this connection no protein was observed. Experiments to determine the binding capacity of the column were carried out. Increasing amounts of supernatant tissue proteins were chromatographed on the specific adsorbent column. Quantities of proteins recovered in the eluate were in agreement with a typical saturation curve. The experiments reported below were carried out in non-saturating conditions.

Results and discussion. In the table are reported the amounts of proteins of various tissues specifically eluted from the resin. It is remarkable that liver, kidney and spleen contain higher amount of proteins specifically adsorbed on the adriamycin-Sepharose. A small fraction of plasma proteins was retained by the resin. These data are in fairly good agreement with previous results of Kimura et al.[3], on blood levels and tissue distribution of adriamycin in rats, and of Lenaz et al.[4], who studied its distribution in mouse tissues.

Use of affinity chromatography to study the binding of drugs to proteins not only gives an indication of the presence of tissue proteins capable of binding, but also allows one to isolate and compare the proteins which bind drugs. The proteins specifically adsorbed by adriamycin and eluted were examined by electrophoresis on polyacrylamide gel in the presence of urea and SDS. (For experimental details see figures 1 and 2.) From this study, the following conclusions are drawn: 1. In the tissues examined various proteins capable of binding to adriamycin are present. 2. The pattern is characteristic for every tissue. In fact, differences are pointed out between spleen, thymus and heart, as shown in figure 1 and 2. 3. In spleen and heart eluates, some proteins are concentrated by the column; in particular, in the heart eluate the presence of a protein, not detectable in the supernatant, is shown. The presence of this protein, highly concentrated by the adriamycin-Sepharose column, indicates that in the heart 'in vivo', the binding of the drug to this protein could be one of the reasons for the particular cardiotoxicity of the drug. Studies are in progress to identify this protein. 4. Albumin appears as the only plasma protein, responsible of the slight binding to adriamycin.

1 A. Lucacchini, R. Barsacchi and C. Martinelli, J. Solid-Phase Biochem. *2*, 79 (1977).
2 E. Hurwitz, R. Levy, R. Maron, M. Wilchek, R. Arnon and M. Sela, Cancer Res. *35*, 1175 (1975).
3 K. Kimura, H. Fujita and Y. Saki, in: International Symposium on Adriamycin, p. 124, Milan, Sept. 1971. Springer, Berlin 1972.
4 L. Lenaz, G. Di Fronzo and R. Molinari, in: EORTC International Symposium on Adriamycin Review, p. 89. Brussels, May 1974. European Press, Ghent, 1975.
5 P.M. Sender, FEBS Lett. *17*, 106 (1971).

The amino acid composition of histidine ammonialyase from *Pseudomonas putida* NCIB 10807

A.A. Rokosu[1]

Department of Biochemistry, University of Benin, P.M.B. 1154, Benin City (Nigeria), 12 December 1978

Summary. The amino acid composition of histidine ammonia-lyase from *Pseudomonas putida* NCIB 10807 suggests that this enzyme may be different from the *Pseudomonas testosteroni* NCIB 10808 histidine ammonia-lyase, whose amino acid composition is known[2].

Amino acid composition and sequence can provide information on regions of homology between related proteins. *Pseudomonas putida* NCIB 10807 belongs to the fluorescent group of Pseudomonas[3]. The aerobic *Pseudomonas* species constitute a large and fairly diverse array of bacteria. Histidine ammonia-lyase has been isolated and purified from *P. putida* NCIB 10807[4], but the amino acid composition was not determined. The histidine ammonia-lyase from *P. putida* NCIB 10807 does not form multiple polymers[4], while the histidine ammonia-lyase from *P. testosteroni* NCIB 10808, a nonfluorescent *Pseudomonad* forms multiple polymers[5]. It is quite likely that the ability of histidine ammonia-lyase to form multiple polymers or not may be dependent on the nature of its amino acid composition.

Materials and methods. P. putida NCIB 10807 was grown on a medium containing (g/l), L-histidine-HCl, 3; sodium succinate, 3; and KH_2PO_4, 5; with 0.02% (w/v) $MgSO_4$, and pH adjusted to 7.2 with 5 M NaOH as previously described[6]. Cells were harvested in the late exponential phase. Histidine ammonia-lyase was isolated from the cells of *P. putida* NCIB 10807 by the method described previously[6]. The procedure used for the purification of histidine ammonia-lyase was based on the method[7], and was similar to that employed by other workers for the purification of the enzyme[4,6]. Histidine ammonia-lyase activity was assayed by measuring spectrophotometrically the production of urocanate from L-histidine at 277 nm[7]. The pure histidine ammonia-lyase displayed a single homogeneous band, which had enzymic activity, on polyacrylamide gel electrophoresis as reported before[4].

Quantitative amino acid analysis. Histidine ammonia-lyase samples were hydrolyzed in 6 M-HCl before quantitative amino acid analysis. The protein solution (5 ml) was dialyzed extensively (24 h) at 0 °C against 5 mM-KH_2PO_4 buffer, pH 7.0 in double distilled water. Duplicate samples, containing 0.8–1.0 mg protein were placed in separate hydrolysis tubes and freeze dried. Aliquot, 2 ml of 6 M-HCl was added to each sample, and the tubes flushed with nitrogen gas, evacuated and sealed. The tubes were placed in an oven at 110 °C for 48 and 72 h respectively. This procedure ensures that all peptide bonds are broken and that the values obtained for amino acids partially destroyed during hydrolysis (serine and threonine) may be extrapolated to zero time. After hydrolysis, the tubes were cooled, opened and the HCl removed under vacuum; water was then added and the samples freeze-dried to ensure complete removal of acid.

