The Metabolic Interrelationship and Physicochemical Analysis of C-reactive Protein and Hepatic Catalase

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SUMMARY

The relationship of the appearance of rabbit C-reactive protein (CxRP) and the depression of enzymatic activity of hepatic catalase in rabbits following 3-amino-1,2,4-triazole (AT) administration has been examined sequentially. A rapid depletion of rabbit hepatic catalase was accompanied by a corresponding appearance of CxRP in the serum of AT-treated animals. Conversely, the restoration of catalatic activity resulted in a disappearance of CxRP. Similar relationships were obtained following subcutaneous talc or Freund’s adjuvant injections of Swiss mice. In addition, the antigenic relationship of human C-reactive protein (CRP) to mouse acute phase protein (M-APP) was shown in double diffusion tests with sheep anti-CRP antiserum (S-CRPA). Physical-chemical evidence which suggests a close relationship of CRP and human hepatic catalase (HHC) is presented. This is shown by a striking similarity in tryptic peptide maps of CRP and HHC following high-voltage paper electrophoresis. Additionally, CxRP incorporated into tissue culture medium containing rabbit liver slices enhanced the catalatic activity and reversed significantly the inhibitory effect of AT. It is suggested that the appearance of CRP may represent changes in metabolic events of the oxidative system, specifically that of catalase.

INTRODUCTION

Acute and chronic injuries induced by a variety of agents and disease processes are attended by the appearance of CRP in the plasma of man and experimental animals (7, 13, 18, 21). Under similar adverse conditions, the animal also exhibits a pronounced depletion of the liver enzyme, catalase (1, 15, 17). Although no enzymatic function has been attributed to CRP, a recent study by Hokama et al. (10) demonstrated the occurrence of common antigenic components in purified preparations of CRP and human liver and erythrocyte catalases.

Since antigenic similarities exist between CRP and catalases, the logical contention would be to assess concurrently the relationship of CRP and liver catalase activity in rabbits and mice following administration of a catalase inhibitor (AT) and nonspecific inflammatory agents.

Additionally, immunologic, electrophoretic, and tryptic peptide maps of CRP and liver catalase are presented to indicate their remarkable similarities.

MATERIALS AND METHODS

Effect of 2-Amino-1,2,4-triazole Administration in Rabbits. Albino rabbits weighing 2.5 to 3.0 kilograms were administered AT suspended in physiologic saline intraperitoneally using a dosage of 1 gm/kg body weight. The rabbits were exsanguinated by cardiac puncture at time intervals varying from 1 hr up to 120 hr after the administration of AT. The serum obtained from each rabbit was assayed for the presence of CxRP utilizing H-CxRPA and S-CRPA respectively. The liver and kidney of each animal were removed immediately after sacrifice, and a portion of each tissue was homogenized with a Ten Broeck homogenizer and assayed for catalatic activity.

Effect of Talc and Freund’s Adjuvant on Liver Catalase and APP in Mice. Female Swiss mice weighing 25 to 30 gm were inoculated subcutaneously in the dorsum with 0.5 ml of a 40% suspension of talc in saline and sacrificed at various time intervals following the injections. Blood samples were obtained by sectioning the brachial artery under ether anesthesia. The serum obtained was analyzed for the presence of acute phase protein with S-CRPA.

Individual livers were removed immediately after sacrifice and homogenized in buffered physiologic saline. The supernatant solution following centrifugation was assayed for catalatic activity. Groups of female mice were similarly examined following the administration of Freund’s complete adjuvant subcutaneously using a dosage of 0.5 ml per mouse.

Acute Phase Protein Assay. CxRP, CRP, and M-APP were analyzed by the capillary precipitation procedure described previously (10). In some instances, a two-fold dilution of acute
phase serum was prepared with physiologic saline, and the reciprocal of the highest dilution showing a precipitate was scored as the log₂ titer of the serum.

