C_{55}-ISOPRENOID ALCOHOL PHOSPHOKINASE: THE FORMATION OF A TERNARY LIPOPROTEIN COMPLEX

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1. Introduction

The butanol-soluble enzyme C_{55} -isoprenoid alcohol phosphokinase catalyzes the ATP-dependent formation of C_{55} -isoprenoid alcohol monophosphate, which functions as a carrier molecule in the biosynthesis of several bacterial polysaccharides [1]. A previous analysis of Arrhenius plots for the reactivation of IPA * by synthetic lecithins suggested that the enzyme reaction might occur at the aqueous interphase of mixed micelles consisting of the lecithin cofactor, the C_{55} -isoprenoid alcohol substrate and IPA [2]. In the present communication the preparation of the postulated ternary complex and its isolation by isopycnic centrifugation are reported.

2. Experimental

IPA, ficaprenol and [γ-^{32}P] ATP were prepared as indicated previously [2]. Egg lecithin (Merck, puriss.) was purified on a column of alumina and was homogeneous on thin-layer chromatography.

3. Results

A ternary complex of egg lecithin, ficaprenol and IPA, which was visible after centrifugation as a solid layer on top of a sucrose gradient, was isolated (fig. 1). The very low density of the complex ($\leqslant 1.02$ g cm^{-3}) reflects the high content of lecithin and of ficaprenol. The expected density of IPA alone is about 1.28 g cm^{-3}, as calculated from the amino acid composition

* *Abbreviation:*
IPA = C_{55} -isoprenoid alcohol phosphokinase apoprotein.

Table 1
Isopycnic centrifugation.

Substances examined	Present in film	in gradient
Phosphokinase apoprotein (20 μg)	+	–
Ficaprenol (0.3 μmole)	+	–
Egg lecithin (1.52 μmole)	–	+
Phosphokinase apoprotein (20 μg)	+	–
+ Ficaprenol (0.3 μmole)	+	–
Phosphokinase apoprotein (20 μg)	–	+
+ Egg lecithin (1.52 μmole)	–	+
Phosphokinase apoprotein (20 μg)	–	+
+ Ficaprenol (0.3 μmole)	–	+
+ Egg lecithin (1.52 μmole) (see fig. 1)	–	+

Samples were prepared and centrifuged as described in the legend of fig. 1. Immediately after layering the samples on top of the gradients, material present as a film on the inner surface of the sample tubes was dissolved in 300 μl methanol/n-butanol (1:1), 50 mM ammonium acetate. These solutions as well as aliquots of all fractions from the sucrose gradients were tested for phosphokinase activity with the addition in each case of the components necessary for a complete assay system. The analysis of aliquots for lecithin phosphate and ficaprenol was as described in fig. 1. The substances examined were either virtually exclusively present in the film layer on the inner wall of the sample tubes or in the top fraction on the sucrose gradients.

