# EFFECT OF DISULFIDE CLEAVAGE ON THE MOLECULAR WEIGHT OF ONE OF THE MAJOR POLYPEPTIDES OF HUMAN SERUM HIGH DENSITY LIPOPROTEIN

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Received 16 August 1971

## 1. Introduction

In previous studies we have shown that apo HDL contains two major polypeptide classes which can be separated by gel chromatography in 8 M urea [1]. These two polypeptides, operationally termed III and IV, were found to have distinct physical, chemical and immunological properties [1] and to be comparable, though not identical, in amino acid composition with fractions 4 and 3 of Shore and Shore [2] and II and III of Rudman et al. [3]. Among its distinctive features, III contains no cysteine or cystine, contrary to IV where cystine is present and appears to have a significant conformational role [1, 4]. As a part of a study directed at the elucidation of the role of disulfides in HDL structure, both III and IV were systematically examined before and after reduction or reduction and carboxymethylation. We wish here to report on the aspect of the studies dealing with the analysis of these fractions by Sephadex column chromatography in 8 M urea and SDS polyacrylamide gel electrophoresis. It will be shown that IV, which, in its unreduced state, has a MW of 16–17,000 daltons, is converted by disulfide cleavage into units half of its weight, thus providing evidence for a dimer-monomer relationship.

## 2. Experimental

$HDL_2$ was separated and purified from normal sera by ultracentrifugal flotation [5]. Apo $HDL_2$ was obtained by delipidation of $HDL_2$ with 3:2 ethanol–ethyl ether at $-10°$ [5]. Fractions III and IV were separated at 12° by Sephadex G-200 chromatography using 0.01 M Tris-HCl, $10^{-3}$ M EDTA, pH 8.2, 8 M urea as the eluting buffer [1]. The same procedure was followed for specimens of apo $HDL_2$, which had been reduced with β-mercaptoethanol and then alkylated with iodoacetamide following the procedure previously described [4]. When reduced but not alkylated apo $HDL_2$ preparations were subjected to column chromatography, the eluting buffer contained 0.1% β-mercaptoethanol. Reduction or reduction and carboxymethylation of III and IV were carried out as for apo $HDL_2$. Each Sephadex fraction was dialyzed until urea-free and then concentrated in an Amicon Diaflo ultrafiltration cell, Model 52, using a UM-2 membrane (MW cutoff at 1000). Their purity was tested by analytical PAGE-8M urea [1], PAGE-isoelectric focusing [1] and by their reaction with specific antibodies made against fraction III or IV. PAGE-SDS in the presence or absence of reducing agent was carried out essentially according to Weber and Osborn [6] and the system calibrated with pro-

* Operated by the University of Chicago for the United States Atomic Energy Commission.

*Abbreviations:* HDL, high density lipoprotein; $HDL_2$, HDL of d 1.063–1.125 g/ml; apo $HDL_2$, protein moiety obtained by delipidation of $HDL_2$; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

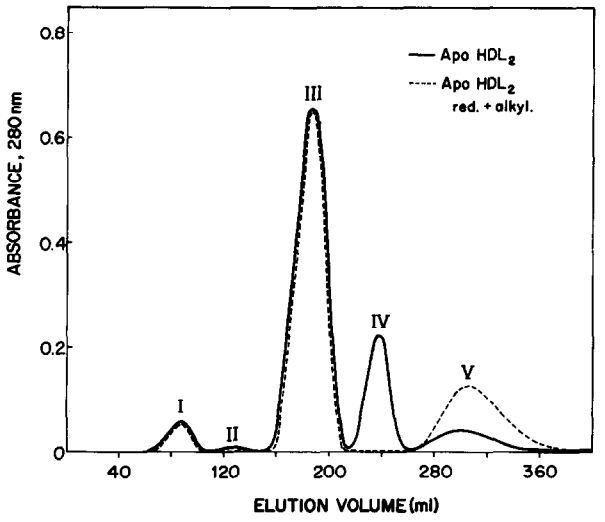


Fig. 1. Sephadex G-200 chromatography in 8 M urea of apo $HDL_2$ before and after reduction and carboxymethylation. The experimental conditions were those reported before [1].

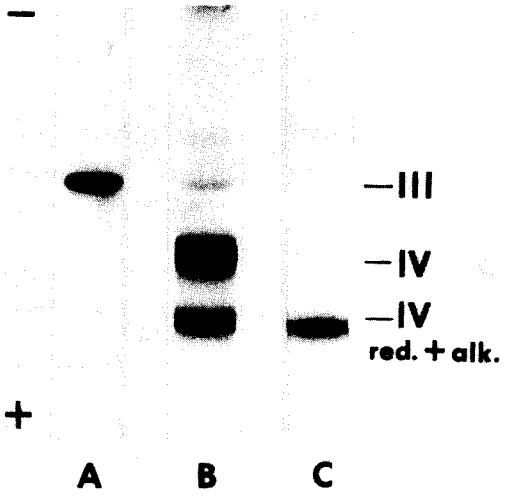


Fig. 2. PAGE-SDS patterns of: A. Fraction III; B. Mixture of III (used as a marker), IV and IV, reduced and carboxymethylated; C. IV, reduced and carboxymethylated. The experimental conditions were those described by Weber and Osborn [6] but with omission of reducing agent ($\beta$-mercaptoethanol).

teins of known molecular weight. Molecular weight calculations were carried out according to Weber and Osborn [6].