**Antiserum.** H-CRPA was prepared against chromatographically purified CRP as described previously (11) and was obtained from Waimanalo Research Laboratory, Waimanalo, Hawaii. Rabbit AHHC serum was obtained from rabbits which were administered purified HHC subcutaneously 2 to 3 times a week for three weeks at a dosage of 250 µg/injection. Freund's complete adjuvant was administered subcutaneously with the initial injection of HHC. The animals were sacrificed seven days after the last injection. S-CRPA and H-CxRPA were purchased from Hyland Laboratories, Los Angeles, California.

**Enzymatic Analysis of Catalase.** The crude liver and kidney tissue homogenates and purified HHC were assayed by the procedure of Beers and Sizer (2). The enzymatic activity of catalase examined is reported as the first order reaction constant, K, divided by the mg protein nitrogen of the sample examined and hence expressed as K/N. An automated Gilford 200 recorder was used for recording the immediate drop in optical density of the H₂O₂ substrate at 240 µM. The concentration of the sample, contained in approximately 20 µl, selected for these analyses was such that the destruction of H₂O₂ closely followed first order kinetics. The concentration of H₂O₂ employed for the assay was adjusted to give an initial O.D. of 0.5 at 240 µM. K was determined from the formula:

\[
K = \frac{1}{t} \log \frac{E_0}{E_t}
\]

where t is the time in fractions of a minute and E₀ and Eₜ the O.D. at time O and time t, respectively.

**CxRP and CRP Isolation.** Purified CxRP was isolated from rabbit serum obtained 48 hr after the subcutaneous injection of 4 ml of Freund's complete adjuvant. The method used was that recently reported from this laboratory (5). Purified CRP was isolated from pooled acute phase serum of tuberculosis patients or from samples of postmortem blood of individuals who had succumbed to heart failure or cancer. The procedure, slightly modified, after Kokama et al. (9) was used. The untreated serum sample containing CRP in 40 ml volume was passed directly through a Sephadex G 200 column bed of 1800 ml in 0.05 M citrate buffer in 0.1 M NaCl, pH 7.0. CRP found in the second peak associated with the immunoglobulin G was then chromatographed as indicated previously (11).

**Human Hepatic Catalase Isolation.** HHC was isolated from livers obtained at autopsy from individuals who had succumbed to cardiac or renal failure. Purification of HHC was carried out by an initial ammonium sulfate treatment of the liver homogenate according to the method of Saha et al. (23). The active HHC fraction which precipitated between 30 and 50% ammonium sulfate concentration was then passed through Sephadex G-200 columns twice according to a procedure described earlier (16).

**Immunoelectrophoresis and Disc Electrophoresis.** HHC and CRP used in these studies were analyzed by the method of Scheidegger (25) in immunoelectrophoresis using 1.5% agarose in barbital buffer, pH 8.6. Characterization of purified HHC and CRP by disc electrophoresis was carried out using the procedure of Davis (6) in 7.5% polyacrylamide gel at pH 8.2 with tris-hydroxyethylaminomethane-glycine buffer.

**Tryptic Peptide Map.** Analysis of the tryptic digests of purified HHC and CRP was performed according to the procedure of Schwartz and Edelman (26). The purified preparations were reduced with β-mercaptoethanol, alkylated with iodoacetamide, and treated with trypsin, and the polypeptides were separated by two-dimensional high-voltage paper electrophoresis. The first dimension was run at pH 4.7 in pyridine-acetic acid buffer and the second dimension at pH 1.9 in formic acid-acetic acid buffer.

**Liver Tissue Slices in Culture Medium 199.** Normal rabbits were sacrificed by cardiac puncture and the serum collected under aseptic conditions. The abdominal cavity was opened immediately and the liver removed aseptically and rinsed with sterile saline three times. The liver tissue was sliced into 3 by 10 by 15 mm sections and then transferred to sterile tissue culture medium contained in 150-ml plastic tissue culture bottles.