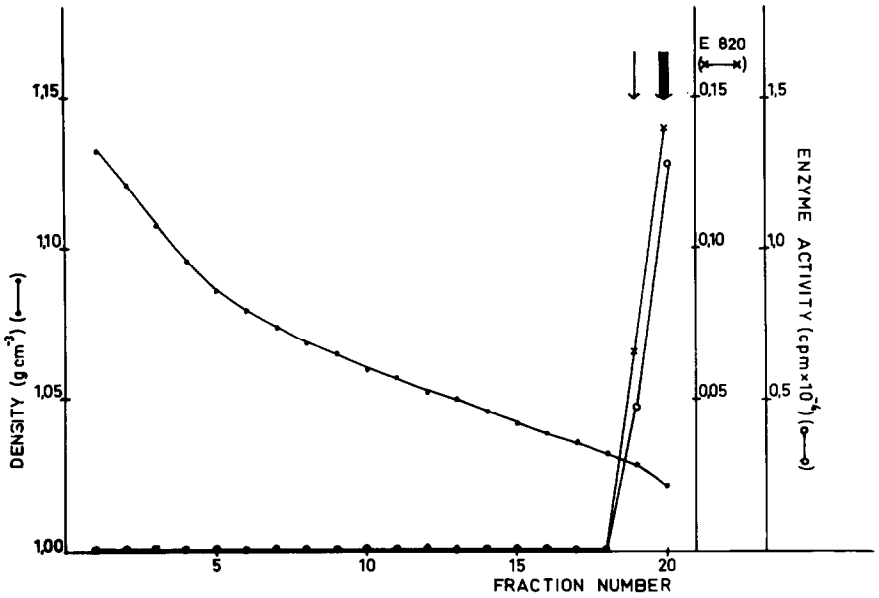

Fig. 1. Isolation of a ternary complex. Solutions containing 0.3 μmole ficaprenol and 1.52 μmoles egg lecithin in organic solvents were dried in vacuo in a glass test tube. Enzyme apoprotein (20 μg, see [2]) was added in organic solvent. After redrying, the residue was suspended by means of a capillary tube in 300 μl buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, pH 8.5). The suspension was layered on top of a linear gradient of 5 to 20% sucrose on a cushion of 300 μl 40% sucrose. All sucrose solutions contained 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 8.5. Centrifugation was performed with a Spinco SW-40 swinging bucket rotor at 20° (40 hr, 35000 rpm) in a Spinco model L2 65B ultracentrifuge. Fractions of about 0.5 ml were collected from the bottom of the centrifuge tubes by means of a sampling needle. A part of the solid layer on top of the gradient adsorbed to the inner wall of the centrifuge tube during fractionation. This material was added to fraction 20 after a wash with the above buffer. The density (●–●–●) of individual fractions was determined from the refractive index. Enzyme activity (○–○–○) was determined by adding 10 μl of 1 M Tris–HCl, 10 mM $MgCl_2$, pH 8.5 to 100 μl of the fraction to be tested. 2 μl of 17 mM [γ-^{32}P]-ATP (5.5×10^5 cpm) was added and incubation was for 15 min at 35°. After addition of 100 μl chloroform/methanol, 2:1, (v/v), the radioactivity associated with [^{32}P] ficaprenol monophosphate was determined by chromatography [2]. In a parallel set of incubations 30 nmoles ficaprenol and 158 nmoles egg lecithin were dried in the test tubes and then dispersed in the above components of the assay. No increase in enzyme activity was observed. The recovery of enzyme activity in fractions 19 and 20 was about 45%. 1 ml chloroform/methanol, 2:1 (v/v), was added to the gradient fractions. Aliquots of the chloroform layers were assayed for total phosphate [9]. This is plotted as optical density at 820 nm (X–X–X). The recovery of lecithin phosphate in fractions 19 and 20 was about 90%. Several aliquots of the chloroform layers were chromatographed in parallel with known amounts of ficaprenol on silica gel thin layer plates using benzene/methanol 98.5:1.5 (v/v) as a solvent. The bulk of the ficaprenol applied to the gradient was found in fraction 20 (located with iodine vapor), a smaller amount being present in fraction 19. These results are symbolized on the graph by arrows.

[3] by the method of Cohn and Edsall [4]. On addition of ATP the isolated complex showed full enzyme activity. Further addition of lecithin and ficaprenol to the isolated complex did not increase enzyme activity.

The C_{55}-isoprenoid alcohol and IPA alone or admixed formed a film on the inner wall of the tube used for the sample preparation. They were exclusively found in this film layer after the experiment (table 1). These components of the assay mixture were clearly unable to disperse in aqueous buffer under the conditions of the experiment. In the presence of the active lipid cofactor, egg lecithin, however, binary (table 1) and ternary (fig. 1) complexes were formed.

4. Discussion

After solubilization by egg lecithin IPA was found in a sucrose gradient at the position of the lecithin phase. Interestingly, both ficaprenol and IPA [3] will also partition into the butanol phase of a butanol/

water two-phase mixture. The solubilization observed might thus be considered as the partitioning of ficaprenol and of certain regions of IPA into the hydrophobic interior of the cofactor lipid phase. No data are as yet available on the sequence of events or on the extent of the partitioning process, although an analogy between the processes of solubilization and partitioning has been shown for other lipids [5, 6]. Partition coefficients determined with water/organic solvent two-phase mixtures certainly are of great significance for the process of solubilization.

However, there are two further and interrelated requirements for solubilization to occur [5–7]:

(i) The solubilizing lipid has to be above its Krafft temperature, that is, its fatty acid chains have to be "molten", (ii) The solubilizing lipid has to be swollen, that is, it has to allow the penetration of water between the lipid polar groups.

A previous attempt to show the significance of the Krafft temperature for the reactivation of IPA by synthetic lecithins failed [2]. It has now been found that this negative result was possibly due to the fact that the thermal phase transition of lecithins is largely suppressed and shifted in the presence of the substrate, ficaprenol (H.S., unpublished data).

With regard to the second requirement for solubilization it is of interest that all active lipid cofactors found so far, although varying greatly in chemical structure, are able to swell and disperse in water, while non-swelling lipids appear to be devoid of cofactor activity (H.S., manuscript in preparation).

In the experiments described in table 1 ficaprenol and IPA behaved as non-swelling lipophilic substances. In the presence of the active lipid cofactor egg lecithin solubilization occurred. Egg lecithin is well above its Krafft temperature at the temperature of the experiment [8] and it easily swells and disperses in aqueous buffer (observed with a polarizing microscope, data not shown). Solubilization as specified above is proposed to be of general importance in the function of lipid-dependent enzymes and transport proteins as well as in the biosynthesis of membrane lipoproteins.

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