Biochemistry and Genetics of Esterase-20 (ES-20), a Second Trimeric Carboxylesterase of the House Mouse (Mus musculus). I. Purification and Characterization of ES-20C\(^1\) from Male Kidney

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ES-20\(^1\) was isolated from male mouse kidney and purified 350-fold by ion-exchange chromatography, isoelectric focusing, and gel filtration. The resultant product was apparently homogeneous by the criteria of polyacrylamide gel electrophoresis and immunodiffusion and represented a major fraction of male mouse kidney esterase. Sodium dodecyl sulfate gel electrophoresis revealed the presence of a single subunit band, molecular weight 59,500; the molecular weight of the native protein was found to be 179,000. Titration of the active site yielded an equivalent weight of about 175,000. The enzyme was further characterized by its kinetic parameters for the hydrolysis of a series of 4-nitrophenyl esters and was classified as a carboxylesterase (EC 3.1.1.1). ES-20C\(^1\) bound to concanavalin A, indicating that it was a high-mannose-type glycoprotein; the role of terminal β-N-acetylglucosamine residues in the carbohydrate side chains for stabilization of the quaternary structure of the trimer was revealed. Extensive biochemical and immunological similarities to ES-9C supported an earlier suggestion that the Es-9\(^{e}\) gene product is a component of the ES-20C\(^1\) trimer.

KEY WORDS: Mus musculus; carboxylesterase (EC 3.1.1.1); glycoprotein; purification; locus Es-9.

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INTRODUCTION

Among the bewildering number of mouse nonspecific esterases, an isozyme system can be recognized whose components are controlled by one or two closely linked multigene families on chromosome 8 (reviewed by Peters, 1982) and are, without exception, carboxylesterases (EC 3.1.1.1). The complexity of this isozyme system, consisting of many components of widely overlapping biochemical properties, has presented a major obstacle to our understanding of the biological function of the carboxylesterases. As stressed by Peters and Nash (1978), progress in this direction, as well as in identifying the number of loci coding for these nonspecific enzymes, may be aided by isolating and characterizing the individual esterases, an approach we have adopted in recent years (Göppinger et al., 1978; Lexow et al., 1980; Otto et al., 1981; Oehm et al., 1982).

Esterase-20 (ES-20), a prominent mouse kidney carboxylesterase, has so far been defined electrophoretically only as a series of bands migrating just anodally to ES-6 (Nash and von Deimling, 1982), from which it had hitherto been unresolvable. It was clear that ES-20 represented a polymorphism separate from ES-6, on the basis of substrate specificity, organ distribution, and differential control by testosterone (ES-20, unlike ES-6, was expressed much more strongly in male mice than in females). Less clear was the relationship between ES-20 and ES-9, which resembled ES-20 in the above properties as well as in the allele distribution among strains. By analogy with the ES-6 monomer/trimer system (Oehm et al., 1982), it was suggested that ES-20 may represent an aggregated product of the Es-9 locus (Nash and von Deimling, 1982).

The present work was undertaken in order to clarify the genetic and biochemical status of ES-20, in particular its relationship to other mouse chromosome 8 esterases. This paper describes a purification procedure for ES-20 and its biochemical analysis. Most common laboratory strains (e.g., C57 strains) express the ES-20A phenotype. However, the successful isolation of ES-20 depended upon the occurrence of the much more strongly expressed ES-20C, seen in Mus musculus molossinus. In addition to the major band ES-20C¹, this phenotype also included a less prominent anodal band, ES-20C². During purification, ES-20C¹ was separated from ES-20C², which was not further investigated in the present work.

MATERIALS AND METHODS

Source of ES-20C¹

Male mice of our stock NMRI/Fre.mo-Es-1¹ (Otto et al., 1981) were used as a source of ES-20. These animals were produced by transferring the carboxyl-