Surfactant-like particles in the intestine

A project on the study of surfactant-like particles (SLPs) was funded by NIH, USA, for nearly 10 years with the aim to characterize the SLPs, secreted by the small intestine in response to fat feeding and to determine their physiological functions. The work started in 1989, when David Alpers and his colleagues from the Department of Gastroenterology, Washington University Medical School, St Louis, USA, published a paper in the *Journal of Biological Chemistry*[1]. The published findings showed the presence of a thick membranous layer on the intestinal surface in rats fed with corn oil (Figure 1). This was collected by scraping the surface with a filter sheet and upon centrifugation in NaBr gradient (0.49–1.46 M (molar)), it formed a translucent layer in fraction 3 from the top (Figure 2).

One of us (A.M.) joined Alpers' laboratory in 1990 (on leave from Panjab University, Chandigarh, for a couple of years) and opted to work on this project. At that time, alkaline phosphatase (AP) was the only marker for these structures. We wanted to find out the other proteins present in SLPs and also determine their functions, particularly in relation to fat absorption from the intestine. We started getting more pure SLPs, so antibodies could be raised to identify other proteins present therein and clone them. Also, the presence of these structures in other animal species, including the human intestine, could be studied.

Since SLPs are rich in lipids (triglycerides/phospholipids/cholesterol, FFA, etc.), they appear in the NaBr gradient centrifugation at a density of 1.07–0.08 g/ml, which is identical to lung surfactants rich in proteins B/C. Thus they have been named as surfactant-like particles[2,3]. On SDS PAGE, SLPs yielded several protein bands ranging in molecular mass from 28 to 168 kDa (Figure 3). However, using Western blot analysis only 4–6 proteins were visible (Figure 4)[4].

Several research papers were published under the pretext that SLP contains some novel proteins. They represent unique structures involved in the absorption of lipids from the intestine. Our efforts to identify these proteins were unsuccessful, as many other researchers did. These structures were also detected in CaCo-2 cells when grown in lipid-containing media and their role in lipid transport was postulated[5]. The binding of ECM proteins and *Escherichia coli* toxin involved in urinary tract infection were also demonstrated[6,7].

On his revisit to Washington University Medical School, St Louis, in 1999, A.M. was keen to identify the protein present in the SLPs along with AP. The SLP proteins were separated on 2D gel and a few protein bands were selected to determine the N-terminal amino acid sequence. Unfortunately, each time the results were negative. We also analysed the internal amino acid sequence of the proteins by proteolytic hydrolysis, but with no success. Since the protein : acryl amide ratio was small, it seemed to interfere with the amino acid sequence analysis. This problem was solved by removing the excess of unstained gel from the stained protein band before sequence analysis.

In the meantime, A.M. discussed this problem with Abdul Waheed, who works at St Louis University. Waheed kindly gave

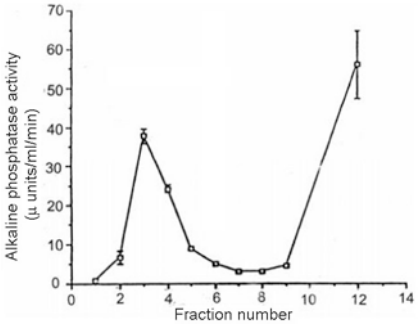

Figure 2. Alkaline phosphatase activity as a marker of surfactant-like particle (SLP) in NaBr gradient fractions in rat intestine.

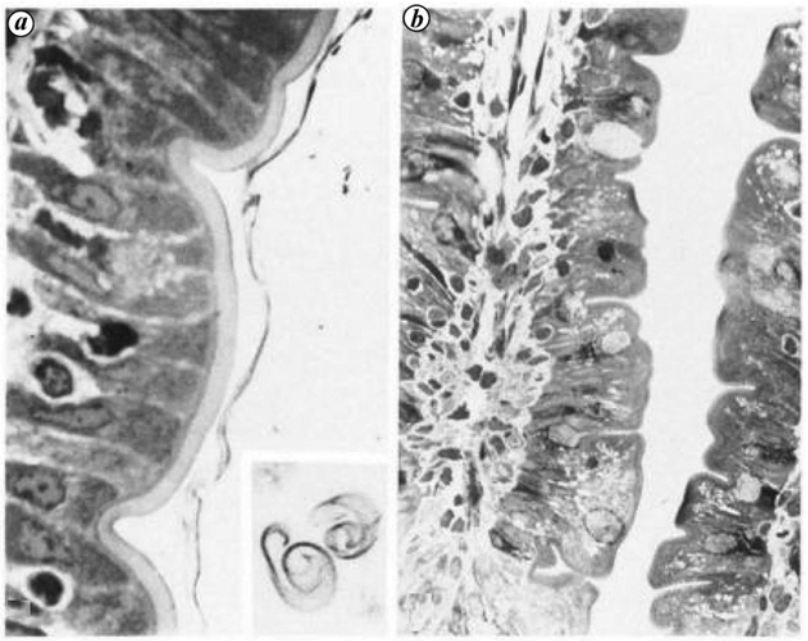

Figure 1. Appearance of intestine before and after light scraping in rats fed with corn oil. (Taken from ref. 1 with permission from the publisher).

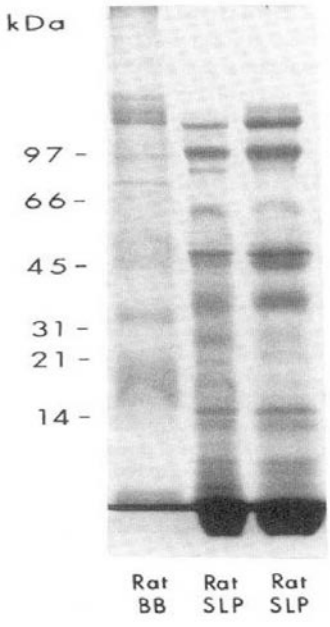

Figure 3. SDS PAGE analysis of SLPs and brush border membranes isolated from rat intestine.

