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NUCLEOTIDE PYROPHOSPHATASE ACTIVITY OF RAT LIVER PLASMA MEMBRANES

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SUMMARY

1. Palmityl-CoA is metabolised to *S*-palmityl pantetheine by plasma membrane preparations. The metabolism of other nucleotide pyrophosphates by the plasma membrane of liver cells was found to be similar. UDPG was converted to glucose 1-phosphate and UMP; NAD^+ and NADH were hydrolysed at the pyrophosphate bond.

2. The subcellular distribution of the nucleotide pyrophosphatase was studied with NADH as substrate. The similarity in distribution to 5'-nucleotidase, a plasma membrane marker enzyme, indicates that nucleotide pyrophosphatase is an authentic plasma membrane enzyme.

3. NADH breakdown was competitively inhibited by UDPG, CoA and NAD^+, and the properties of the enzyme were very similar to those of other mammalian nucleotide pyrophosphatases.

INTRODUCTION

It was found that palmityl-CoA was metabolised by plasma membrane preparations to form *S*-palmitylpantetheine[1]. The initial reaction was thought to be a hydrolytic cleavage of the pyrophosphate bond of the CoA moiety, since there had been previous reports that NAD^+ was metabolised by plasma membranes. Two enzymes have been reported to catalyse the hydrolysis of NAD^+ in animal tissues[2]: dinucleotide nucleotidohydrolase (EC 3.6.1.9), trivial name nucleotide pyrophosphatase; and NAD^+ glycohydrolase (EC 3.2.2.5), trivial name NAD^+ nucleosidase. LANSING *et al.*[3] showed NAD^+ was rapidly metabolised by a rat liver plasma membrane preparation, but did not characterise the enzyme. EMMELOT *et al.*[4], on the basis of lack of nicotinamide inhibition of NAD^+ metabolism and of the effects of Mg^{2+}, F^-, and EDTA on the enzyme, concluded that nucleotide pyrophosphatase was the enzyme present. In this study we have attempted to characterise the plasma membrane enzyme involved in the metabolism of nucleotide coenzymes.

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MATERIALS

Sources for materials were as follows: NADH (disodium salt, chromatopure), CoA (lithium salt, or free acid, chromatopure), NAD^+, UDP (disodium salt) and UMP (disodium salt): P–L Biochemicals; UDPG (disodium salt), glucose 1-phosphate (disodium salt), sodium pyruvate, lactate dehydrogenase (rabbit muscle, 5 mg/ml), alcohol dehydrogenase (yeast, 30 mg/ml): Boehringer Mannheim Corporation; UDP-[$^{14}C_6$]glucose (204 mC/mmole in 50 mM phosphate buffer (pH 6.4)): Amersham Searle; AMP, glucose 6-phosphate and deoxyguanosine: Sigma Chemical Company; L-glutamic acid, uridine, and nicotinamide: Nutritional Biochemicals Corporation; Triton X-100 and sucrose (density gradient grade): Mann Research. Other chemicals were analytical reagent grade.

DEAE-cellulose thin-layer chromatographic plates were prepared according to Fahn *et al.*[5]. MN Polygram polyamide 11 UV_{254} plates were from Brinkmann Instruments Inc., and MN 300 cellulose 250-μ plates from Analtech Inc.

METHODS

Fractionation of rat liver

Subcellular fractions of rat liver were prepared essentially according to DE DUVE *et al.*[6]. Livers from 50-g, fed, male Sprague–Dawley rats were homogenised in 9 vol. of 0.25 M sucrose in a glass tube with a teflon pestle (radial clearance 0.051–0.076 mm) with 3 strokes at 1300 rev./min. Nuclear (N), mitochondrial, light mitochondrial, microsomal and supernatant fractions were isolated by successive centrifugations at 600 × *g* for 10 min; 3300 × *g* for 10 min; 25 000 × *g* for 10 min and 105 000 × *g* for 60 min. Each fraction was washed once and the washings combined with the corresponding supernatant and processed for the next fraction. The first supernatant *plus* wash was termed the cytoplasmic extract[6] (E). The pellets were suspended in 0.25 M sucrose, and stored at —20°.

