Phosphodiesterase and Ribonuclease Activities of the Plasma Membrane of Rat Liver and Hepatoma

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Plasma-membrane fractions isolated from the crude nuclear fraction of liver homogenates possess considerable alkaline phosphodiesterase and ribonuclease activities (Emmelot, Bos, Benedetti & Rümke, 1964; Lansing, Belkhome, Lynch & Lieberman, 1967; Erecinska, Sierakowska & Shugar, 1969). Until recently, however, methods were not available to isolate the 40% of the plasma-membrane fragments that sediment as microsomal fraction, or to separate plasma-membrane sheets simultaneously with other cell organelles. We have used zonal rotors to investigate the subcellular distribution of phosphodiesterase and ribonuclease activities, employing techniques that separated plasma-membrane sheets from the crude nuclear fraction (Hinton, Fitzsimons, Dobrota & Reid, 1970) and plasma-membrane vesicles from the microsomal fraction (El-Aaser, Norris & Reid, 1970; Hinton, Norris & Reid, 1971), while simultaneously giving other elements in good condition. Methods developed for liver microsomal fraction were equally applicable to hepatoma, but attempts to separate large sheets of membrane from hepatoma failed.

The results indicate that about one-half of the particulate alkaline ribonuclease and a considerably higher proportion of alkaline phosphodiesterase (assayed with bis-*p*-nitrophenyl hydrogen phosphate) are associated with the plasma-membrane. The distribution of enzymes in microsomal sub-fractions indicates that a major part both of microsomal ribonuclease (de Lamirande, Boileau & Morais, 1966) and microsomal phosphodiesterase (Razzell, 1961; de Lamirande *et al.* 1966) is due to plasma-membrane fragments.

Comparison of 'nuclear' and 'microsomal' plasma-membrane phosphodiesterase and ribonuclease activities revealed no significant differences in amounts or properties. The pH optimum of the phosphodiesterase was between 7.4 and 7.8, but with considerable residual activity in the acidic region. These determinations were, however, complicated by the inhibitory action of some buffers, notably phosphate and 3,3-dimethylglutarate. The phosphodiesterase was inhibited by EDTA and activated by Mg^{2+} , the activity in the presence of 5mM-magnesium chloride being four times that in the presence of 5mM-EDTA. The pH optimum of the ribonuclease was very similar, but this enzyme was less activated by Mg^{2+} than was the phospho-

diesterase. Preliminary experiments indicate that the properties of tumour and liver plasma-membrane ribonuclease activities are similar. Liver plasma-membrane ribonuclease had little activity towards poly(A), activity towards this substrate being largely confined to mitochondria (cf. Morais, Blackstein & de Lamirande, 1967).

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Amino-Azo-Dye-Binding Protein in the Soluble Cytoplasm of the Rat Liver

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It has been the belief for some time that amino-azo-dye carcinogens bind covalently to proteins of the soluble cytoplasm of the rat liver and that the major part is bound to a basic protein fraction (Sorof, Young, McCue & Fetterman, 1963). Further, a basic amino-azo-dye-binding protein, thought to be the major dye-binding component, has been isolated as a pure substance and some of its properties have been described (Ketterer, Ross-Mansell & Whitehead, 1967).

A claim that the principal amino-azo-dye-binding protein is not basic has been made (Sugimoto & Terayama, 1970), and this is based on experiments in which liver extracts are subjected to mild heat treatment followed by several chromatographic separations. However, we have found that if the heat treatment is followed by electrophoresis in an

inert medium most of the amino-azo-dye-bound protein is basic, just as it is in fresh soluble cytoplasm. It has been found that the heat treatment used partially denatures the purified basic protein, and this may account for the abnormal behaviour of amino-azo-dye-bound protein in the heat-treated extracts observed by Sugimoto & Terayama (1970).

The physicochemical properties of the purified basic protein have now received further study. Its molecular weight by meniscus-depletion approach-to-equilibrium sedimentation in aqueous buffer is 45900 and in 6M-guanidine hydrochloride in the presence of thiol-blocking agents is 23000. The same value of 23000 is obtained by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate-8M-urea. The protein is thus composed of similar subunits and these are not held together by disulphide links (there are four cysteine residues/molecule of protein).

The amino-azo-dye derivative is bound to a cysteinyl residue (Ketterer & Christodoulides, 1969), but in two forms. Mild denaturing agents release one form as an amino-azo-dye-bound peptide (Glu, Cys, Gly), but most of the dye remains covalently bound to the protein.

The electrophoretic properties of the protein vary from batch to batch. This is probably due to its affinity for charged compounds. It has been shown to bind both bromosulphophthalein and bilirubin. Litwack & Morey (1970) have drawn parallels between it and a binding protein for charged cortisol metabolites. This may be related to its physiological role: it has also been suggested that it may be involved in bilirubin excretion (I. M. Arias & J. Levi, personal communication).

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Rotational Mobility of a Fluorescent Rhodopsin Derivative in the Rod Outer-Segment Membrane

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In the simultaneous presence of light and sodium borohydride, rhodopsin is converted into a fluorescent derivative known as *N*-retinyl-opsin (Bownds, 1967). From 'decay-of-fluorescence-anisotropy' (Stryer, 1968; Tao, 1969) measurements the rotational mobilities were obtained for the following materials: (1) *N*-retinyl-opsin solubilized by digitonin; (2) *N*-retinyl-opsin solubilized by cetyltrimethylammonium bromide; (3) membrane-bound *N*-retinyl-opsin. For cases (1) and (2) the measured rotational relaxation times (148 ns and 52 ns respectively) were consistent with the sizes of the corresponding rhodopsin-detergent micelles. For case (3) no anisotropy decay was observed within the limits of detection. A lower limit of 300 ns can be set for the rotational relaxation time of membrane-bound *N*-retinyl-opsin. Other reports suggest that membrane rhodopsin possesses both translational (Blasie & Worthington, 1969) and rotational (Hagins & Jennings, 1959) mobility on a millisecond time-scale. This work indicates that rhodopsin in the rod outer-segment membrane is rotationally immobile on a nanosecond time-scale.

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