***N*-Formyl-L-methionine Deformylase Activity in Human Leucocytes and Platelets**

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(Received 13 June 1979)

Extracts of human neutrophils, lymphocytes and platelets enzymically deformylate *N*-formyl-L-methionine. Enzyme activity is stimulated by Co^{2+} , inhibited by bivalent-cation chelators and unaffected by inhibitors of serine, thiol and carboxyl proteinases. Leucocyte or platelet *N*-formylmethionine deformylase may be important in modulation of neutrophil responses to chemoattractant formylmethionyl peptides or similar compounds.

Enzymes able to catalyse deacylation of *N*-acylated amino acids are widely distributed in Nature (Greenstein & Winitz, 1961). Enzymes particularly active on *N*-formylmethionine or *N*-formylmethionyl dipeptides have been found in bacteria (Fry & Lambourg, 1967; Adams, 1968), in protozoa (Aronson & Lugay, 1969), in plants (Lugay & Aronson, 1967) and in extracts of numerous mammalian tissues (Grisolia *et al.*, 1977). Although their function in bacteria may relate to recycling of initiator formylmethionine (Adams, 1968) or to specific metabolic pathways (Fry & Lambourg, 1967), the function(s) of these enzymes in higher organisms is not known. It has been demonstrated that naturally occurring bacterial *N*-blocked peptides and synthetic *N*-formylmethionyl oligopeptides are highly chemotactic for neutrophils (Schiffman *et al.*, 1975). Since in the model compounds this activity depends on the presence of the formyl group (Showell *et al.*, 1976), we assayed several cell types involved in the inflammatory response for the presence of *N*-formylmethionine deformylase activity, which might potentially be of physiological importance in modulation of neutrophil responses. We report here that extracts of human neutrophils, lymphocytes and platelets possess *N*-formylmethionine deformylase activity.

A portion of this work has been presented in abstract form (Ackerman & Douglas, 1978).

Methods

Heparinized blood from normal volunteers was subjected to Ficoll/Hypaque centrifugation (Boyum, 1968). Interface mononuclear cells containing lymphocytes, monocytes and platelets were washed, resuspended in lysis buffer {250 mM-sucrose/25 mM-NaCl/25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/5 mM-MgCl₂, pH 7.3}, incubated at 4°C for 30 min and then subjected to nitrogen cavitation at 5.2 MPa (750 lbf/in²) in a Parr

nitrogen cavitation device (Parr Instruments, Chicago, IL, U.S.A.). All mononuclear cells were disrupted and 85–90% of cell nuclei were morphologically intact. This crude cell homogenate was spun at 500g for 10 min to sediment nuclei and cell debris, which were discarded. The supernatant was dialysed overnight against 5 mM-sodium phosphate buffer, pH 7.5, and then used in the assay. Those extracts made from the unfractionated Ficoll/Hypaque interface cells are denoted 'Ficoll/Hypaque cell extracts'. To make 'polymorphonuclear-leucocyte extracts', Ficoll/Hypaque pellets containing polymorphonuclear leucocytes and erythrocytes were twice haemolysed with distilled water, then washed, and the leucocytes were resuspended in lysis buffer. These were approx. 95–100% neutrophils and 0–5% eosinophils. Nitrogen cavitation was performed at 6.2 MPa (900 lbf/in²) and cell homogenates were treated as above. To prepare 'lymphocyte extracts', normal venous blood was defibrinated before Ficoll/Hypaque centrifugation; the resultant interface was devoid of platelets and markedly depleted of monocytes. It consisted of 95–98% small lymphocytes by morphological criteria. Preparation of extracts was done as for Ficoll/Hypaque cell extracts. To prepare 'platelet extract', freshly drawn platelet packs virtually free of leucocytes were obtained from the hospital blood-bank, then washed twice, and extracts were prepared as for Ficoll/Hypaque cell extracts.

To assay *N*-formylmethionine deformylase activity, dialysed extracts were added to solutions of *N*-formylmethionine (Sigma Chemical Co., St. Louis, MO, U.S.A.) (final concn. of substrate 20 mM) in various buffers as indicated. Tubes were incubated at 37°C for the designated times, and reactions were terminated by addition of cold trichloroacetic acid to a final concentration of 10% (w/v). Tubes were incubated at 4°C for 30 min, then spun at 500g for 20 min. Supernatants were partially neutralized with NaOH and subjected to colorimetric ninhydrin assay (Spies, 1957), with L-leucine as standard. Control

tubes received extract plus buffer without *N*-formyl-methionine and generally showed negligible autolysis. The results were calculated as the difference between values for experimental and control tubes, each in triplicate.

Iodoacetamide, phenylmethanesulphonyl fluoride, pepstatin, leucine, EDTA, *o*-phenanthroline, dithiothreitol and Ficoll were from Sigma Chemical Co. Hypaque was from Winthrop Laboratories (New York, NY, U.S.A.).