The nuclear fraction was further separated into a nuclear pellet and a partially purified plasma membrane fraction as follows. The nuclear fraction was suspended in 2.2 M sucrose and centrifuged at 40 000 × *g* for 60 min to give a nuclear pellet and a float[7]. The float was further fractionated on a linear sucrose gradient[8]; and the fraction immediately above the 50% sucrose cushion (partially purified plasma membrane fraction (PM1)) was retained.

Rat liver plasma membranes were prepared routinely from 125–150-g, 12-h fasted, male Sprague–Dawley rats by the method of NEVILLE[8]. The final pellet was suspended in 1 mM sodium bicarbonate and stored at —20° (PM2). Plasma membranes were washed with saline to remove adsorbed soluble proteins, according to the method of EMMELOT AND BOS[9].

Metabolism of NAD^+ and NADH

NAD^+ nucleosidase was measured by the cyanide method of KAPLAN[10]. The combined activities of NAD^+ nucleosidase and NAD^+ pyrophosphatase were measured by the alcohol dehydrogenase method of KORNBERG[11], and the activity of NAD^+ pyrophosphatase was calculated by difference. NADH pyrophosphatase was assayed essentially according to the method of KESSELRING AND SIEBERT[12], based on the

principle of JACOBSON AND KAPLAN[2]. The incubation medium usually contained 100 mM buffer (Tris–HCl at pH 7.5–8.95; or 2-amino-2-methyl-1,3-propanediol–HCl at pH 8.5–9.6 or glycine–NaOH at pH 8.8–10.3) and 2.0 mM NADH. The reaction was run at 37°, and 0.1-ml samples were removed at 0, 2, 4 and 6 min into cuvettes containing 0.9 ml of a solution of 44.5 mM Tris–HCl (pH 7.5), 1.48 mM pyruvate and 5 μl lactate dehydrogenase. The absorbance at 366 nm was measured (ε_{mM}(366 nm) NADH = 3.3)[13]. To increase the sensitivity for measurement of the K_m and K_i values, the sample volume was increased to 1.0 ml, and was added to cuvettes containing 30 μl 50 mM pyruvate and 5 μl lactate dehydrogenase. The maximum pH at which such reactions could be measured was pH 9.0 in order to obtain a favourable equilibrium for lactate dehydrogenase[14]. When assaying the enzyme in other subcellular fractions, the incubation medium contained 100 mM buffer (pH 9.6), 2.0 mM NADH and also 3.3 mM azide and 10 mM $MgCl_2$ (the activity *minus* $MgCl_2$ averaged 90 % of the activity *plus* $MgCl_2$). The incubation time was increased to 40 min, and the cuvettes into which samples (0.1 ml) were transferred contained 0.9 ml 44.5 mM Tris–HCl (pH 7.5), 1.48 mM pyruvate, 5 μl lactate dehydrogenase and 0.1 % Triton X-100.

Assay of marker enzymes DNA and protein

5'-Nucleotidase (a plasma membrane marker[15]) was assayed by the method of EMMELOT AND BOS in MEDIUM A_1 (ref. 9) (pH 7.4) in a final volume of 1.0 ml. 100-μg samples of protein were incubated for 5 or 10 min at 37°, the reaction stopped with 0.5 ml 15 % (w/v) trichloroacetic acid and P_i in the supernatant determined by the method of AMES AND DUBIN[16]. Glutamic dehydrogenase (a mitochondrial marker[17,18]) was assayed for the forward reaction (NAD^+ reduction) by the method of BEAUFAY *et al.*[17]. Acid phosphatase (a light mitochondrial marker[18,19]) was determined by the method of WATTIAUX AND DE DUVE[20], in a 50-μl volume with 200 μg protein and 5- and 10-min assays. The reaction was stopped and P_i determined as for 5'-nucleotidase. Glucose-6-phosphatase (a microsomal marker [6,18]) was assayed by the method of STAHL AND TRAMS[21] in a 50-μl volume with 100 μg protein and 5-, and 10-min assays. The reaction was stopped and P_i determined as for 5'-nucleotidase. DNA was determined by the diphenylamine procedure[22], and protein by the method of LOWRY *et al.*[23].