The tissue culture medium consisted of 25 ml of Medium 199 (Hyland Laboratories, Los Angeles, California) containing 5 ml of normal rabbit serum obtained from the same animal. In one series, AT at 1.67 mg/ml of culture medium was added. In another series, purified CxRP was added to the medium at 6.0 µg protein N/ml. Finally, CxRP and AT were added to the medium in the third set at 6.0 µg N/ml and 1.67 mg/ml concentrations respectively. These studies were carried out concurrently with tissue slices from the same liver as controls, and all culture bottles were incubated at 37°C in 5% CO₂ atmosphere. Liver slices were removed at intervals of 2, 5, 10, and 24 hr and homogenized; the homogenates were assayed for catalase activity. The studies were repeated several times with different rabbit livers, and the results are summarized in Table 1.

**RESULTS**

**Effect of 3-Amino-1,2,4-triazole Administration in Rabbits.** The appearance of CxRP in the plasma and the corresponding depletion of liver catalatic activity are depicted and summarized in Chart 1A. The activity of catalase, expressed as K/N, shows a rapid drop to approximately 10% of the normal rabbit liver catalatic activity as early as 6 hr after AT administration. The depression persists for 24 hr, then the catalatic activity gradually returns to normal levels at approximately 4 days after AT administration. Attendant with the drop in catalatic activity is the corresponding appearance of CxRP in serum of the same animal. Minimum levels of CxRP are detected at about 6 hr after AT, with the maximal levels appearing at 24 hr and persisting for another 24 hr with a sudden decline thereafter.

A similar interrelationship between CxRP and kidney catalatic activity was noted following exposure of rabbits to AT. This is summarized in Chart 1B. The results obtained in this study represent 3 to 8 rabbits at each point depicted in the graph.

**Effect of Talc and Adjuvant Administration in Mice.** Using S-CRPA to assay M-APS, we extended the examination of the CRP-catalase interrelationship in Swiss mice which had re-
Table 1

<table>
<thead>
<tr>
<th>Tissue culture system</th>
<th>Time in hours at 37.0°C</th>
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<tbody>
<tr>
<td>Control: Liver Slices plus Medium</td>
<td>42.0</td>
</tr>
<tr>
<td>CxRP: 6.0 μg N/ml of medium</td>
<td>42.0</td>
</tr>
<tr>
<td>AT: 1.67 mg/ml of medium</td>
<td>42.0</td>
</tr>
<tr>
<td>AT, 1.67 mg/ml plus CxRP, 6.0 μg N/ml of medium</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Catalase activity as K/N of rabbit liver slices in tissue culture Medium 199. CxRP, Cx-reactive protein; AT, 3-amino-1,2,4-triazole.

*Average K/N liver catalatic value of 8 normal rabbits is given at zero time.

Chart 1. Effect of intraperitoneal administration of 3-amino-1,2,4-triazole into rabbits: A, depression of liver catalase activity (0—0) with corresponding appearance of CxRP (+—+); B, depression of kidney catalase activity (0—0) with corresponding appearance of CxRP (+—+). See Footnote 2 for abbreviations.

Chart 2. Effect of inflammation inducing agents on mouse liver catalase and the "acute phase protein" response: A, depression of liver catalase activity (0—0) and the corresponding appearance of M-APP (+—+) following a single subcutaneous talc injection; B, depression of liver catalase activity (0—0) and the corresponding appearance of M-APP (+—+) following a single subcutaneous injection of Freund’s complete adjuvant. See Footnote 2 for abbreviations.

Received subcutaneous administration of talc or Freund’s complete adjuvant. The findings are summarized in Charts 2A and 2B. Mouse liver catalase activity following talc administration, unlike the response to AT in rabbits, shows a gradual decline of the enzyme with a maximum depression noted at Day 2 and, thereafter, a gradual increase to normal levels. These results are similar to those reported by Nishimura et al. (15).

