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# STRUCTURAL ALTERATIONS IN ALPHA$_1$-ANTICHYMOTRYPSIN FROM NORMAL AND ACUTE PHASE HUMAN PLASMA

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Summary: Human alpha$_1$-antichymotrypsin, isolated at pH 8.0 from both normal and acute phase plasma, has been found to have two different amino terminal sequences despite the fact that inhibitory activities are unchanged. In normal plasma over 90% of the protein has an amino terminal sequence beginning with aspartic acid and less than 10% with arginine. However, in acute rheumatoid arthritis plasma 55% of the inhibitor begins with arginine and the remainder with aspartic acid. Sequence studies indicate that a fifteen amino acid peptide fragment has been cleaved to yield the arginine protein. Human alpha$_1$-proteinase inhibitor also shows this heterogeneity, but the ratios do not change between normal and acute phase plasma. It may well be that the missing peptide has some biological activity manifested only in the acute phase state.

Human alpha$_1$-antichymotrypsin ($\alpha_1$-Achy) is a plasma proteinase inhibitor with a specificity believed to be directed entirely towards enzymes with chymotrypsin-like activity (1). This protein, first described as $\alpha_1$-X glycoprotein, was originally isolated by Heimburger and Haupt (2). However, its significance in controlling proteolytic events has received little attention despite the fact that it is a major acute phase reactant whose concentration may quadruple within eight hours after the initiation of trauma (3).

Previously (4), this laboratory described a simplistic procedure for the isolation of $\alpha_1$-Achy and also characterized the inhibitor in terms of structural and functional properties. More recently (5), an improved purification scheme was developed. However, while the inhibitory activity of this latter preparation was identical to that described earlier, its amino terminal sequence differed considerably depending on the source of

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the plasma used. Since our primary purpose was to delineate the physiological role of $\alpha_1$-Achy in controlling chymotrypsin-like enzymes, through mechanism studies and including the delineation of the reactive site sequence, it became necessary to investigate in some detail the reasons for differences in the structure of $\alpha_1$-Achy prepared by the new procedure, relative to that originally used.

## Materials and Methods

Human $\alpha_1$-Achy was purified from both normal and acute rheumatoid arthritis plasma by a modification of that originally described from this laboratory (4) and involved two chromatographic passes through Cibacron Blue Sepharose at pH 8.0 (5,6), rather than ion-exchange chromatography at pH 5.5. Human $\alpha_1$-proteinase inhibitor ($\alpha_1$-PI) was prepared from both types of plasma by procedures described earlier (7). Carboxypeptidase A-DFP was from Sigma Chemical Co. and carboxypeptidase Y from Pierce Chemical Co.

The amino terminal sequence of $\alpha_1$-Achy and $\alpha_1$-PI was determined using a Beckman Model 890C Sequencer and the 0.1M Quadrol program described by the manufacturer. Phenylthiohydantoin derivatives obtained from sequence analysis were identified by either high pressure liquid chromatography (8) or by amino acid analysis after back hydrolysis of the derivatives to free amino acids (9).

The carboxy terminal sequence of $\alpha_1$-Achy was investigated by hydrolysis with either carboxypeptidase A-DFP or carboxypeptidase Y. Protein was dissolved in a 0.05M sodium acetate buffer, pH 5.5 (for carboxypeptidase Y) or in 0.05M sodium veronal buffer, pH 8.5 (for carboxypeptidase A). After incubation with enzyme for various time intervals at 25°, aliquots were withdrawn and the reaction terminated by the addition of an equal volume of 20% trichloroacetic acid. The released amino acids present in the supernatant after centrifugation of the trichloroacetic acid precipitated protein were identified by amino acid analysis.

## Results and Discussion

The isolation of $\alpha_1$-Achy and $\alpha_1$-PI from 500 ml of normal plasma by the newer procedure which we have developed (5) resulted in the recovery 90 mg and 210 mg of each inhibitor, respectively. However, the yields from rheumatoid arthritis plasma were significantly higher, with 380 mg of $\alpha_1$-Achy and 525 mg of $\alpha_1$-PI being obtained from 500 ml of plasma. Since this disease state causes the development of an acute phase condition whereby increased synthesis of certain plasma proteins occurs (3), it was not unexpected to recover higher quantities of these two acute phase reactants. Furthermore, both inhibitors prepared from the acute rheumatoid arthritis plasma had identical inhibitory activities towards chymotrypsin-like enzymes compared to those isolated from normal plasma despite the structural differences described below.

Amino and Carboxy Terminal Sequences of Normal and Rheumatoid $\alpha_1$-Achy and $\alpha_1$-PI

When either $\alpha_1$-Achy preparation was subjected to amino terminal sequence analysis there appeared to be heterogeneity in the preparations used since two sequences were detected. However, based on the amino terminal sequence of $\alpha_1$-Achy reported earlier (4), it was possible to discern that the two sequences present in these preparations overlapped each other. In fact, as shown in Table 1, it was found that a sequence beginning with aspartic acid predominated in normal plasma (91%), while the sequence beginning with arginine and representing that originally published from our laboratory was only present in minor quantities (9%). Significantly, this ratio changed in rheumatoid synovial plasma to a 55%-45% ratio in favor of the arginine sequence. In both sets of experiments the initial yields were near 84% and the repetitive yields about 93% (leu 4 to leu 9 in the aspartic acid sequence of normal plasma; val 5 to val 14 in the arginine sequence of rheumatoid plasma). It was not possible to sequence beyond residue 8 in the arginine sequence present in normal plasma because of the low levels present in this preparation. However, it was possible to go through 41 cycles of the major