RESULTS

Metabolism of nucleotide pyrophosphates by plasma membranes

In our original observations we found that plasma membrane preparations metabolised long chain acyl-CoA to the corresponding *S*-acylpantetheine. Since 4'-phospho-*S*-acylpantetheine did not accumulate, the initial site of the CoA cleavage remained uncertain[1]. Similarly, when UDPG or UDPGal was incubated with plasma membrane preparations, only uridine and the corresponding hexose were found. Subsequently it was observed that the yield of the respective intermediates or products depended on the pH of the incubation mixture. If UDPG was incubated at pH 9.6 (instead of 7.5) or if other phosphatases were inhibited by the addition of P_i to the incubation mixture, UMP and glucose 1-phosphate accumulated (Table I), indicating cleavage of UDPG at the pyrophosphate bond.

TABLE I

METABOLISM OF UDPG BY PLASMA MEMBRANES (NEVILLE[8] PREPARATION): IDENTIFICATION OF PRODUCTS BY THIN-LAYER CHROMATOGRAPHY

(a) Incubations for identification of UMP (Systems 1 and 2) were run as follows: plasma membranes (13 μg protein) were incubated in 90 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.6) containing 10 mM UDPG in a 100-μl volume for 60 min at 37°. The reactions were stopped with 50 μl 30% (w/v) trichloroacetic acid, and after removal of the protein by centrifugation, the supernatants were washed 3 times with 1.5 ml diethyl ether. Samples of supernatant (8–15 μl) and standards were chromatographed as follows: System 1, DEAE-cellulose plates washed in methanol for 5 min and developed in 10% formic acid. System 2, MN polygram polyamide 11 UV_{254} developed in butan-1-ol–glacial acetic acid–water (5:2:3, by vol.)[33]. Spots were visualised under ultraviolet light. (b) Incubations for identification of glucose 1-phosphate (System 3) were run as follows: plasma membranes (227 μg protein) were incubated in 90 mM Tris–HCl (pH 7.5) containing 0.024 mM UDP-[$^{14}C_6$]glucose and 12 mM P_i in a 100-μl volume for 30 min at 37°. The reaction was stopped with 50 μl 30% (w/v) trichloroacetic acid, 1 μmole each of carrier glucose, glucose 1-phosphate and UDPG were added, and the washed supernatants prepared as in (a). Samples of supernatant (25 μl) were chromatographed in duplicate on MN cellulose plates in ethyl acetate–90% formic acid–water (11:5:3, by vol.)[34] 3 times. One set of each duplicate was visualised with $AgNO_3$ spray[35], and the other scanned for radioactivity. The radioactive spots were scraped into scintillation vials, and counted in 1 ml hyamine 10 ml scintillation fluid according to BRAY[36], in a Packard scintillation counter equipped with external standardisation. The efficiency was determined by reference to a standard quench curve.

(*a*) *Identification of UMP*

Thin-layer chromatographic System 1			*Thin-layer chromatographic System 2*		
Standard R_F	*Incubation obs.* R_F	*Incubation control* obs.* R_F	*Standard* R_F	*Incubation obs.* R_F	*Incubation control* obs.* R_F
UDPG 0.07	0.07	0.08	UDPG 0.32	0.38	0.34
UMP 0.49	0.49		UDP 0.18		
Uridine 0.88			UMP 0.45	0.54	
			Uridine 0.80		

(*b*) *Identification of glucose 1-phosphate***

Thin-layer chromatographic System 3			
Standard R_F	*Incubation obs.* R_F	*Incubation controls* obs.* R_F	
		(*i*)	(*ii*)
UDPG 0.06	0.05 (0.05 nmole)	0.04 (1.38 nmoles)	0.05 (1.62 nmoles)
Glc-1-*P* 0.28	0.26 (1.28 nmoles)	0.28 (0.24 nmole)	0.27 (0.24 nmole)
Glucose 0.64	0.64 (0.10 nmole)		

* Incubation controls in (a) were run with boiled membranes; and in (b) at zero time (*i*) or with no membranes (*ii*).