Attendant with the fall of catalase activity is the appearance of M-APP which attains a maximum level in 24 hr and gradually declines during the catalase-depressed period with an abrupt drop following recovery of catalase activity to normal levels. This is illustrated in Chart 2A.

Mice administered adjuvant show a lesser depression of liver catalase activity, and also the activity appears to fluctuate.
Similarly, the M-APP appears and seems to fluctuate inversely with the level of catalase activity. This is shown in Chart 2B.

The Immunoelectrophoretic and Disc Electrophoretic Analyses of CRP and HHC. Purified CRP and HHC used for the tryptic peptide mapping were examined with their corresponding antisera, CRP with H-CRPA or S-CRPA and HHC with R-AHHC respectively, by immunoelectrophoretic procedure. The immunoelectrophoregrams of these examinations are shown in Fig. 1A and 1B for CRP and HHC respectively.

Against H-CRPA, purified CRP shows bands in the γ-region and a broad band in the α2-region, while against S-CRPA, two distinct bands are shown in the γ-region.

Under a similar condition of examination, purified HHC reacted with R-AHHC to give two precipitation bands, a heavy band in the β-region and a lesser band showing partial reaction of identity in the γ-region close to the longitudinal antibody well. This is shown in Fig. 1B.

Disc electrophoretic patterns of purified CRP and HHC are shown in Fig. 1C. These gels were stained with Coomassie blue (4). The major bands appear in the γ-region or upper portion of the disc gel and are quite similar to those reported earlier (10).

Tryptic Peptide Maps of CRP and HHC. Results of the analysis of purified CRP and HHC by two-dimensional high-voltage electrophoresis following reduction, alkylation, and tryptic hydrolysis of the proteins are shown in Fig. 2. The tryptic peptide map obtained by us for purified CRP is very much like the pattern described by Gotschlich and Edelman (8). Similarly, the tryptic peptide map of purified HHC, to a remarkable degree, is essentially indistinguishable from that of the CRP map.

Effect of Purified CxRP on Catalase Synthesis by Liver Slices in Tissue Culture. Table 1 summarizes the study of catalase synthesis by normal liver tissue slices in tissue culture Medium 199 containing 15% normal rabbit serum. Liver catalase activity showed a gradual increase during incubation and attained approximately a 2.5-fold increase over the initial value. The addition of purified CxRP to the control mixture showed a faster rate of increase in catalase activity, with the lowest level seen at 24 hr and having approximately one-half of the control value. Incubation of liver slices with AT in the presence of purified CxRP resulted in an increase in catalase activity during the first 10 hr. Following this initial rise, the activity decreased until, after 24 hr, the depression of catalase activity was approximately one-half that of the control and slightly more than twice that of AT alone. Purified CxRP appears to reverse somewhat the effect of AT. The results at each time interval are averages of 10 determinations for control and AT mixtures; where CxRP was used, the results are averages of 3 to 4 determinations.

DISCUSSION

The rapid depletion of liver and kidney catalatic activities and the subsequent alteration in the kinetics of catalase synthesis of these organs, after AT administration, have been demonstrated previously in mice and rats by Price et al. (19), Margoliash et al. (14), and Rechcigl et al. (20). In the present study, rabbits administered AT showed similar rapid depletion of liver and kidney catalatic activity following AT administration. Associated with the initial depression of catalatic activity was the constant appearance of CxRP in the peripheral blood of these animals. The subsequent recovery of catalatic activity was accompanied by a corresponding and rapid disappearance of CxRP from the peripheral blood.

Mice administered talc or adjuvant showed a gradual depletion of liver catalatic activity with a corresponding appearance of an acute phase protein in the peripheral blood during the acute injury. This depletion of liver catalatic activity and the associated alterations in the ultrastructure of hepatic cells had been reported previously by Nishimura et al. (15) and Baum and Nishimura (1). The occurrence of an acute phase protein in mice analogous to CRP following bacterial infection has been reported earlier by Patterson and Higgenbotham (18).