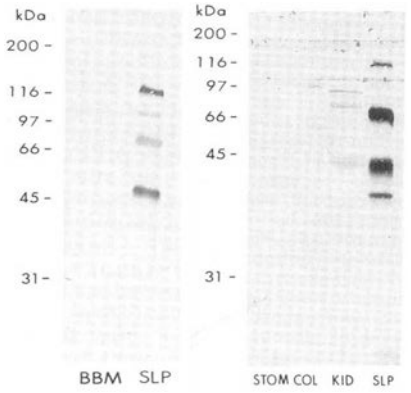

Figure 4. Western blot analysis of brush border membranes and SLPs isolated from the intestine of rat fed with corn oil using SLP antibodies. STOM, Stomach; COL, Colon; KID, Kidney.

A.M. a few microlitres of rat albumin antibodies, which were utilized for Western blot analysis using SLP proteins along with the standard rat albumin. To our surprise, all 4–6 SLP protein bands were strongly identified by rat albumin antibodies. A reverse experiment was also carried out, where using SLP antibodies, rat albumin was identified by Western blot analysis. Experiments were repeated using human albumin antibodies against human albumin and SLPs isolated from the human intestine. Essentially, similar results were obtained.

Amino acid analysis of the protein samples also showed that ten N-terminal amino acids (Asp–Ala–His–Lys–Glu–Val–Ala–His–Arg–Phe) were of albumin. This was further confirmed by a review of literature[8,9]. The amino acid sequence of the proteins matched exactly with those reported in the literature[8,9]. Thus the nature of these novel proteins was solved after nearly 10 years of discovery of SLPs.

With the knowledge that albumin is a part of SLPs in association with AP (they have similar molecular mass; 64–65 kDa), some new questions arise. For example, what are the functions of albumin in SLPs? It is well known that albumin can bind to many ligands, including a variety of lipids, bile salts/pigments, toxins and metal ions. Thus a new chapter has begun to define the role of albumin in the intestine. The role of serum albumin in intestine can be further explore the significance of SLPs upon fat-feeding.

- Is there a separate lipid absorption pathway involving albumin in the intestine?
- Is it involved in the absorption of other ligands which bind to albumin in the intestine?
- What is its association with AP in the intestinal absorption of fats?
- What is its significance in adherence to other basolateral proteins?

These and many more questions will need answers in the future.

1. Eliakim, R., Deshryver-Keeschkemetic, K., Nagee, L., Stenson, W. F. and Alpers, D. H., *J. Biol. Chem*., 1989, **264**, 20614–20619.
2. Deschryver-Kecskemetic, K., Elaikim, R., Carrol, S., Stenson, W. F., Moxley, M. A. and Alpers, D. H., *J. Clin. Invest*., 1989, **84**, 1355–1361.
3. Mahmood, A., Yamagishi, F., Elaikim, R., Deschryver-Kesckemetic, K., Gramlich, T. L. and Alpers, D. H., *J. Clin. Invest*., 1994, **93**, 70–80.
4. Mahmood, A., Mahmood, S., DeSchryver-Kecskemetic, K. and Alpers, D. H., *Arch. Biochem. Biophys*., 1993, **300**, 280–286.
5. Engle, M. J., Mahmood, A. and Alpers, D. H., *Biochim. Biophys. Acta*, 2001, **1511**, 369–380.
6. Goetz, G. S., Mahmood, A., Hultgren, S. J., Engle, M. J., Dodson, K. and Alpers, D. H., *Infect. Immun.*, 1999, **67**, 6161–6163.
7. Mahmood, A., Engle, M. J., Hultgren, S. J., Goetz, G. S., Dodson, K. and Alpers, D. H., *Biochim. Biophys. Acta*, 2000, **1523**, 49–55.
8. Meloun, B., Moravek, L. and Kostka, V., *FEBS Lett*., 1975, **58**, 134–137.
9. Isemiva, S. and Ikenaka, T., *J. Biochem*., 1978, **83**, 35–48.

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Modelling electric permittivity of ice–rock mixtures and implications regarding permittivity-based ice detection techniques in the 1–1000 Hz range

Potential resources for future lunar exploration can be identified and further quantified by studying the near-surface structure of the Moon, up to depths of hundreds of metres. The thermal and geological history of the Moon can also be deciphered from such studies. The lunar volatiles are expected to be preserved in cold traps or buried beneath the surface layer near the poles[1]. The Moon was considered to be entirely dry after the lunar sample return missions (Apollo, Luna) in the 1960s and early 1970s. Infrared mapping by the Moon Mineralogy Mapper (M^3) on Chandrayaan-1 resulted in the detection of hydroxyl molecule (OH) and water on the uppermost few millimetres of the lunar surface[2]. Recent efforts to study the lunar subsurface include various instruments such as the Kaguya lunar radar sounder[3] (LRS), the Chang-E1 microwave radiometer[4], Mini-SAR[5] onboard Chandrayaan-1, Lunar Reconnaissance Orbiter[6] (LRO) and DFSAR[7] on-board Chandrayaan-2 orbiter. This study presents the characterization of ice embedded in regolith materials and discusses a model for evaluating the real component of electric permittivity for the lunar subsurface. The frequency dependence of the real component of the electric permittivity is determined at temperatures 190 and 220 K, over a frequency range 1 Hz to 1 kHz for pure ice. The electric permittivity of two-component