Letter to the Editor

Neutral Phospholipids Do Not Buffer at Neutral pH

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Some years ago, Grzesiek and Dencher (1986) reported that the buffering power of the interior of lipid vesicles can differ markedly from the buffering power of the internal solution and concluded that the internal lipid surface contributes to the buffering power. This result was astonishing because most lipids are not expected to exert a buffering power at neutral pH. Nonetheless, it led to speculations about specific headgroup interactions at the lipid surface.

In the context of studies on membrane permeability for protons (Kiefer et al., 1991), we determined the buffering power of phospholipids extracted from *E. coli* by the same technique as Grzesiek and Dencher, i.e., measuring the kinetics of proton permeation into vesicles at different phosphate buffer concentrations and extrapolating to zero buffer concentration. We also found a contribution of the internal lipid surface to the buffering power inside the vesicles. The buffering power of the inner surface of the *E. coli* lipid vesicles corresponded to a phosphate buffer of 14 mM at neutral pH. Because the headgroup concentration (number of headgroups divided by the inner volume) is about 110 mM, this means that about 10% of the lipid behaves as a phosphate buffer. This result was also found by direct titration of lipid vesicles containing gramicidin for rapid pH equilibration.

To find out the reason for the buffering power of *E. coli* lipid, we titrated the individual main components of *E. coli* lipid, which are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL). The data were fitted to the theoretical curve for a buffer containing one or two pK values, as described recently in an illuminating article by Darvey and Ralston (1993). For all lipid headgroups, we found a pK in the range 2–3, which obviously results from the phosphate group. No buffering power could be observed at neutral pH, at least not for PE and CL. The same observation was made for phosphatidylcholine (PC), in contrast to the finding of Grzesiek and Dencher. Only PG showed some buffering power at neutral pH, but this effect may be attributed to its chemical instability at low pH. TLC analysis after the titration experiment revealed that about 20% of the PG had hydrolysed to phosphatidic acid (PA). We were not able to suppress this effect completely. When PA was titrated, a second pK was found at 8.5 providing some buffering power at neutral pH. However, PA could not be detected in *E. coli* lipid by TLC analysis, the sensitivity being 1%. These results let us conclude that phospholipids are not the cause of the buffering power of *E. coli* lipid; another compound must be responsible for this effect.

The molar lipid headgroup concentration in small vesicles is rather high (110 mM in our case; about 500 mM in small sonicated vesicles of 50 nm diameter). Hence, even small impurities or hydrolytic products of phospholipids such as fatty acids will contribute to the internal buffering power if present.

In conclusion, we could say that the internal buffering power of vesicles made of extracted lipids may be much higher than expected and must be determined accurately for certain types of experiments, but this effect is not caused by the phospholipids.

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