P_i \quad (1)$$

$$\text{Carbamyl-P} + \text{Glc} \rightarrow \text{Glc-6-P} + \text{carbamate} \quad (2)$$

$$\text{Mannose-6-P} + \text{Glc} \rightarrow \text{Glc-6-P} + \text{mannose} \quad (3)$$

$$\text{Mannose-6-P} + \text{Glc} \rightarrow \text{Mannose} + P_i \quad (4)$$

These newly-defined phosphotransferase and phosphohydrolase activities of the enzyme contrast with Glc-6-P phosphohydrolase activity in being to a considerably greater extent latent when measured in isolated, but otherwise

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†Abbreviations used are: Glc-6-P, glucose-6-phosphate; Glc, D-glucose; DOC, deoxycholate; HEPES, 2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

untreated, liver (2-5) or kidney (6) microsomal preparations. These apparently in-some-way constrained activities may be fully manifest with the aid of a variety of disruptive techniques, including detergent supplementation (2-6), exposure to ammonium hydroxide at pH 9.8 (7), mechanical shearing (8), phospholipase C-exposure plus phospholipid supplementation (9), and the like.

The significant question has remained: Is this differential latency as observed with isolated fragments (microsomes) of the membrane of a discrete cellular structure (endoplasmic reticulum) directly reflective of the behavior of this membrane-bound enzyme in the intact membrane in situ, or is it largely or totally an artifact created in the manufacture and isolation of these fragments?

Since endoplasmic reticulum cannot be isolated intact, we decided to gain insight regarding this fundamental, physiologically important question by investigating the properties of this multifunctional enzyme in the membrane of a cellular organelle which, in marked contrast with endoplasmic reticulum, can be recovered intact from liver or kidney homogenates---the nucleus.

Results presented in this paper indicate that both hydrolytic and synthetic activities of various sorts previously attributed to the microsomal enzyme are present in hepatic (and kidney) nuclear membrane*, and that all such activities are totally, or nearly totally, maximally manifest in the membrane of the intact nucleus without the need for preliminary activation of any sort. Further, disruption and isolation of the membrane of this organelle leads to the development of detergent sensitivity generally reminiscent of that seen with isolated fragments of endoplasmic reticulum. A brief discussion of the possible physiological implications of these observations is given.

MATERIALS AND METHODS: Sources of substrates and other chemicals (2,3), as well as methods for isolating microsomal fraction and for measuring enzymic activities (2,16) and protein concentration (17,18) were as previously described. Livers were from White Leghorn cockerels obtained from Jack Frost Hatchery, St. Cloud, MN, which were maintained on commercial chick grower, and

*Hydrolysis of Glc-6-P by nuclear membrane has previously been reported by several groups of workers (see, for example, refs. 10-15).

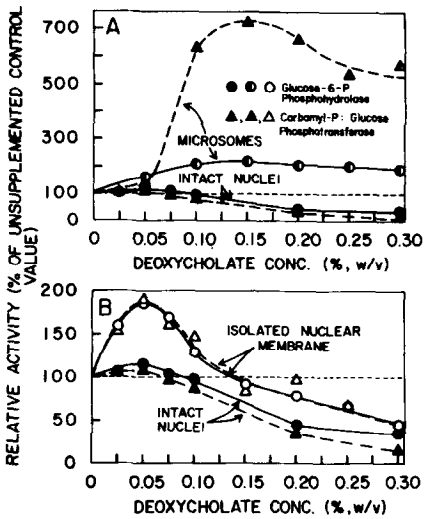

FIG. 1: Comparative effects of various DOC concentrations on Glc-6-P phosphohydrolase and carbamyl-P:Glc phosphotransferase activities of microsomal, intact nuclear, and nuclear membrane preparations. Details of assay are given in the text and the legend to Table I.