This treatment causes the total destruction of tryptophan and may bring about partial oxidation of cysteine and methionine residues. To determine the accurate value for cysteine and cystine residues, a small sample (0.5 mg) was oxidized with performic acid before hydrolysis to convert cysteine and cystine residues to cysteic acid[8]. The solution of performic acid was prepared as described[9] and 0.15 ml of this solution was added to 1–2 mg protein dissolved in 0.15 ml of a solution of 0.87 ml 98% (w/v) formic acid and

Amino acid composition of histidase

	μmole in acid hydrolysate (obtained from mole residue values as amount present in hydrolyzed samples) 48 h	72 h	mole residue/ mole enzyme for *P. putida* NCIB 10807	mole residue/ mole enzyme for *P. testosteroni* NCIB 10808[2]
Aspartic acid	0.1792	0.1864	182	164
Threonine	0.0621	0.0612	63	79
Serine	0.0740	0.0715	76	122
Glutamic acid	0.2014	0.2026	198	195
Proline	0.0753	0.0672	71	80
Glycine	0.1661	0.1620	164	159
Alanine	0.2675	0.02800	273	274
Valine	0.1361	0.1481	145	147
Methionine	0.0088	(0.0330)	22	26
Isoleucine	0.0952	0.0920	93	93
Leucine	0.2581	0.2453	251	224
Tyrosine	0.0245	0.0327	27	24
Phenylalanine	0.0542	0.0522	53	36
Lysine	0.0752	0.0790	76	43
Histidine	0.0485	0.493	49	46
Arginine	0.1410	0.0970	126	85
Cysteine	0.0028	(0.0027)	28	16

* The mol.wt of the enzyme is 200,000 and the calculations were based on this value.

0.17 ml absolute methanol cooled to 10 °C. The mixture was maintained at this temperature for 2 h and then an excess of water (10–15 ml) was added to terminate the reaction. The solution was freeze-dried and the sample hydrolyzed with 6 M-HCl as described above. Quantitative amino acid analysis was carried out by using an automatic analyser (Beckman Unichrome amino acid analyser with a high sensitivity flow cell).

Results and discussion. The amino acid composition of histidine ammonia-lyase from *P. putida* NCIB 10807 is presented (table). When the amino acid composition of *P. putida* NCIB 10807 is compared with that of *P. testosteroni,* the former has more basic amino acid residues, lysine and arginine than the latter. The cysteine content in *P. putida* enzyme tends to be higher than that of *P. testosteroni* enzyme. The variations in the amino acid compositions of the 2 enzymes may play a part in the ability of the enzyme to have multiple polymers or not. It is significant to note that the two histidine ammonia-lyases are not identical immunologically[10]. It is reasonable to suggest the absence of homologous regions between the 2 enzymes. Taxonomically the *Pseudomonas* are nutritionally diverse, and the ability to form pigment is variable. *P. putida* NCIB 10807 belongs to the fluorescent group, while *P. testosteroni* NCIB 10808 belongs to the nonfluorescent group. The observed variations in amino acid composition and the possibility of nonhomologous regions in the amino acid sequences of the histidine ammonia-lyases from the two organisms further separate the 2 organisms in terms of evolution of histidine ammonia-lyase in *P. putida* NCIB 10807 and *P. testosteroni* NCIB 10808.

1 Acknowledgment. I wish to thank the Departments of Biochemistry and Biophysics, Leeds University, England, for the use of their excellent laboratory facilities.
2 A.K. Soutar, Thesis, Leeds University 1971.
3 R.Y. Stanier, N-J. Palleroni, and M. Duodoroff, J. gen. Microbiol. *43,* 159 (1966).
4 H. Hassal, Analyt. Biochem. *35,* 335 (1970).
5 A.K. Soutar and H. Hassall, Biochem. J. *114,* 79 (1969).
6 H. Hassall and A.K. Soutar, Biochem. J. *137,* 559 (1974).
7 H. Tabor and A.H. Mehler, J. biol. Chem. *210,* 559 (1954).
8 F. Sanger, Biochem. J. *44,* 126 (1948).
9 C.W.H. Hirs, Meth. Enzymol. *11,* 197 (1967).
10 H. Hassall and A.A. Rokosu, J. gen. Microbiol., in press (1979).

Chloroperoxidase-catalyzed oxidation of N-methyl-4-chloroaniline[1]

M.D. Corbett and B.R. Chipko

Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami (Florida 33149, USA), 28 November 1978

Summary. Chloroperoxidase catalyzed the H_2O_2 oxidative conversion of N-methyl-4-chloroaniline to 4-chloronitrosobenzene, 4-chloroaniline and a mixture of complex products.

We recently reported the facile oxidation of primary arylamines to nitrosoaromatics by hydrogen peroxide in the presence of chloroperoxidase[2]. This conversion is unique in that it is the only known enzymatic redox process that gives the nitroso oxidation state as the major product. We now report that chloroperoxidase also oxidizes secondary arylamines, and that a significant product of this conversion is the corresponding nitrosoaromatic. This study was conducted with N-methyl-4-chloroaniline as the model substrate for N-methyl arylamines.

Experimental. A solution of chloroperoxidase (Sigma Chemical Co.) consisting of approximately 9.6 μg/ml of protein in 0.05 M, pH 4.4 phosphate buffer was assayed for enzymatic activity according to the standard method[3]. In