

ANALYSIS OF SOLUBLE PROTEINS IN COMEDONES

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Abstract. The detergent-soluble fraction of purified, concentrated comedo samples was analysed for protein by disc-gel electrophoresis and fluorescamine assay. Spectrophotometer scanning revealed a characteristic pattern of 14 peaks which was essentially uniform in comedones from individuals having acne, Favre-Racouchot syndrome, or prepubertal comedones. The pattern was different in material derived from keratinous cysts and the contents of follicles from the nose. The presence of small amounts of serum proteins, notably globulins, was detected by immunodiffusion.

Key words: Acne; Electrophoresis disc; Skin-sebaceous glands; Folliculitis

A prevalent theory of the pathogenesis of acne involves the action of lipases which hydrolyse triglycerides in the pilosebaceous follicle, with the formation of free fatty acids (10). These fatty acids are then both comedogenic (6) and capable of inducing inflammation (11). In a recent critique of the fatty acid thesis (12), the possibility has been raised that enzymes other than lipases may also play a role in the etiology of acne. These proteins should be contained in the soluble fraction of comedones. In preliminary studies, we found that about 1% of the contents of whole pooled comedones consisted of proteins of molecular weight greater than 15 000, soluble in a dilute solution of non-ionic detergent (Triton X-100). This paper describes further study of the soluble protein fraction extracted from comedones.

MATERIAL AND METHODS

Comedones were collected from individual subjects at one time, pooled and 1-2 mg of each sample was removed for lipid analysis. The remainder (5-50 mg) was homogenized

under sterile conditions in a 5-ml hand homogenizer at a concentration of 10 mg/ml in a 0.1% Triton X-100 solution containing 0.05 M Tris-HCl and 5 mM CaCl₂ at a pH of 7.9. While quantitative microbiology is usually performed on comedones using detergent phosphate buffer homogenates (13), the substitution of Tris and CaCl₂ in the above homogenizing solution gave comparable results. The Tris and CaCl₂ were used as they resulted in higher levels of lipase activity, as measured by a new, sensitive lipid assay which will be discussed in a subsequent paper.

The homogenate was made up to 3 ml with 0.05 mM Ca buffer (without Triton X-100) and centrifuged at 37 000 g at 3°C for 15 min. Occasionally a lipid layer formed at the meniscus. This layer was stirred gently to dislodge any trapped particles and recentrifuged. The supernatant (including any lipid layer present) was concentrated to 0.1 ml in a Minicon B-15 macrosolute concentrator at 5°C, brought to 5 ml by the addition of 0.05 M Tris, 5 mM Ca buffer, and the lipids extracted with 0.5 ml of hexane. Lipid extraction at earlier stages of the procedure could not be performed, as the presence of Triton X-100 resulted in stable emulsions.

After re-concentration to 0.2-0.5 ml, aliquots were taken for protein determination and disc electrophoresis. Separation of interfering Tris was accomplished by gel-filtration on G-15 Sephadex, and the protein was then analysed by fluorescamine (2) using a Perkin-Elmer fluorescence spectrophotometer. By this method as little as 0.5 µg of protein can be detected, using bovine serum albumin as a standard. After disc-gel electrophoresis according to the method of Davis (4), the gels were stained with Coomassie blue (5) and scanned at 580 nm using a Gilford recording spectrophotometer. In a few cases, when larger samples were available, additional electrophoresis gels were run in parallel and were sectioned for lipase assay or immunodiffusion.

RESULTS

In almost every sample the soluble protein comprised less than 1% by weight and rarely amounted to more than 0.5%. Therefore, a typical comedo

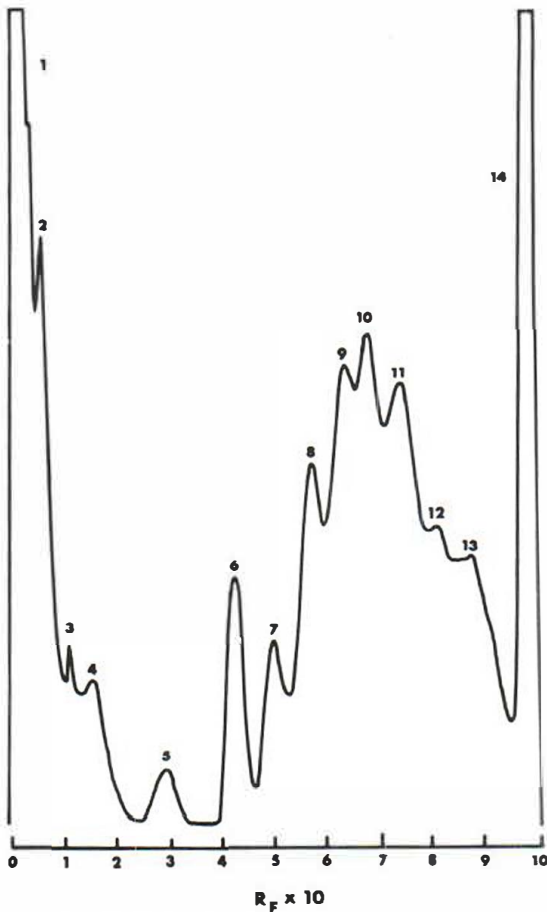


Fig. 1. Disc electrophoretic pattern of soluble protein of comedones from patient with acne.

