Di- and Tri-nucleotidase Activities of Rat Liver Cytomembranes[1]

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Abstract

Enzymatically active fractions of endoplasmic reticulum, Golgi apparatus and plasma membrane were isolated from rat liver. Without exception, the di- and trinucleotide phosphatase activities of the Golgi apparatus fraction were intermediate between those of the endoplasmic reticulum and plasma membrane. These data support the proposal that the Golgi apparatus serves as a site of endomembrane differentiation from endoplasmic reticulum-like to plasma membrane-like.

Morphological evidence has provided the basis for a proposal that Golgi apparatus function as sites of cytomembrane differentiation in the formation of membranes which are plasma membrane-like, beginning with an input of membrane constituents from endoplasmic reticulum and implying a progressive change in the composition or arrangement of constituents within the membrane (5, 10). An understanding of the functional significance of the transitional nature of the Golgi apparatus requires information on the chemical and enzymatic composition of Golgi apparatus relative to membranes of the endoplasmic reticulum and plasma membrane.

In this report, we compare nucleoside di- and triphosphatase activities of endoplasmic reticulum, Golgi apparatus and plasma membrane. With every nucleotide tested, the activity of the Golgi apparatus is intermediate between that of the endoplasmic reticulum and plasma membrane.

Materials and Methods

Methods for isolation (2, 9, 10), yield and purity (8) of the endoplasmic reticulum, Golgi apparatus and plasma membrane fractions have been described. Enzyme assays were at 37° C under conditions where activity was proportional to time of incubation and protein concentration. Protein was determined by the method of Lowry *et al.* (7) and inorganic phosphate by the method of Fiske and Subbarow (4). All assays were with nucleotides as sodium salts in medium A-1 of Emmelot *et al.* (3), pH 7.4.

Substrates were of the highest purity obtainable from the suppliers indicated: inosine-5'-diphosphate (IDP); thymidine-5'-diphosphate (TDP); thymidine-5'-triphosphate (TTP) and guanosine-5'-diphosphate (GDP) [Calbiochem]; adenosine-5'-diphosphate (ADP), cytidine-5'-diphosphate (CDP), uridine-5'-diphosphate (UDP), inosine-5'-triphosphate (ITP), uridine-5'-triphosphate (UTP) [Sigma]; adenosine-5'-triphosphate (ATP), guanosine-5'-triphosphate (GTP) [Nutritional Biochemicals].

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Results and Discussion

The relative nucleoside di- and triphosphatase activities of the isolated fractions are shown in Figure 1. The specific activity (μmoles P_i/hr/mg protein) of each fraction was divided by the specific activity of the corresponding total homogenate. This corrects for fluctuations in feeding and age of the animals and for variations in the isolation procedures. The activity of the Golgi apparatus-rich fraction is intermediate between that of endoplasmic reticulum- and plasma membrane-rich fractions even though the activity is increasing, as with uridine triphosphatase; decreasing, as with guanosine diphosphatase; or unchanging, as with inosine diphosphatase; in going from endoplasmic reticulum to plasma membrane.

Morphological evidence for a transitional nature for the Golgi apparatus has come from studies with the fungus *Pythium ultimum* (5)

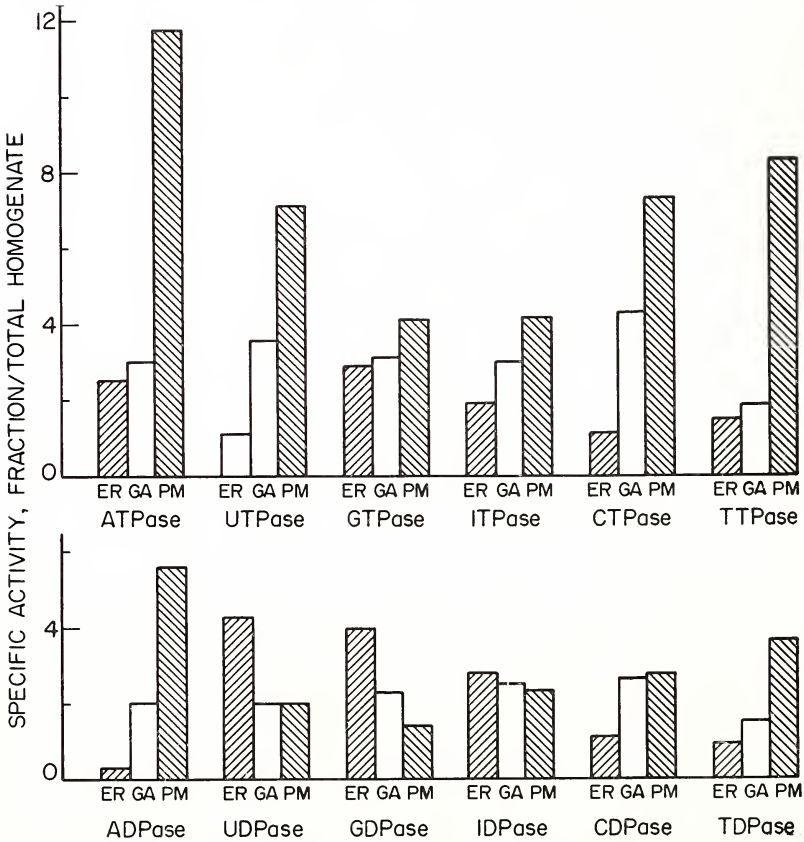

FIGURE 1. *Relative specific activities of hydrolysis of nucleoside di- and tri-phosphates at pH 7.4 by endoplasmic reticulum (ER)-, Golgi apparatus (GA)- and plasma membrane (PM)-rich cell fractions from rat liver. Relative specific activity is the ratio of specific activity (μmoles iP/hr/mg protein) of each fraction to that of the total homogenate. (Abbreviations for substrates are explained in the text.)*

and rat liver (10). These studies show a progressive change in both membrane staining and membrane thickness across stacked cisternae from a morphology which resembles that of the endoplasmic reticulum to a morphology which is plasma membrane-like. The transitional nature of the Golgi apparatus is also reflected in the lipid composition of these fractions where the Golgi apparatus is again intermediate between the endoplasmic reticulum and the plasma membrane (10). The enzyme activities presented here lend further support to our proposal that the Golgi apparatus functions in the transformation of membranes from endoplasmic reticulum-like to plasma membrane-like.

Golgi apparatus are not known to be sites of protein synthesis (6) but enzyme proteins might be added to or removed from the membranes during transformation. If transfer of proteins from endoplasmic reticulum to Golgi apparatus occurs in the formation of plasma membrane-like secretory vesicles, then it appears that the process must involve a selective transfer so that certain proteins become concentrated in the secretory vesicle membranes whereas others are not incorporated (1). As an alternative, certain activities of enzyme proteins derived from endoplasmic reticulum might be progressively activated or inhibited concurrent with the changes in lipid and carbohydrate composition of the membranes (1). Hopefully, pulse labeling studies involving one or more of the protein constituents of plasma membrane can be used to provide a direct test of the concept of membrane flow in rat liver.

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