** The nmoles product are given in parentheses.

By measuring NAD^+ disappearance by two different methods (see METHODS) it was possible to show that NAD^+ was also cleaved at the pyrophosphate bond, but that some glycohydrolase activity was also present (Table II). This is a similar finding to that of EMMELOT *et al.*[4]. Since NADH is not a substrate for NAD^+ nucleosidase[10], NADH disappearance can be used to measure NADH pyrophosphatase activity (see METHODS). By this method plasma membrane preparations showed considerable NADH pyrophosphatase activity (Table II).

TABLE II

METABOLISM OF NAD+ AND NADH BY PLASMA MEMBRANES (NEVILLE[8] PREPARATION)

The total rate of NAD+ metabolism, and NAD+ nucleosidase were measured as described in METHODS. NADH pyrophosphatase was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.6) or Tris–HCl (pH 7.5) containing 2.0 mM NADH. (Different membrane preparations were used for the NAD+ and NADH breakdown studies.)

Enzyme		*Activity* (*μmoles/mg protein per h*)
Total NAD+ metabolism	(pH 7.0)	5.7
NAD+ nucleosidase	(pH 7.0)	1.6
NAD+ pyrophosphatase	(by difference)	4.1
NADH pyrophosphatase	(pH 7.5)	9.5
	(pH 9.6)	84.0

Subcellular distribution of NADH pyrophosphatase

Tables III and IV compare the subcellular distribution of nucleotide pyrophosphatase in rat liver with that of 5′-nucleotidase, a plasma membrane marker enzyme, and with nuclear, mitochondrial, light mitochondrial and microsomal marker enzymes. Nucleotide pyrophosphatase is distributed very similarly to 5′-nucleotidase. Moreover, nucleotide pyrophosphatase shows a 13-fold and 5′-nucleotidase a 16-fold increase in specific activity in plasma membranes above that in the homogenate.

It has been found during the preparation of plasma membranes in hypotonic media, soluble enzymes may become adsorbed on to the membrane[24]. Two lines of evidence, however, indicate that nucleotide pyrophosphatase is not an adsorbed soluble enzyme: the partially purified plasma membrane fraction prepared under isotonic conditions (PM1) also had a high specific activity of nucleotide pyrophosphatase (Table IV); and the nucleotide pyrophosphatase activity of plasma membranes prepared under hypotonic conditions (PM2) could not be released by treatment with isotonic saline (Table V). Although in the latter experiment saline treatment inactivated the enzyme about 50%, the specific activity of the soluble protein was less than 1/10th that of the insoluble residue.

Properties of NADH pyrophosphatase

Table VI shows that neither Mg^{2+}, at concentrations up to 10 mM, nor Co^{2+}, at concentrations up to 1.0 mM had any effect on the nucleotide pyrophosphatase activity of plasma membranes, at pH 9.6 or 7.5 (these concentrations of Mg^{2+} and Co^{2+} have been found to activate other mammalian nucleotide pyrophosphatases[12,25,26]). However, preincubation of membranes for 5 min in the presence of 1.0 mM EDTA, and subsequent assay at the same EDTA concentration inhibited the activity by 80%. It will be shown elsewhere that nucleotide pyrophosphatase does require a divalent cation for activity.

The effect of pH on the activity of nucleotide pyrophosphatase is illustrated in Fig. 1. The pH optimum is between 9.3 and 9.6.

The effect on the plasma membrane enzyme of a variety of other compounds which have been shown to inhibit other mammalian nucleotide pyrophosphatases is shown in Table VII. Both 100 mM nicotinamide and 10 mM PP_i produced considerable inhibition. The reason for the small activation by P_i is not known.

TABLE III

THE DISTRIBUTION AND RECOVERY OF NUCLEOTIDE PYROPHOSPHATASE, MARKER ENZYMES, DNA, AND PROTEIN IN SUBCELLULAR FRACTIONS OF RAT LIVER

Subcellular fractions were prepared from rat liver; and nucleotide pyrophosphatase, marker enzymes, DNA, and protein assayed as described in METHODS. Relative specific activity (R.S.A) is obtained by dividing the activity (or DNA content) of the fraction by the corresponding activity of the N + E. Absolute values and percentage recoveries are related to the activity of N + E (nuclear fraction *plus* cytoplasmic extract). Absolute values are given as μmoles/mg protein per h at 37° (25° for glutamic dehydrogenase); DNA is expressed as μg/mg protein.