sample weighing about 10 mg contained approximately 100 μ g of soluble protein. The peaks on scans of electrophoresis gels corresponding to 14 protein bands were numbered consecutively, starting at the origin. A typical scan pattern from an acne patient is shown in Fig. 1. For purposes of comparison, in our system human serum albumin ran at peak 13 (R_f 0.87) and $5\alpha_2$ -globulin between peaks 5 and 6 (R_f 0.34). When unstained gels from 2 acne patients were analysed by immunodiffusion, α_1 -globulin was found associated with band 12 in one and β -globulins with bands 7 and 8 in the other. Sufficient material was collected from 3 acne patients to permit lipase activities to be assayed in parallel unstained gels. Lipase activity was found associated with band 6 in one and in the region of bands 7, 8 and 9 in the other two.

Table I lists the frequency of protein bands observed in comedo samples compared with material from keratinous cysts and nasal follicles. After examination of 44 electrophoresis gels from 23 patients with acne, 3 with Favre-Racouchot syndrome and one with prepubertal comedones, the data were pooled, as the band patterns were essentially the same. Bands 6, 8, 9, 10 and 14 were almost always present (90–100%) and bands 1 and 11 were often present (84%, 86%). Seven gels representing material from nasal follicles (obtained by squeezing) and keratinous cysts showed patterns similar to each other but different from comedones. There was a relatively greater predominance of band 4 and a lesser amount of bands 6, 8 and 10.

DISCUSSION

The major constituents of comedones are water, lipids, proteins and microorganisms. The water content varies from 20–60%, and the lipid moiety comprises roughly 20% of the comedo. Our studies indicate that comedones contain approximately 15% nitrogenous components (Kjeldahl). Two-thirds of this represents proteins insoluble in dilute aqueous Triton X-100 at 5°C, which make up the keratinous framework of the comedo. Essentially all the rest is comprised of amino acids except for a small amount of soluble proteins representing

Table I. Frequency of soluble protein bands as determined by disc electrophoresis

Numbering starts from the origin

Band	R_f	Comedones (44 gels) % positive	Keratinous cysts and nasal follicles (7 Gels) % positive
1	0-0.02	84	57
2	0.03	24	29
3	0.06	30	29
4	0.15	53	100
5	0.27	61	57
6	0.34	100	57
7	0.45	73	86
8	0.54	91	57
9	0.59	98	100
10	0.64	93	57
11	0.69	86	43
12	0.73	67	17
13	0.84	75	50
14	0.94-1.00	93	100

0.5–1.0% total fresh sample weight. Since the soluble protein fraction is so small, any study of these components will be difficult. In order to collect enough material for one electrophoretic analysis, at least 20 individual comedones are required, a quantity larger than readily obtainable routinely from individual acne patients. More extensive examination such as immunodiffusion or enzymatic assays requiring several parallel electrophoresis gels will necessarily be restricted to patients with an unusually large number of comedones.

It was hoped that mucopolysaccharides might be present in sufficient quantity to be studied, but after removal of detergent by dialysis, acid mucopolysaccharides were found to comprise less than 0.1% fresh weight by the carbazole method (1) and carbohydrates less than 0.01% by the orcinol method (8).

Disc electrophoresis patterns have recently been published for neonatal rat epidermal cell fractions by Stern et al. (9, 3). Our results seem most similar to their keratinized fraction (as might be expected) and, taking differences in technique into account, their peak "1" (9), or the regions "e,f,g" (3), may be comparable to our peak 6.

The presence of certain serum α - and β -globulins in comedones from the 3 acne patients examined is intriguing. A simple explanation might be that a small amount of dermal extracellular fluid is included in the samples during the trauma of collection. More likely, this might be the result of direct secretion of those globulins into the pilosebaceous lumen. Immunoglobulin A has been well established as a secretory product of the gastrointestinal tract, and immunoglobulins have also been detected in cerumen (7).

It is disappointing that only one type of electrophoretic pattern was found for the soluble protein fractions from all comedo samples examined. However, this should not be taken as ruling out a significant role for proteins in the pathogenesis of acne. First, important changes may be found in the insoluble protein fraction, which has yet to be examined. Second, it is important to emphasize that each band in the electrophoretic pattern is not necessarily a homogenous protein species, but probably represents a group of different protein molecules having about the same size and charge density. Further fractionation, if it were possible, might reveal marked variations in the amounts of significant enzymes such as lipases and proteases,

which presumably are obscured by other proteins present in the same band in relatively larger amounts. An alternative approach, which we have now adopted, is to assay the soluble protein fraction directly for enzymatic activity.

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