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weighed approximately 1.5 kg at death. Nuclei were isolated from liver homogenates by the method of Blobel and Potter (19), including an additional final washing in 0.25 M sucrose solution. Electron microscopic inspection indicated the integrity of nuclei obtained, and that such preparations contained less than 2 % contamination with other cellular organelles and debris. Nuclear membranes were isolated as described by Berezney et al. (11). Sixty-five to 75 % of total nuclear activity routinely was recovered in isolated nuclear membrane preparations. Microsomes were finally suspended in 10 ml of 0.25 M sucrose solution per g original wet liver; nuclei and nuclear membrane preparations were suspended in 1 ml of this sucrose solution per g wet liver.

One vol. of stock DOC solution (or distilled water) was added to 9 vol. of such preparations to attain the final concentrations of this detergent indicated in Fig. 1 and Table I. Such preparations were allowed to stand for 10 min at 0° prior to assay for enzymic activity. Assay mixture compositions, definitions of activity units, and other details are given in the legend to Table I.

RESULTS AND DISCUSSION: The effects of various concentrations of supplemental deoxycholate on Glc-6-P phosphohydrolase and carbamyl-P:Glc phosphotransferase activities of intact nuclei are contrasted with those of microsomal preparations in Fig. 1A. In Fig. 1B, the effects of varied concentrations of the detergent on both activities of isolated nuclear membrane are similarly compared with those of intact nuclei.

It is apparent from Fig. 1A that both Glc-6-P phosphohydrolase and carbamyl-P:Glc phosphotransferase of microsomal preparations are highly responsive to DOC-activation, the latter considerably more so than the former. In marked contrast, also as shown in Fig. 1A, these activities of intact nuclear preparations are almost totally manifest without the need for detergent supplementation, and increase only minimally (8 to 17 %) even with optimal DOC levels (0.05 %, w/v) present.

When nuclei are disrupted through treatment with DNase and exposure to high Mg^{2+} concentrations and nuclear membrane then isolated (11), a significant degree of detergent sensitivity is attained, as indicated in Fig. 1B. A near-doubling of both activities may be brought about through DOC-supplementation of such preparations in which the integrity of the intact nuclear membrane has been disrupted.

In Table I, levels of Glc-6-P phosphohydrolase, carbamyl-P:Glc phosphotransferase, and two additional activities of multifunctional Glc-6-phosphatase---mannose-6-P phosphohydrolase and mannose-6-P:Glc phosphotransferase---in three types of preparations (microsomes, nuclei, nuclear membrane), as assayed in the absence of detergent and after supplementation with maximally-activating levels of DOC (see Fig. 1), are presented. Data in this table serve a threefold purpose: 1) Specific activity values are directly provided for comparative purposes; 2) Information in the third, sixth, and ninth horizontal lines ("Activity without DOC/activity observed after optimal DOC supplementation") provides an index of that fraction of total intrinsic enzymic activity which is manifest with each preparation without preliminary activation; and 3) "Activity Ratio" values in the third, fifth, and seventh vertical columns of numbers provide an index of the relative extent of manifestation of each activity, as compared with Glc-6-P phosphohydrolase, with the various preparations in the absence and presence of detergent.

All activities of microsomal preparations are to a considerable extent latent in the absence of detergent, Glc-6-P phosphohydrolase (33 % of total

TABLE I: COMPARATIVE LATENCY OF GLC-6-P PHOSPHOHYDROLASE, CARBAMYL-P: GLC PHOSPHOTRANSFERASE, MANNOSE-6-P PHOSPHOHYDROLASE, AND MANNOSE-6-P:GLC PHOSPHOTRANSFERASE OF MICROSOMES, NUCLEI, AND NUCLEAR MEMBRANE

Assay mixtures, pH 7.0, contained in 1.5 ml, 20 mM sodium cacodylate, 20 mM HEPES, 5 mM phosphate substrate (Glc-6-P, carbamyl-P, or mannose-6-P), 100 mM Glc (phosphotransferase only), sufficient supplemental NaCl such that μ=0.1, 10 mg bovine serum albumin when DOC was present, and enzyme preparations (0.1-ml aliquots of the supplemented preparations described in the text). Incubations were for 10 min at 30±0.1°, with shaking. One unit of activity = 1 μmole of P_i liberated (phosphohydrolase) or 1 μmole of Glc-6-P synthesized (phosphotransferase) per 10 min under these conditions; specific activity = units per mg protein.

