The Chemistry of Xanthine Oxidase

12. THE AMINO ACID COMPOSITION

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In this paper we report the amino acid composition of highly purified xanthine oxidase from cow's milk. The results have been used for calculating the partial specific volume of the enzyme (Andrews, Bray, Edwards & Shooter, 1964) and may serve as a basis for future work on the detailed structure of the molecule.

MATERIALS AND METHODS

The analysis was carried out by the method of Moore, Spackman & Stein (1958). Two xanthine oxidase samples (cf. Bray, Pettersson & Ehrenberg, 1961) with purities in the ultracentrifuge of 97 and 94% respectively were used for the main analyses. The first sample was used without pretreatment but the second was oxidized with performic acid (Hirs, 1956). Both were hydrolysed for 20 and 50 hr. (Nyman & Lindskog, 1964). Longer hydrolysis times were avoided because of possible interference by the metals of the enzyme. Portions of the hydrolysates corresponding to about 1 mg. of xanthine oxidase were chromatographed. The concentration of xanthine oxidase was in all cases determined from Kjeldahl nitrogen values, the enzyme nitrogen content being assumed to be 16.3% (Ball, 1939).

The value found for the methionine content of the native enzyme agreed with that for methionine sulphone in the oxidized enzyme (Table 1). This indicates that over-oxidation had not occurred in the performic acid treatment (Moore, 1963), and it was therefore considered valid to combine results from the native and oxidized samples for all except the basic amino acids and threonine, serine and tyrosine.

A third and fourth sample of xanthine oxidase (Palmer, Bray & Beinert, 1964) were used for tryptophan analysis by the colorimetric method of Spies & Chambers (1949) (procedure L).

RESULTS AND DISCUSSION

The amino acid composition is given in Table 1. The values correspond to $101.27 \pm 1.67\%$ recovery of nitrogen, and the sum of the weights of the amino acid residues corresponds to 94.89% of the weight of xanthine oxidase that was nominally used. Since the FAD, iron and molybdenum account for only about a further 0.65% (Avis,

Bergel & Bray, 1956b), it seems likely that the rather old value for the nitrogen content of the enzyme that we have used may be in error and that the true value is probably about 17.1%. In view of the difficulties of determining the true dry weights of proteins, no attempt was made to check this, but if this is indeed the case all the values in all the columns of Table 1 will be low in the ratio 16.3/17.1.

The most noteworthy feature of the analysis is the low tryptophan content, corresponding to only 9 out of the total of 2378 residues. A second sample of the enzyme gave a somewhat higher value for this amino acid, but this could well be due to traces of impurities with higher tryptophan contents. No attempt has been made to calculate an exact molecular weight from the amino acid composition (cf. Nyman & Lindskog, 1964), because of the high molecular weight of the enzyme. The value of 275 000 employed in Table 1 is the average result of Andrews *et al.* (1964).

The results indicate 180 acidic groups (aspartic acid + glutamic acid - ammonia) and 322 basic groups (lysine + histidine + arginine), i.e. an excess of 142 basic groups/molecule. Though some of these may be involved in binding metal ions, and though the exact number of basic groups in excess would be rather lower if the ammonia value on the oxidized enzyme were accepted in place of that on the native, this still means that an isoelectric point above neutrality would be predicted. This prediction contrasts with the value of 5.3-5.4 at *I* 0.2 found by Avis, Bergel, Bray, James & Shooter (1956a). Possible explanations of similar observations on other proteins have been discussed by Malmström, Kimmel & Smith (1959) and by Mahowald, Noltmann & Kuby (1962).

No analyses for thiol groups have been carried out in the present work, but earlier data (Bergel & Bray, 1959) suggest that most of the cysteic acid found must have been present as cysteine.

