des études réalisées sur cette glycoprotéine réduite et alkylée[9] en présence d'urée et à différents pH, il n'est pas possible d'obtenir des préparations de poids moléculaire plus faible que celui de l'orosomucoïde natif.

Ainsi, un peptide acétylé est présent dans l'orosomucoïde et puisqu'aucun amino acide N terminal n'a jamais été décelé dans cette glycoprotéine native ou dénaturée, il paraît très vraisemblable que cet acétyl peptide constitue l'extrémité N terminale de l'orosomucoïde.

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An improved method for preparing agarose

Agarose, the neutral polysaccharide of agar[1], has recently been established as a useful matrix for immunodiffusion[2], electrophoresis[3], and the gel filtration of viruses and sub-cellular particles[4,5]. Agarose gels are transparent, have superior mechanical properties at very low concentrations, and, because of their low content of sulfated polysaccharides, exhibit practically no adsorption phenomena or electroendosmosis. Although polyacrylamide gels have to some extent satisfied the need for non-ionic matrices in chromatography[6,7] and electrophoresis (see ref. 8), the recent description of two entirely different procedures for fractionating refined commercial agars[9,10] has made the application of agarose gels economically feasible.

HJERTÉN[9] has removed the sulfated polysaccharides (agaropectin[1]) from hot solutions of Difco Bacto-Agar (Difco Laboratories, Detroit, Michigan, U.S.A.) by precipitation with the quaternary-ammonium detergent, cetylpyridinium chloride; after centrifugation of the cetylpyridinium complexes, the supernatant (agarose) is treated with Fuller's earth to bind residual detergent. High yields of agarose may be obtained by this method, although the frequent washings required and the capacities of conventional centrifuges allow only small batches to be processed, making the procedure technically laborious. In addition, the treatment of some other commercial agars with cetylpyridinium chloride may result in poor flocculation of the agaropectin.

RUSSELL, MEAD AND POLSON[10] have successfully fractionated Ionagar No. 2 (OXO Limited, London, Great Britain) by repeated precipitation of agarose from

hot solutions with polyethyleneglycol 6000. This method allows larger batches of agar to be processed more rapidly than does the technique of HJERTÉN[9], but the overall yield is lower. Our attempts to apply this procedure to Difco Bacto-Agar resulted in low recoveries of agarose with a high sulfate content; applied even to Ionagar No. 2, the resulting agarose frequently produced some turbidity when treated with cetylpyridinium chloride, although no sulfate content was detectable.

It seemed to us that our failure to arrive at acceptable agarose preparations by the method of RUSSELL, MEAD AND POLSON[10] resulted from the difficulty of washing out contaminants occluded in the agarose precipitate. On this assumption we modified their procedure by producing a precipitate which could be washed exhaustively in a finely granular form by conventional filtration. This modification also reduced the number of precipitations required to achieve preparations free of sulfate and cetylpyridinium-precipitating contaminants, and allowed both Difco Bacto-Agar and Ionagar No. 2 to be fractionated rapidly in large batches with equal effectiveness.

In the following procedure, tap water and technical-grade chemicals were found satisfactory. The initial agar solution is prepared by suspending 100 g of Difco Bacto-Agar or Ionagar No. 2 (distributed in the U.S.A. by Consolidated Laboratories, Chicago Heights, Ill.) in 2 l of boiling 1 % sodium citrate dihydrate solution, melting at 15 lb/in^2 for 30 min in an autoclave or pressure boiler, and stirring to ensure a homogeneous solution. The agar solution is cooled to 70–75° in a warm-water bath, after which precipitation of the agarose is accomplished by adding 250 g of polyethyleneglycol 4000 (Carbowax 4000, Union Carbide Corp., South Charleston, W. Va., U.S.A.) in small portions with continuous manual stirring. A flocculent, opalescent precipitate separates immediately, and the suspension is allowed to stand at room temperature until the gelatinous mass has sedimented to a minimal volume (usually after 5–10 min). The supernatant is poured off, and 60 ml of hot (50–60°) Tween 80 (Atlas Chemical Industries, Wilmington, Del., U.S.A.) are stirred vigorously, with copious foaming, into the hot, sticky precipitate. Stirring is continued until the precipitate is reduced to a homogeneous suspension of fine granules. The container is then agitated for several minutes under cold running water to ensure complete gelation of the agarose. This suspension is transferred to a 2-l coarse sintered-glass Buchner funnel, washed under suction with about 2 l of cold water to remove the bulk of free detergent, then with 10–15 l of hot (55–65°) water until little or no foaming is observed in the wash solution. Hot water serves to melt and dissolve the large amount of polyethyleneglycol that is co-precipitated with the agarose and permits a faster filtration rate. The moist granules are transferred to a container, 20 g of sodium citrate dihydrate are added, and the volume is brought to 2 l with water. This crude agarose is melted under pressure, stirred until homogeneous, processed at about 70° with the same quantities of Carbowax 4000 and Tween 80, and washed thoroughly as described. The moist granules may be dried on the filter by stirring with small portions of acetone, hot methanol (to dissolve traces of polyethyleneglycol), and ether. Excess solvent may be evaporated under warm forced-air. Agarose may be obtained in yields of 35–45 % from Ionagar No. 2 or 25–30 % from Difco Bacto-Agar. This discrepancy in yield may reflect the purity of the starting material (*cf.* ref. 9). Crude agar shreds from two suppliers were very poor sources of agarose.

Although somewhat higher concentrations were required for complete precipi-

tation, Carbowax 4000 was more readily removed from the agarose and was generally more effective for this procedure than Carbowax 6000 (equivalent to the grade employed by RUSSELL, MEAD AND POLSON[10]). Many of the Triton non-ionic surfactants (Rohm and Haas Corp., Philadelphia, Pa., U.S.A.), *e.g.*, X-100, may be substituted for Tween as efficient dispersion agents for the agarose precipitates. Non-ionic detergents also appear to facilitate the fractionation by impairing the gelation of agaropectin. The quality of the agarose obtained by this procedure depends largely on the temperature at which the precipitation with polyethyleneglycol is carried out. High temperatures (80–100°) favor the co-precipitation of agaropectin, but precipitation at intermediate temperatures (40–60°) results in poor flocculation and consequent low recoveries.

Before using agarose prepared by any of the methods described, it is desirable to ensure the complete removal of salts, polyethyleneglycol, detergents, and debris. This can be accomplished conveniently in the following manner: The agarose is prepared at a known concentration—usually at 4 %, since higher concentrations may be difficult to melt—by soaking the dry powder in a 1 % sodium citrate dihydrate solution and melting under pressure. The hot solution is filtered through a coarse sintered-glass funnel under mild suction to remove suspended contaminants and gelled by refrigeration. The agarose block is diced into 1-cm cubes, washed in running tap water at room temperature for 24 h, then in frequent changes of distilled water over a period of days. Stored under water, the agarose cubes are stable indefinitely in the cold. The agarose cubes are readily dissolved on a boiling saturated sodium chloride bath; and, by appropriate adjustment of stock cube weight and solvent volume and concentration, an agarose matrix can be prepared at any of the concentrations ordinarily employed for electrophoretic or immunodiffusion studies. Alternatively, the washed agarose may be precipitated from warm solutions with ethanol[9], collected by filtration, and dried in the usual manner.

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