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Determination of the activity of diamine oxidase in extremely small tissue samples

H. G. Schwelberger, J. Klocker, J. Sattler, and E. Bodner

Abteilung für Theoretische Chirurgie, II. Univ.-Klinik für Chirurgie, Universität Innsbruck, Schöpfstraße 41, A-6020 Innsbruck, Austria

Introduction

Diamine oxidase (DAO; EC 1.4.3.6) degrades histamine and other biogenic amines [1]. DAO activity has been reported for many mammalian tissues with highest activities being found in the intestine [2]. We are interested in exploring DAO as a tool for the diagnosis and follow-up of gastrointestinal diseases associated with lesions of the intestinal mucosa as well as in allergic reactions caused by an altered DAO activity in the gut. The clinical situation requires the measurement of the enzymatic activity in minute amounts of biopsy specimens. Here we describe a highly sensitive radiometric micro-assay for the determination of DAO activity in extremely small tissue samples.

Materials and methods

Tissue samples (human duodenal biopsies) of 10–100 mg were homogenized for 20 s in 1 ml 20 mM Bis-Tris hydrochloride pH 7.0 containing 1 mM phenylmethane sulfonyl fluoride with an Ultra-Turrax T8 (Janke and Kunkel) using a S8N-5G probe at 20,000 rpm. The homogenates were cleared by centrifugation at 23,000 × g and could be stored at −75 °C without loss of activity. The protein concentration was determined according to Bradford [3].

The activity of DAO was determined by a modification of procedures described previously [4, 5] which are based on the DAO catalyzed conversion of ^{14}C-putrescine to γ-aminobutyraldehyde. The latter compound is spontaneously converted to Δ_1-pyrroline which can be extracted into toluene for quantification. The whole assay is performed in 2 ml screw-capped microcentrifuge tubes. Up to 80 µl of cleared tissue homogenate are mixed with 10 µl 1 M sodium phosphate buffer of optimum pH for the respective source of enzyme. The reaction is started by addition of 10 µl ^{14}C-putrescine (1,4-diamino-[1,4-^{14}C]butane dihydrochloride, 0.222 Ci/mol, 1 nCi/µl, Amersham) and the reaction mixture is incubated at 37 °C in a total volume of 100 µl. The reaction is stopped by addition of 10 µl 1 M perchloric acid which irreversibly denatures DAO. Following adjustment of the pH to ca. 10 by adding 50 µl 0.6 M sodium carbonate pH 12, the aqueous solution is extracted with 1.6 ml toluene containing 0.35% 2,5-diphenyloxazole by mixing vigorously for 30 sec. The phases are separated by centrifugation at 10,000 × g and 1.4 ml of the organic phase are transferred to a vial for the determination of radioactivity by liquid scintillation counting. Based on the specific radioactivity of the substrate, and on the known fraction of product recovered in the scintillation vial, DAO activities are calculated in international units per milligram protein (U/mg) where one unit converts one micromole of putrescine per minute at 37 °C.

Correspondence to: H. G. Schwelberger

Results and discussion

For the evaluation of the assay we used a commercially available crude preparation of diamine oxidase from pig kidney (pkDAO, Sigma) containing approximately 0.1% DAO. The optimum conditions for measuring this enzyme were found to be 100–250 mM phosphate buffer and pH 7.2–8.0. The reaction was linear for several hours if there was no substrate limitation. In the standard assay we could detect 1 ng pkDAO and sub-nanogram amounts were detectable by increasing the specific radioactivity of the substrate. Aminoguanidine [6] totally inhibited the activity at 1 µM, but was almost without effect at 10 nM. Blank values determined by addition of either aminoguanidine or perchloric acid before the start of the reaction or by omitting DAO were found to be identical and varied considerably with the batch of ^{14}C-putrescine used.

For any type of sample we routinely determine the pH-optimum and make sure that the measurement is carried out in the linear range of the reaction. We also check whether a one-step purification on a small high performance anion exchange column or the addition of the aldehyde dehydrogenase inhibitor acetaldehyde [7] results in an increase of the DAO activity thus indicating masking of the activity in the sample by enzyme inhibition or secondary reactions [8]. Finally, all DAO activity must be inhibited by addition of the specific inhibitor aminoguanidine [6].

In order to demonstrate the applicability of our test to small tissue samples we determined the DAO activity in human biopsy specimens collected during diagnostic endoscopy of the duodenum (Table 1). The DAO activities in all twelve patients who were diagnosed as free of duodenal diseases lie within a narrow range. This indicates that there are only slight variations in the normal values of DAO activity which should facilitate the detection of changes resulting from lesions of the intestinal mucosa.

Table 1. Determination of the DAO activities in human duodenal biopsy samples.

Sample (Patient)	Sample weight [mg]	Protein concentration [mg/ml]	Product extracted [dpm/15 min]	DAO activity [mU/mg protein]
1	14.5	0.80	131	0.60
2	15.8	1.73	228	0.48
3	23.1	1.77	360	0.73
4	20.3	1.54	331	0.78
5	22.6	1.79	383	0.77
6	21.4	1.67	306	0.66
7	12.8	0.89	172	0.70
8	23.0	1.91	356	0.67
9	18.9	1.37	235	0.62
10	19.2	2.10	413	0.71
11	11.7	1.03	180	0.63
12	15.6	1.52	212	0.50
Median	19.1	1.61	271	0.67
Range	11.7–23.1	0.80–2.10	131–413	0.48–0.78

DAO activities were determined in human biopsy specimens collected during diagnostic endoscopy from the duodenum of patients later classified as normal. The tissue samples were homogenized as described in *Materials and methods*. DAO assays were performed in triplicate in 100 mM phosphate buffer pH 7.0 with 50 µl homogenate and incubating for 15 minutes.

In summary, we describe a simple and sensitive method for the determination of soluble DAO in the nanogram-range requiring only milligrams of tissue material. The assay gives highly reproducible results calculated in international units per milligram protein. The small assay volume results in a considerable reduction of radioactive waste produced and organic solvent used. Employing disposable goods allows the simultaneous processing of a large number of samples. The procedure is easily adapted to measuring DAO activity in serum and represents an excellent tool for the routine testing of patients with various gastrointestinal and allergic symptoms.

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