SUMMARY

1. The amino acid composition of xanthine oxidase from cow's milk is presented and discussed briefly.

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Table 1. *Amino acid composition of xanthine oxidase*

The 20 hr. hydrolysis was carried out in duplicate on the native enzyme and once on the oxidized enzyme, and results were averaged unless otherwise stated. The 50 hr. hydrolysis was carried out once on the native enzyme and once on the oxidized enzyme, and, again, the results were averaged. In some cases chromatography of the hydrolysates was also carried out in duplicate. The 'best' values are generally the average from all the experiments. The deviations given are the average values of the absolute deviations from the mean.

	Amino acid content (μ mole/mg. of xanthine oxidase)			Amino acid residues (%)	No. of residues/molecule of mol. wt. 275 000
	20 hr. hydrolysis	50 hr. hydrolysis	Best		
Lysine*	0.588 $\pm$ 0.011	0.593	0.590 $\pm$ 0.008	7.56	162
Histidine*	0.201 $\pm$ 0.004	0.203	0.201 $\pm$ 0.003	2.76	55
Ammonia†	0.944 $\pm$ 0.004	0.964	0.951 $\pm$ 0.009	—	(262)‡
Arginine	0.383 $\pm$ 0.003	0.383 $\pm$ 0.001	0.383 $\pm$ 0.002	5.98	105
Tryptophan	—	—	0.033§	0.61	9
Aspartic acid	0.728 $\pm$ 0.026	0.718 $\pm$ 0.007	0.724 $\pm$ 0.019	8.33	199
Threonine*	0.595 $\pm$ 0.013	0.566	0.615 $\pm$ 0.013	6.22	169
Serine*	0.525 $\pm$ 0.013	0.475	0.561 $\pm$ 0.013	4.89	154
Glutamic acid	0.887 $\pm$ 0.024	0.877 $\pm$ 0.004	0.833 $\pm$ 0.016	11.40	243
Proline	0.465 $\pm$ 0.017	0.487 $\pm$ 0.012	0.474 $\pm$ 0.017	4.60	130
Glycine	0.714 $\pm$ 0.021	0.708 $\pm$ 0.002	0.712 $\pm$ 0.014	4.07	196
Alanine	0.654 $\pm$ 0.016	0.650 $\pm$ 0.010	0.652 $\pm$ 0.014	4.64	179
Valine	0.572 $\pm$ 0.022	0.594 $\pm$ 0.005	0.594 $\pm$ 0.005¶	5.89	163
Methionine**	0.165 $\pm$ 0.005	0.178	0.172 $\pm$ 0.005	2.26	47
Isoleucine††	0.404 $\pm$ 0.015	0.436 $\pm$ 0.010	0.436 $\pm$ 0.010¶	4.94	120
Leucine	0.749 $\pm$ 0.014	0.751 $\pm$ 0.006	0.750 $\pm$ 0.011	8.49	206
Tyrosine‡‡	0.219 $\pm$ 0.003	0.231	0.223 $\pm$ 0.006	3.64	61
Phenylalanine	0.431 $\pm$ 0.006	0.426 $\pm$ 0.002	0.429 $\pm$ 0.005	6.31	118
Cystine (half)§§	0.228	0.221	0.225 $\pm$ 0.004	2.30	62
Total				94.89	2378

* Values on the oxidized samples were slightly (3–10%) lower than those on the native and were omitted.

† Values on the oxidized sample of 0.752 (20 hr.) and 0.796 (50 hr.) were omitted.

‡ Omitted from total.

§ Analysis by the method of Spies & Chambers (1949). A second sample gave a value of 0.054.

|| Extrapolated to zero hydrolysis time assuming first-order kinetics (Noltmann, Mahowald & Kuby, 1962).

¶ 50 hr. value taken as 'best' value.

** Values on native samples only are given in the 20 hr. and 50 hr. columns. Values of 0.172 (20 hr.) and 0.178 (50 hr.), obtained for methionine sulphone on the oxidized sample, were included for the 'best' column.

†† Result may be slightly low, since a trace of alloisoleucine was also detected in some runs.

‡‡ The values for the oxidized sample were very low and were omitted.

§§ Values for the oxidized sample only, measured as cysteic acid.

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