Fraction	*Protein* (%)	*Nucleotide pyrophosphatase*		*5'-Nucleotidase*		*DNA*		*Glutamic dehydrogenase*		*Acid phosphatase*		*Glucose-6-phosphatase*	
		R.S.A.	%	*R.S.A.*	%	*R.S.A.*	%	*R.S.A.*	%	*R.S.A.*	%	*R.S.A.*	%
N + E	100	1.0	100	1.0	100	1.0	100	1.0	100	1.0	100	1.0	100
Nuclear	12.8	2.6	33.7	3.6	45.6	6.2	79.0	0.8	10.6	0.8	10.0	0.9	10.8
Mitochondrial	21.4	0.6	12.0	0.7	15.4	0.2	3.2	2.8	60.0	1.3	28.8	0.5	9.6
Light mitochondrial	3.7	2.4	8.7	2.9	10.8	0.09	0.4	0.5	2.0	5.5	20.4	1.6	6.0
Microsomal	18.2	1.9	35.4	1.5	26.6	0.06	1.2	0.1	2.5	0.7	12.6	2.7	48.3
Supernatant	35.6	0.2	7.9	0.3	10.4	0.1	3.4	0.02	0.6	0.4	14.5	0.06	2.0
Recovery (%)	91.7		97.7		108.8		87.2		75.7		86.3		76.7
Absolute activity of N + E		6.6		3.7		9.3		1.7		3.2		5.8	

TABLE IV

THE RELATIVE SPECIFIC ACTIVITY OF NUCLEOTIDE PYROPHOSPHATASE AND 5'-NUCLEOTIDASE IN FRACTIONS PREPARED FROM THE CRUDE NUCLEAR FRACTION, AND IN PLASMA MEMBRANES (NEVILLE[8] PREPARATION)

The crude nuclear fraction was separated into a nuclear pellet and a partially purified plasma membrane fraction (PM1), and plasma membranes (PM2) were prepared (from rats of the same age and nutritional state), and enzymes were assayed, as described in METHODS. Relative specific activity is obtained by dividing the activity of the fraction by the corresponding activity of N + E (nuclear fraction *plus* cytoplasmic extract).

Fraction	*Relative specific activity*	
	Nucleotide pyrophosphatase	*5'-Nucleotidase*
Crude nuclear fraction	2.6	3.6
Nuclear pellet	1.7	1.3
PM1	3.9	6.3
PM2 (NEVILLE[8] preparation)	13.1	15.8

TABLE V

SALINE SOLUBILITY OF NUCLEOTIDE PYROPHOSPHATASE OF PLASMA MEMBRANES (NEVILLE[8] PREPARATION)

Freshly prepared plasma membranes were suspended in 0.9% NaCl and treated as described in METHODS. Nucleotide pyrophosphatase activity in each fraction was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.6) containing 2.0 mM NADH and 10 mM $MgCl_2$. The addition of 0.9% NaCl to the assay mixture had no effect on the activity.

Fraction	*Protein* (%)	*Specific activity* (*μmoles/mg protein per h*, 37°)
Original plasma membranes	100	138
Insoluble fraction	80	80.5
Soluble fraction	11.7	7.5

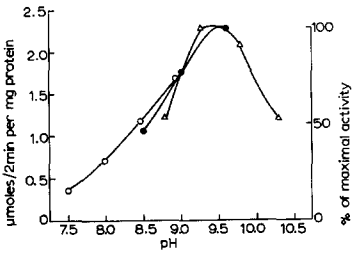
TABLE VI

THE EFFECT OF CATIONS AND EDTA ON NUCLEOTIDE PYROPHOSPHATASE OF PLASMA MEMBRANES (NEVILLE[8] PREPARATION)