In a preliminary examination, occurrence of common related antigens showing reactions of partial identity were observed between purified CRP and M-APS against S-CRPA and H-CRPA. Samples of GP-CxRPA, G-CxRPA, H-CxRPA, R-CRPA, and G-CRPA antisera employed gave no precipitation in immunodiffusion or immunoelectrophoresis with M-APS. Whether the protein(s) in M-APS reacting with S-CRPA and H-CRPA are related to CRP and to the "acute phase proteins" found in rat and mouse sera reported by Weimer and Benjamin (27) and Rowen and Wiest (22) remains to be determined.

Margoliash et al. (14) have shown that AT at optimum conditions binds the protein moiety of the enzyme catalase-peroxide complex I to form irreversible complexes and thus nullifies the activity of the enzyme. Hence, a rapid depletion of catalatic activity follows AT administration. This is unlike the action following talc or adjuvant treatment, in which a gradual depression of catalatic activity occurs. Nevertheless, in both systems, regardless of the rate of catalase degradation, the rate of appearance of the acute phase protein in the peripheral blood is similar. This probably precludes the assumption that the "acute phase protein" is a catabolic product of catalase, since it would be expected that CxRP would appear earlier in the AT-administered animals. Furthermore, synthesis of "acute phase protein" by liver tissues has been demonstrated by Hurliman et al. (12) for CRP and CxRP and by Benjamin and Weimer (3) and Sarcone and Boyd (23) for the "acute phase protein" of the rat, using radioautographic and immunoelectrophoretic procedures. These observations of the relationships between CRP and liver catalase are compatible with our earlier report on the occurrence of common antigenic determinants in CRP and human catalases (10). In addition, the examination of tryptic digests of CRP and HHC by two-dimensional high-voltage paper electrophoresis suggests occurrence of similar peptide units. The tryptic peptide map obtained for our preparation of CRP is comparable to that found previously by Gotschlich and Edelman (8), although our preparation was isolated from a serum of an individual by a recently described procedure (9). Similarly, the tryptic peptide map of a representative preparation of HHC appeared indistinguishable from that of CRP. Further peptide mapping and amino acid analysis of CxRP is required to resolve this point.

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acid analysis of several preparations of individual CRP and HHC are being carried out to elucidate the structural relationships and will be reported in detail later.

Addition of purified CxRP to liver slices in tissue culture medium containing 15% normal rabbit serum resulted in an enhancement of catalytic activity. CxRP appeared to reverse significantly the effect of AT in the tissue culture system. Since common antigens occur in CRP and HHC, it is possible that the presence of CxRP in the culture medium could have reversed the AT effect by competitive binding. An alternative explanation would reiterate our previous contention that CR2 reversed the AT effect by competitive binding. An alternative explanation would reiterate our previous contention that CR2 reversed the AT effect by competitive binding.

Data from these studies and of previous observations by Hokama et al. (10) and Nishimura et al. (16) strongly suggest that the acute phase proteins elaborated by animals in pathologic conditions are in some way associated with the liver oxidative metabolism of the animal and that the measure of CRP in peripheral blood may be a reflection of the state of liver oxidative function, specifically that of catalase.

ACKNOWLEDGMENTS

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REFERENCES


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Fig. 1. Immunoelectrophoretic and disc electrophoretic analyses of purified CRP and HHC: 
A, immunoelectrophoretic pattern of P-CRP against H-CRPA and S-CRPA; 
B, immunoelectrophoretic pattern of HHC against its homologous AHHC; 
C, disc polyacrylamide gel patterns of purified CRP and HHC stained with Coomassie blue. See Footnote 2 for abbreviations.
Fig. 2. Tryptic peptide maps of P-CRP and P-HHC obtained by two-dimensional high-voltage paper electrophoresis; the peptide spots showing similarities in the two preparations are circumscribed. See Footnote 2 for abbreviations.
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