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THE LOW-MOLECULAR WEIGHT CONSTITUENTS PRESENT AFTER POLYMERISATION OF ACRYLAMIDE IN SOLUTION

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Little attention has been paid to the monomers which fail to be incorporated into a polyacrylamide gel; when mentioned at all they are referred to as 'unpolymerised monomers'. Preliminary experiments suggested that species other than monomers might contribute, and we have commenced characterisation of the materials seen after polymerisation of acrylamide alone, i.e. without any cross-linker.

The system involves varying concentrations of acrylamide in de-gassed aqueous solution, polymerisation being initiated with 0.1% w/v each of ammonium persulfate and TEMED. The eventual high-molecular weight product is precipitated with 100 mls methanol to 6 mls of polymer solution. Our studies are on the methanol-soluble material.

The proportion of free acrylamide found by HPLC varied from 10% at an initial acrylamide concentration of 1% w/v to 2% at an initial acrylamide concentration of 4.5% w/v.

Sephadex G 10 chromatography with water as eluent and absorbance recorded at 254 nm showed a peak close to the void volume (A) and another close to the total column volume (B). Where benzamide was used as an internal standard, a third, much later peak was also seen.

B may represent free acrylamide, but we don't yet have proof. A has been re-run on Sephadex G 25 and shows two peaks, A1 close to the void volume and A2 with K_{av} approximately 0.7.

The results thus summarised exhibit several curious and interesting features.

ICHF: INTERNALLY-CLAMPED HOMOGENEOUS FIELD APPARATUS FOR ELECTROPHORESIS OF LARGE DNAS.

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The physical characterization of large genomes requires techniques whereby large DNAs (usually linear) and intact chromosomes in the size range of 100 kb to several megabase pairs may be electrophoretically separated. This is accomplished by the use of periodically-reorienting electric fields.

We describe a pulsed-field gel electrophoretic apparatus which has been used to separate intact chromosomes and fragments up to at least 7 Mbp. Excellent resolution is obtained and DNAs migrate in straight tracks.

Field homogeneity is achieved with six field-correcting electrodes, four of which are active at any time. The voltages at these accessory electrodes are electronically controlled and track the main power supply voltage. Several reorientation angles are possible. The use of a low ionic strength buffer allowed passive temperature control by reducing Joule heating.

An unexpected finding was that yeast chromosomes migrated ~20% faster in field-corrected gels than in identical gels when the fields were non-homogeneous.