Nucleotide pyrophosphatase in plasma membranes was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.6), or Tris–HCl (pH 7.5), containing 2.0 mM NADH with the additions indicated. To test the effect of EDTA the membranes were preincubated with EDTA for 5 min before starting the reaction by addition of substrate.

pH	*Addition*	*Concentration* (*mM*)	% *of control*
7.5	$MgCl_2$	0.1	96
	$MgCl_2$	1.0	96
	$MgCl_2$	10.0	104
9.6	$MgCl_2$	0.1	96
	$MgCl_2$	1.0	112
	$MgCl_2$	10.0	112
7.5	$CoCl_2$	0.1	102
	$CoCl_2$	1.0	100
9.6	$CoCl_2$	0.1	84
	$CoCl_2$	1.0	82
9.6	EDTA	1.0	20

The effect of other possible substrates of nucleotide pyrophosphatase on NADH breakdown was studied. Fig. 2 and Table VIII show that CoA, UDPG and NAD^+ all inhibited NADH breakdown competitively.



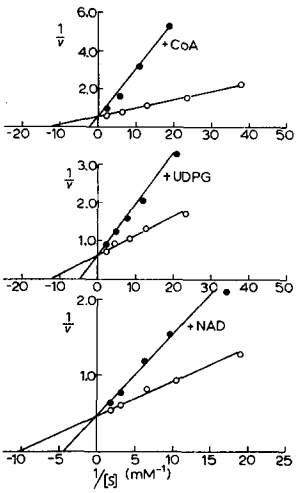


Fig. 1. The effect of pH on the activity of nucleotide pyrophosphatase of plasma membranes (NEVILLE[8] preparation). Nucleotide pyrophosphatase in plasma membranes was assayed in 100 mM Tris–HCl buffers (pH 7.5–8.95) or 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffers (pH 8.5–9.6) or 100 mM glycine–NaOH buffers (pH 8.8–10.3) containing 2.0 mM NADH. The activity is expressed both as a percentage of the maximal activity, and as μmoles/mg protein per 2 min. The figure has been corrected for the effect of different buffers at the same pH: 2-amino-2-methyl-1,3-propanediol–HCl figures are 79% of the actual specific activity; and glycine–NaOH figures 105%. ○, Tris–HCl; ●, 2-amino-2-methyl-1,3-propanediol–HCl; △, glycine–NaOH.

Fig. 2. Effect of CoA, UDPG and NAD^+ on the nucleotide pyrophosphatase activity of plasma membranes (NEVILLE[8] preparation). Nucleotide pyrophosphatase in plasma membranes was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.0) containing various concentrations of NADH, and in the absence and presence of CoA (0.2 mM), or UDPG (0.92 mM) or NAD^+ (0.4 mM). $1/v$ is expressed as $(\mu moles/mg\ protein\ per\ 2\ min)^{-1}$.

TABLE VII

PROPERTIES OF NUCLEOTIDE PYROPHOSPHATASE OF PLASMA MEMBRANES (NEVILLE[8] PREPARATION)

Nucleotide pyrophosphatase in plasma membranes was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.6) containing 2.0 mM NADH and the additions indicated.

Addition	*Concentration (mM)*	*% of control*
Nicotinamide	50	67
Nicotinamide	100	46
PP_i	10	56
F^-	10	101
P_i	20	127

TABLE VIII

NUCLEOTIDE PYROPHOSPHATASE OF PLASMA MEMBRANES (NEVILLE[8] PREPARATION): KINETIC DATA

Nucleotide pyrophosphatase in plasma membranes was assayed in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9.0), or 100 mM Tris–HCl (pH 7.5) containing various concentrations of NADH in the presence and absence of the various inhibitors. The data were calculated by the method of LINEWEAVER AND BURK[37].

pH		K_m (*mM*) *NADH*	*Inhibitor*	K_i (*mM*)	*Type of inhibition*
9.0		0.089	CoA	0.046	Competitive
		0.081	UDPG	0.58	Competitive
		0.097	NAD^+	0.31	Competitive
	Average	0.089			
7.5		0.030	CoA	0.042	Competitive
		0.032	UDPG	0.095	Competitive
	Average	0.031			