## 3. Results and discussion

The pattern of elution of apo $HDL_2$ from Sephadex G-200 columns exhibited the previously described five components [1]; peak II, however, was seen only occasionally. Apo $HDL_2$, reduced and carboxymethylated, behaved as unreduced apo $HDL_2$ in terms of peaks I and III. In turn, peak IV was not seen in its ordinary elution volume and appeared to overlap with peak V (fig. 1). Reduced apo $HDL_2$, eluted in the presence of $\beta$-mercaptoethanol had the same elution profile as reduced and carboxymethylated apo $HDL_2$. By PAGE-8 M urea, the fraction III, which had been obtained from the above experiments, had an identical electrophoretic mobility. On the other hand, reduced and carboxymethylated IV had a rate of migration faster than unreduced IV, giving additional bands in the area occupied by fraction V. The same observation was made with reduced preparations of IV analyzed in gels containing $\beta$-mercaptoethanol.

These results and the values of molecular weight computed from a calibrated Sephadex G-200–8 M urea column [1] suggested the existence of a dimer-monomer relationship between unreduced and reduced IV. This conclusion was corroborated by the study of fractions III and IV and of their reduced, or reduced and carboxymethylated, derivations by analytical PAGE-SDS. Whereas III was not affected by chemical modifications, reduced and carboxymethylated fraction IV exhibited a band with electrophoretic mobility significantly faster than that of the unreduced product (fig. 2). This was also observed with reduced preparations of IV separated in SDS gel containing 0.1% $\beta$-mercaptoethanol. From the calibration plot, constructed with known standards (fig. 3), the apparent molecular weights were: 25–27,500 daltons for III, 16–17,000 daltons for IV and 7,500–8,500 daltons for the chemically-modified products. The values for III and unreduced IV were in good agreement with the chromatographic, electrophoretic and sedimentation equilibrium data previously obtained in this laboratory [1]. In our earlier report [1] we observed a smaller molecular weight component (16–17,000 daltons) in the preparations of frac-

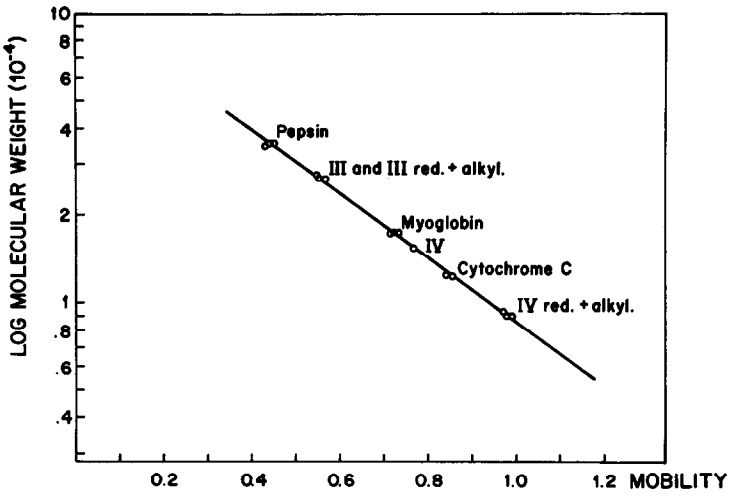


Fig. 3. Molecular weight plots of markers and apo $HDL_2$ fractions vs. electrophoretic migration. PAGE-SDS was carried out according to Weber and Osborn [6] both in the presence and absence of reducing agent ($\beta$-mercaptoethanol).

tion III used. Since this component was not detected in the current studies, even in SDS gel containing 8 M urea, it is highly probable that it represented a peak IV contaminant in the preparations of III used. This interpretation is validated by the fact that the immunological cross-reactivity noted earlier was not observed in the current studies. It should be stressed that the presence of urea in the SDS gels did not affect the molecular weight of III.

The above findings indicate that the two major polypeptide classes of apo $HDL_2$ have a protomer molecular weight of 25–27,500 (fraction III) and 7,500–8,500 daltons (fraction IV). The figure of 16–17,000 reported earlier for unreduced IV and confirmed in the present studies, probably represents that of a dimer whose protomeric units are linked by disulfide bridges. This knowledge has proven fundamental for approaching the problem of the previously suspected [1] microheterogeneity of III and IV. Methods for sub-fractionating these polypeptide classes have been developed in this laboratory and their description together with an account of the characteristics of the resulting fractions will be the subject of a separate report.

According to Shore and Shore [2] the two major polypeptides of apo HDL, separated by DEAE chromatography, each have a molecular weight of about 15,000 daltons, a figure seemingly at variance with our present results. It should be stressed, however, that their materials are not strictly comparable with ours both in terms of amino acid composition [1, 2] and *C*-terminals (manuscript in preparation). It is similarly difficult to compare our data with the molecular weight figures recently stated by Kostner and Alaupovic [7], since no supportive technical data for such figures were given.

## Acknowledgements

This work was supported in part by grants from the USPHS (HE-08727-07); American Heart Association (# 70-753) and Chicago and Illinois Heart Association (C71-6). A.M.S. is the recipient of USPHS Research Career Development Award HE-24,867.

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