'A'-esterase activity in the lipoprotein fraction of sheep and human serum

M. I. MACKNESS, S. D. HALLAM and C. H. WALKER

Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ, U.K.

Aldridge (1953) defined two classes of esterase based upon their interaction with organophosphate anticholinesterases: 'A'-esters are inhibited by organophosphates, whilst 'B'-esters are inhibited by them. The 'A'-esterase activity of serum and of liver preparations is much higher in mammals than in birds, and this provides mammals with an effective detoxication mechanism against organophosphates such as pirimiphos-methyl and diazinon. This can explain the marked selectivity of these two compounds between birds and mammals (Brealey et al., 1980).

Most of the 'A'-esterase of sheep serum is separated into the lipoprotein fraction by ultracentrifugation (Mackness & Walker, 1981). This observation prompted further investigation of the separation of 'A'-esterase into lipoprotein fractions as a key step in the purification of the enzyme from mammalian serum.

Lipoprotein fractions were prepared from human and sheep serum by adjusting the density to 1.225 g/ml with potassium bromide and then centrifuging for various periods up to 48h in a Sorvall OTD-50B ultracentrifuge fitted with an 8 x 4.4 ml vertical rotor (TV865); the upper lipoprotein fraction was separated from the soluble fraction. HDL fractions were prepared from sheep serum by using a two-stage centrifugation procedure (Mackness & Walker, 1983). 'A'-esterase activity was assayed at 37°C with paraoxon as substrate (see Mackness & Walker, 1981). The generation of p-nitrophenol at pH7.6 was measured with a recording spectrophotometer. HDL was estimated as HDL-cholesterol with a Sigma cholesterol determination kit. Protein was determined by the dye-binding method (Spector, 1978).

HDL fractions and soluble fractions were subjected to gel filtration using Sepharose 6B (Pharmacia). The column was fitted with an 8 x 4.4 ml vertical rotor (TV865). Individual fractions were assayed for 'A'-esterase activity and protein.

With both sheep and human serum, the amount of 'A'-esterase activity separating into the lipoprotein fraction continued to increase over a period of 24h. Between 24 and 48h further separation into lipoprotein was observed with human serum but not with sheep serum. Total lipoprotein and HDL-cholesterol in lipoprotein also tended to increase with time of centrifugation up to 24h, but the rates of separation differed between these components in both species.

Virtually all of the 'A'-esterase activity of sheep serum lipoprotein separated into the HDL fraction in human and sheep serum (Mackness & Walker, 1983). Both the HDL fraction and the soluble fraction were run on a Sepharose 6B column and showed differences which are reported in Fig. 1.

Centrifugation with the 8 x 4.4 ml vertical rotor for 8h gives similar yields of lipoprotein, HDL-cholesterol and 'A'-esterase in lipoprotein as are obtained by the procedure usually recommended for lipoprotein separation (Havel et al., 1955). With longer centrifugation (<48h) there were substantial improvements in recovery of all three components from both human and sheep serum with about 75% of the 'A'-esterase activity separating into the lipoprotein fraction over the whole period.

The average $M_r$ of 360000 for HDL 'A'-esterase strongly suggests that the activity is in the HDL$_2$ fraction (Tanford, 1980). When partially purified 'A'-esterase fractions from sheep serum lipoprotein were subjected to denaturing gel electrophoresis, the main protein components found corresponded to the core protein of HDL$_2$ and the apoproteins thereof. Thus, it appears that 'A'-esterase activity is expressed by HDL$_2$ itself and is not due to proteins which may have been picked up by the HDL$_2$, during centrifugation (Mackness & Walker, 1983).

The 'A'-esterase of human serum separated more slowly than that of sheep serum during centrifugation. It therefore seems likely that the density and/or particle size of the HDL$_2$ fraction which carries 'A'-esterase activity differs between the two species. This lends further support to earlier evidence for multiple forms of HDL 'A'-esterase (Mackness & Walker, 1983).

Preparation of the HDL$_2$ fractions followed by gel filtration provides a simple and rapid first step in the purification of the major part of serum 'A'-esterase, giving a 54-fold purification.