DISCUSSION

We found a distribution of NADH pyrophosphatase in rat liver very similar to the data of JACOBSON AND KAPLAN[27]. Since the distribution of plasma membrane fragments after homogenisation may vary greatly depending on the mode of homogenisation[15,28] (and compare the data of MICHELL *et al.*[29] and EIBL *et al.*[30] on the subcellular distribution of 5′-nucleotidase), a distribution means little unless compared to that of marker enzymes. The similarity in distribution of 5′-nucleotidase, a plasma membrane marker, and nucleotide pyrophosphatase reported here, and the similar degree of purification of both enzymes in a plasma membrane preparation (16- and 13-fold, respectively) leads us to postulate that nucleotide pyrophosphatase is a plasma membrane enzyme, in line with the results of EMMELOT *et al.*[4] and LANSING *et al.*[3]. Moreover, the activity of the enzyme at its pH optimum compares with the activity of some of the most active plasma membrane enzymes.

The data reported here for UDPG and NAD^+ show that these substrates are cleaved at the pyrophosphate bond. Moreover, the similarity of the properties of the enzyme, with NADH as substrate, to those of nucleotide pyrophosphatase studied in a variety of mammalian tissues, supports this conclusion.

A comparison of the work of KESSELRING AND SIEBERT[12] (NADH pyrophosphatase in rat kidney particles); PATTABIRAMAN *et al.*[25] (UDPGlcNAc metabolism by sheep brain); OGAWA *et al.*[31] (UDPGlcUA pyrophosphatase in rat liver microsomes) and SCHLISELFELD *et al.*[26] (UDPG pyrophosphatase in rat liver nuclei) with the present report indicates that the above authors might have been studying various activities of the plasma membrane enzyme. All the above enzymes are particulate. KESSELRING AND SIEBERT[12] found the highest specific activity in particles sedimenting at 1 050 000 $\times$ g·min, which also had a high specific activity of alkaline phosphatase. Alkaline phosphatase has been considered a plasma membrane enzyme[3,4]. OGAWA *et al.*[31] and SCHLISELFELD *et al.*[26] both report a bimodal distribution of their enzyme in nuclear and microsomal fractions and this distribution may be observed for plasma membrane enzymes[15].

The pH optima, and K_m values reported by these groups, are within the same range as those reported here. The cation requirements, however, show an interesting variability, from no requirement[31] to a 240% stimulation by Co^{2+} (see ref. 25). However, in all cases EDTA was inhibitory. It therefore seems likely that the variability may relate to the degree to which divalent cations are removed during preparation of the enzyme. The inhibition by nicotinamide and PP_i reported here are very similar to those found by KESSELRING AND SIEBERT[12] and SCHLISELFELD *et al.*[26].

So far we have not fully studied the substrate specificity of this enzyme. SCHLISELFELD *et al.*[26] report high activity with nucleoside diphospho sugars, NAD^+, $NADP^+$, ATP and UTP, and OGAWA *et al.*[31] a breakdown of UDP-sugars, NAD^+, NADH, $NADP^+$ and FAD, and a low activity against ATP, and UTP. We have found hydrolysis of palmityl-CoA, UDPG, NADH and NAD^+ by plasma membranes; and competitive inhibition of NADH pyrophosphatase by CoA, UDPG and NAD^+ indicating that these compounds are possibly substrates for the same enzyme. None of the data from mammalian systems has previously implicated CoA or its derivatives as substrates for nucleotide pyrophosphatase.

The position of ATP is interesting; although SCHLISELFELD *et al.*[26] found a rapid breakdown of ATP (to form PP_i) by their purified enzyme, ATP was a non-competitive inhibitor of UDPG breakdown. It is interesting in this context that LIEBERMAN *et al.*[32] have reported an active ATP pyrophosphatase in rat liver plasma membranes, with a pH optimum about 10.

We therefore conclude that rat liver plasma membranes show an active nucleotide pyrophosphatase capable of hydrolysing a variety of nucleotide pyrophosphates at the pyrophosphate bond. The specific localisation of the enzyme, and the central position of its substrates in cellular metabolism, suggest that the enzyme may be of considerable physiological significance.

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