Results and Discussion

Extracts of human Ficoll/Hypaque cells demonstrate significant deformylase activity towards *N*-formyl-L-methionine. In 0.1 M-sodium phosphate buffer, pH 7.5, specific activity was approx. 0.75 μmol of substrate hydrolysed/h per mg of extract at 37°C. Heating of the extract at 100°C for 4 min abolished activity. Fig. 1 shows that in 0.1 M-phosphate buffer optimal activity occurs at pH 7.5, although, as apparent from the broad peak, activity may conveniently be assayed up to pH 8.5. Marked decreases in activity occur in non-phosphate buffers, including Tris, Hepes and barbital, at equivalent pH. Activity in 0.1 M-Tris buffer, pH 8.5, for instance, is 10-fold less than in 0.1 M-phosphate buffer, pH 8.5 (Fig. 1). Investigation of the effect of bivalent cations on enzyme activity showed that *N*-formyl-L-methionine deformylase activity is stimulated 7–10-fold by 100 μM - Co^{2+} , to 5.0–7.0 μmol of substrate hydrolysed/h per mg of protein in 0.1 M-phosphate buffer, pH 7.5, at 37°C. Activity is unaffected by Ca^{2+} , Mg^{2+} , Mn^{2+} or Zn^{2+} at this concentration. Studies of the effect of various proteinase inhibitors on enzyme activity showed 90–100% inhibition by 2 mM-*o*-

phenanthroline and 2 mM-dithiothreitol, and 65–80% inhibition by 2 mM-EDTA. Iodoacetamide and pepstatin, each 2 mM, were without effect, and 2 mM-phenylmethanesulphonyl fluoride inhibited by 10%. In preliminary experiments, ultracentrifugation of Ficoll/Hypaque cell extracts at 10^5g for 1 h before dialysis sedimented only 10–15% of total enzymic activity. This suggests a predominantly cytoplasmic localization of the enzyme.

As noted, Ficoll/Hypaque cell extracts used above contain material from several different cell types. To characterize the specific cell types that possess enzyme activity, we prepared separate extracts of purified lymphocytes and of purified platelets. Both of these extracts demonstrated *N*-formyl-L-methionine deformylase activity. Specific activities in 0.1 M-phosphate buffer, pH 7.5, in the absence of added bivalent cations, were comparable with that of Ficoll/Hypaque cell extracts: 0.55 μmol /h per mg of protein for lymphocyte extracts and 0.40 μmol /h per mg of protein for platelet extract.

Demonstration of activity in purified neutrophil extracts required the addition of phenylmethanesulphonyl fluoride and iodoacetamide, 2 mM each, to the lysis buffer. This prevented autolysis, which interfered with the assay. Also, in contrast with the lymphocyte and platelet extracts, activity was completely dependent on addition of exogenous Co^{2+} . In the presence of 100 μM - Co^{2+} , and with prior addition of phenylmethanesulphonyl fluoride, deformylase activity was easily demonstrable. Specific activity in 0.1 M-phosphate buffer, pH 7.5, was 2.5–3.0 μmol /h per mg of protein; this is approximately half the specific activity of the Ficoll/Hypaque cell extract when the latter was assayed in the presence of 100 μM - Co^{2+} .

The best-studied mammalian acylases are two from pig kidneys, originally described and partially purified by Greenstein and co-workers (Birnbau *et al.*, 1952) (EC 3.5.1.14–15). The relationship between the enzyme activity described in the present paper and these pig kidney acylases is not known, since *N*-formylmethionine has apparently not been well characterized as a substrate for the pig kidney enzymes. Certain attributes of the Ficoll/Hypaque-cell enzyme are, however, strikingly similar to those found in non-mammalian tissues. For instance, the *N*-formylmethionine deformylases of *Escherichia coli* (Adams, 1968), *Euglena* (Aronson & Lugay, 1969) and the Jerusalem thorn (Lugay & Aronson, 1967) are all activated by Co^{2+} (often requiring up to 100 μM), have approximately neutral pH optima and are found in the soluble fraction of cell extracts.

This work was supported by U.S. Public Health Services grant no. AI 12478 and NIH Postdoctoral Fellowship no. AI 05447 (to S. K. A.) and by the Kroc Foundation, Santa Ynez, CA, U.S.A.

Fig. 1. Effect of pH on hydrolysis of *N*-formyl-L-methionine by extracts of human Ficoll/Hypaque cells

Assay conditions were as follows: 0.1 M-phosphate buffer (○) or 0.1 M-Tris buffer (●); 37°C; 100 μM - Co^{2+} ; 3 h incubation. Note the optimum at pH 7.5 and 10-fold greater activity in 0.1 M-phosphate buffer compared with that in 0.1 M-Tris buffer.

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