Table 1: Amino Terminal Sequence of $\alpha_1$-Antichymotrypsin From Normal and Acute Rheumatoid Arthritis Plasma

| Residue Number | Normal Plasma | | Rheumatoid Plasma | |
|---|---|---|---|---|
| | Major (91%) | Minor (9%) | Major (55%) | Minor (45%) |
| 1 | Asp | Arg | Arg | Asp |
| 2 | Pro | Gly | Gly | Pro |
| 3 | Asn | Thr | Thr | Asn |
| 4 | Leu | His | His | Leu |
| 5 | Asn | Val | Val | Asn |
| 6 | Glu | Asp | Asp | Glu |
| 7 | Asp | Leu | Leu | Asp |
| 8 | Asp | Gly | Gly | Asp |
| 9 | Leu | | Leu | Leu |
| 10 | Thr | | Ala | Thr |
| 11 | Glu | | Ser | Glu |
| 12 | Gly | | Ala | Gly |
| 13 | Asp | | Asn | Asp |
| 14 | Glu | | Val | Glu |
| 15 | Asn | | Ser | Asn |
| 16 | Arg | | Phe | Arg |
| 17 | Gly | | Ala | Gly |
| 18 | Thr | | Phe | Thr |
| 19 | His | | Ser | His |
| 20 | Val | | Leu | Val |

protein to confirm the overlap with that previously published for the amino terminal sequence of $\alpha_1$-Achy. With either protein preparation a single carboxy terminal sequence was established as glycyl-serine. This suggests that the differences between the two types of $\alpha_1$-Achy are at the amino terminus.

A double sequence was also found with $\alpha_1$PI isolated from either of the two plasma samples. The major sequence was identified as beginning with Glu-asp-pro-, which was the amino terminal sequence of intact $\alpha_1$-PI reported previously (4). The minor sequence began with Lys-thr-asp and represented the structure beginning at residue ten from the amino terminal of the native inhibitor. The ratio of the quantity of each of these two structures, however, was almost the same (90% to 10%) in favor of the normal sequence, regardless of the type of plasma used. Similar results have been reported earlier (10) with the exception that a glutamyl residue was found at position ten in the native protein and at residue one in the modified form. This may represent microheterogeneity in the $\alpha_1$-PI preparations used both here and previously but clearly indicates that minor proteolysis has occurred to release a nine amino acid peptide fragment.

From the results of the amino terminal sequence of $\alpha_1$-Achy from normal and acute rheumatoid arthritis plasma it can be seen that the inhibitor, when isolated to homogeneity, has two possible sequences. The new sequence is fifteen residues longer than that reported earlier by this group (4), and the amounts of either shift dramatically between the two plasma samples. Significantly, in this case and in that of $\alpha_1$-PI where proteolysis has also occurred but to a lesser extent, the peptide bond broken is between a carboxamide amino acid and a basic amino acid (asn-arg for $\alpha_1$-Achy and gln-lys for $\alpha_1$-PI). This may indicate that a specific enzyme, present in plasma, is responsible for the peptide bond hydrolysis. However, one must still ask the question of the source of the enzyme and the reason for cleavage. For example, are the peptides released biologically active? Since there is

no apparent loss of inhibitory activity between the two sets of inhibitor preparations obtained, it is certainly tempting to suggest such a function, particularly in the case of $\alpha_1$-Achy where up to 55% of the amino terminal peptide is missing in the rheumatoid plasma.

In the original purification procedure described for $\alpha_1$-Achy, apparently normal plasma was utilized and one would have expected to isolate an inhibitor with the extended sequence reported here. However, the method described for the isolation of this protein involved a chromatographic step at pH 5.5, and this could have activated the enzyme responsible for peptide bond cleavage between residues 15 and 16 of the native protein. In the current methodology used for $\alpha_1$-Achy isolation the pH is kept near 8.0 and such peptide bond hydrolysis is obviously reduced. Nevertheless, in the case of $\alpha_1$-Achy from acute phase plasma about one-half of the inhibitor has been converted and, therefore, pH cannot completely account for the heterogeneity in these preparations, especially since $\alpha_1$-PI conversion remains minimal. It may thus be necessary to invoke other considerations for the interpretation of these unusual results, such as the presence of unusual processing enzymes, the presence of enzymes specifically designed to release biologically active peptides, or a second gene for each inhibitor type, any of which could be activated when an acute phase state occurs. We are now attempting to discern whether any of these possibilities can be substantiated.

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References

1. Travis, J., Bowen, J., and Baugh, R. (1978) Biochemistry 17, 5651-5656.
2. Heimburger, N. and Haupt, H. (1965) Clin. Chim. Acta 12, 116-118.
3. Aronsen, K.F., Ekeland, G., Kindmark, C.O., and Laurell, C.B. Scand. J. Clin. Lab. Invest. 29:Suppl. 124, pp. 127-136.
4. Travis, J., Garner, D., and Bowen, J. (1978) Biochemistry 17, 5647-5651.

5. Travis, J. and Morii, M. (1981) Methods of Enzymology 80, 765-771.
6. Travis, J., Bowen, J., Tewksbury, D., Johnson, D., and Pannell, R. Biochem. J. (1976) 157, 301-306.
7. Pannell, R., Johnson, D., and Travis, J. (1974) Biochemistry 13, 5439-5445
8. Zimmerman, C.L., Pisano, J.J., and Appella, E. (1973) Biochem. Biophys. Res. Commun. 55, 1220-1224.
9. Mendez, E. and Lai, C.Y. (1975) Anal. Biochem. 68, 47-53.
10. Morii, M., Odani, S., Koide, T., and Ikenaka, T. (1978) J. Biochem. 83, 269-277.