Abbreviation used: HDL, high-density lipoprotein.

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Effect of a rat plasma high-density lipoprotein subfraction on the synthesis of bile salts by rat hepatocyte monolayers

ROBERT P. FORD, KEITH E. SUCKLING, KATHLEEN M. BOTHAM and GEORGE S. BOYD*

Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh EH8 9XD, U.K.

The liver plays a major role in the homoeostasis of cholesterol, being the major site at which cholesterol is removed from the plasma and the only organ where cholesterol can be degraded to bile salts. It has been suggested that of the plasma lipoproteins, HDL provides a mechanism whereby excess cholesterol from the peripheral tissues is delivered to the liver for its ultimate removal from the body (Glomsett, 1968). This can be achieved by either degradation to bile acids or by direct secretion into the bile. Whilst evidence has shown that the liver is a major site for the catabolism of HDL (Stein et al., 1983) there is no evidence to suggest that HDL can stimulate degradation of cholesterol to bile salts.

We have investigated the synthesis of bile salts by rat hepatocyte monolayers in the presence and absence of a rat plasma HDL subfraction (HDLz).

Rat hepatocytes from donor rats fed a diet supplemented with 4% cholestyramine were prepared as described by Botham et al. (1980) and maintained in monolayers for periods up to 24h. Hepatocytes were incubated in the presence or absence of a physiological concentration of rat HDLz, isolated by rate zonal ultracentrifugation (Oschry & Eisenberg, 1982). The synthesis of conjugated cholic, chenodeoxycholic and β-muricholic acids were determined by specific radioimmunoassays (Beckett et al., 1978, 1979; Botham et al., 1983). Total bile salt synthesis, as represented by addition of conjugated cholic acid + conjugated chenodeoxycholic acid + conjugated β-muricholic acid, was significantly increased in the presence of HDLz (Fig. 1). In a recent report by Davis et al. (1983), HDL isolated by conventional ultracentrifugation procedures had no effect on the synthesis of bile salts by rat hepatocytes in monolayer culture. However, it is important to note that in the present study hepatocytes were obtained from rats fed a diet supplemented with the bile salt sequestrant cholestyramine. The known effects of feeding this resin include stimulation of bile salt synthesis in isolated hepatocytes (Botham et al., 1980; Kempen et al., 1982) and increase in the uptake of LDL from plasma (Shepherd et al., 1980). It is thought that this increased catabolism of LDL is due to an increase in the expression of the apolipoprotein B/E receptor on the hepatocyte plasma membrane. This receptor is also responsible for uptake of HDL. The observed increase in the synthesis of bile salts in the presence of HDLz (Fig. 1) may therefore be due to an increase in the delivery of cholesterol to hepatocytes obtained from cholestyramine-fed rats as compared with control animals, resulting in an increase in the cholesterol available for degradation. It remains to be seen whether an increase in bile salt synthesis is observed in hepatocytes isolated from rats fed a control diet.

The ratios of conjugated cholic acid:conjugated chenodeoxycholic acid:β-muricholic acids and conjugated chenodeoxycholic acid:conjugated β-muricholic acid were also determined. These ratios provide good indications of the rates of 12α-hydroxylation and 6-hydroxylation respectively. 12α-Hydroxylation controls the ratio of conjugated cholic acid to conjugated chenodeoxycholic acid and 6-hydroxylation is an indication of the rate of conversion of chenodeoxycholic acid to its metabolite, β-muricholic acid (Botham & Boyd, 1983). HDLz had no effect on these ratios. We therefore conclude that HDLz-cholesterol enters a common precursor pool which provides cholesterol for the synthesis of the major bile acid conjugates detected.

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Fig. 1. The effect of HDLz on bile salt synthesis by rat hepatocyte monolayers

Hepatocytes in monolayer culture were incubated in the absence (○—○) or presence (●—●) of HDLz (500μg of HDLz protein/ml) for the indicated time. Bile salts detected at 0h represent cell-associated levels. Each point represents duplicate determinations from hepatocytes obtained from six rats. Significance limits: *P<0.05. Error bars show ± S.D.