Preparation	Glc-6-P Phosphohydrolase	Carbamyl-P:Glc Phosphotransferase		Mannose-6-P Phosphohydrolase		Mannose-6-P:Glc Phosphotransferase	
	Sp. Act.*	Sp. Act.	Act. Ratio†	Sp. Act.	Act. Ratio	Sp. Act.	Act. Ratio
MICROSOMES							
- DOC**	0.97	0.30	0.31	0.18	0.19	0.13	0.13
+ DOC	2.52	2.23	0.88	2.71	1.08	1.39	0.55
Act. -DOC / Act. +DOC	0.39	0.13	--	0.07	--	0.09	--
NUCLEI							
- DOC	0.99	0.85	0.86	0.93	0.94	0.66	0.67
+ DOC	1.24	0.96	0.77	1.15	0.93	0.62	0.50
Act. -DOC / Act. + DOC	0.80	0.89	--	0.81	--	1.06	--
NUCLEAR MEMBRANE							
- DOC	8.0	8.5	1.06	6.0	0.70	5.5	0.69
+ DOC	13.2	14.4	1.09	14.2	1.08	10.0	0.76
Act. -DOC / Act. + DOC	0.61	0.59	--	0.42	--	0.55	--

*Sp. Act.; specific activity.

†Act. Ratio; activity ratio = Designated activity/glucose-6-P phosphohydrolase activity.

**"-" indicates DOC absent; "+" indicates DOC present in preparations in maximally-activating (see Fig. 1) levels: 0.2%, w/v, with microsomes; 0.1 %, w/v, with nuclei; 0.05%, w/v, with nuclear membrane preparations.

activity manifest without DOC) less so than the others (7 to 13 % of total activity manifest in the absence of DOC). In sharp contrast, all or nearly all (80 to 106 %) of intrinsic activity of intact nuclear preparations is manifest in the absence of detergent, phosphohydrolases being a bit more detergent-sensitive than phosphotransferase activities. The enzyme of nuclear origin, however, following disruption and isolation of nuclear membrane, acquires a significant degree of detergent-sensitivity. With such preparations, activity levels are approximately doubled following DOC-treatment.

In microsomal preparations, activity ratios (activity levels considered relative to Glc-6-P phosphohydrolase) are significantly elevated following DOC supplementation, while with intact nuclear preparations these already-high ratio values are actually slightly decreased following detergent supplementation. A selective increase by DOC in such activity ratios for mannose-6-P-involving reactions (but not carbamyl-P:Glc phosphotransferase) is seen in nuclear membrane preparations.

In control experiments, activities of nuclei suspended in 0.25 M sucrose at 0^{o} for 2 h, as involved in microsomal isolation, remained detergent-insensitive. And activities of microsomes suspended in 2.3 M sucrose (as employed routinely for isolation of nuclei) remained highly detergent-sensitive.

In supplementary studies, the multifunctional enzyme was found in nuclei of avian kidney, and from liver of rat, rabbit, and guinea pig. Insensitivity of the nuclear enzyme to activation as described here also was observed generally in studies with cholate, taurocholate, cetytrimethylammonium bromide, Triton X-100, stearoyl Coenzyme A, and high-pH treatment which all markedly activate the enzyme of microsomal preparations (2-7). The correspondence of catalytic properties of the nuclear enzyme and that of maximally activated microsomal preparations also was demonstrated.

Conclusions: It is concluded from these studies that synthetic and hydrolytic activities of multifunctional Glc-6-phosphatase-phosphotransferase present in the intact membrane of an isolated cellular organelle---liver (and kidney) nuclei---are nearly completely, or totally, functional without the need for preliminary activation by detergents or other disruptive manipulations. The possibility of physiologically significant roles for the various synthetic (and

hydrolytic) activities of the enzyme in addition to Glc-6-P phosphohydrolase is considerably strengthened by these findings. These observations also raise serious questions as to whether the apparent latency (as indicated-by detergent-sensitivity) of various activities of the enzyme of "intact microsomes" (2-6) might, at least in considerable part, arise as an artifact of manufacture and isolation of these fragments of cellular endoplasmic reticulum.

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