Myoglobin Determination in Muscle Tissue Extracts for Western Blot Dystrophin Analysis

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Abstract

We adapted a nephelometric method for quantitative determination of myoglobin in serum or plasma to standardize the volume of supernatant from muscle tissue extract which should be used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis/Western blot dystrophin analysis. Myoglobin was considered an index of total muscle protein content in biopsy specimens. The procedure allows comparison of dystrophin bands in the diagnosis of Becker muscular dystrophy.

Key words: myoglobin, dystrophin, Becker muscular dystrophy, nephelometric assay.

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The term human dystrophinopaties encompasses different clinical phenotypes of myopathies depending on abnormalities of the dystrophin gene. Within the spectrum of these diseases, Becker muscular dystrophy may be distinguished on the basis of alterations of dystrophin size and quantity detected with Western blot analysis of muscle tissue extracts. This procedure represents a useful laboratory complement to clinical diagnosis. Currently, immunoblots are inspected by comparative analysis of position and intensity of dystrophin bands (i.e., normal control vs suspected pathological specimens) [1]. For this purpose, equal amounts of muscle proteins should be loaded in each lane. Since fibrous connective tissue may replace muscle tissue in patients with myopathies, a laborious procedure for noncollagenous protein determination in muscle tissue extracts is thus required [2]. Dystrophin abundance can be densitometrically quantitated from immunoblots and expressed in percentages by comparing pathological samples with normal controls.

We propose an alternative procedure consisting of the myoglobin determination with nephelometric method and subsequent standardization on the basis of myoglobin content of volumes of muscle tissue extracts that have to be studied with a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/Western blot technique for dystrophin analysis.

Methods

We have previously described the procedure of sample extraction and storage, as well as the SDS-PAGE/Western blot technique [3]. Briefly, frozen muscle biopsy specimens weighing ~20 mg are finely minced with a lancet and placed in 20 volumes of extraction buffer (75 mmol/L Tris-HCl, pH 6.8, 150 g/L SDS, 200 mL/L glycerol, 50 mL/L mercaptoethanol, 0.01 g/L bromophenol blue). Samples are boiled for 3 min and centrifuged at 11000 x g for 3 min. The supernates are taken for immediate analysis or stored in aliquots at -20°C.

Myoglobin was determined in supernates of muscle tissue extracts by nephelometric method (Behring Nephelometer Analyzer; Behringwerke AG, Marburg, Germany) with NA Latex Myoglobin Kit (Behringwerke AG; code no. OWIA). The reagent consists of polystyrene particles coated with rabbit anti-human myoglobin antibody. Prior to analysis, we diluted the samples 1:2000 with N Diluent (Behringwerke AG; code no. OUMT). Next, the samples were automatically diluted 1:20 by the nephelometer.

Results and Discussion

Specific performance characteristics of the test assaying 3 samples with different myoglobin concentrations (12, 120, and 250 mg/L) are shown in Table 1. N Myoglobin Standard and N Diluent (Behringwerke AG) were used for recovery and dilution studies, respectively. Values of myoglobin ranging from 10 to 265 mg/L were found in a

Table 1. Reproducibility, precision, recovery, and dilution studies of NA Latex Myoglobin Kit. Supernates of muscle tissue extracts were used.

Sample #	Myoglobin levels (mg/L)	Between-run (N = 10) CV, %	Within-run (N = 15) CV, %	Recovery +1,+2,+3 mg/L (mean value) %	Dilution x2, x5, x10 (mean value) %
1	12	9.8	6.6	90	102
2	120	10.2	6.0	90	97
3	250	11.5	7.2	88	92

series of muscle tissue extracts (N = 50). Lowest values were from Duchenne muscular dystrophy patients.

At the final sample dilution of 1:40,000, the presence of SDS in extraction buffer does not interfere with myoglobin determination, as we found by repeated testing of an opportunely treated N Myoglobin Standard (data not shown). The 3 min boiling procedure of the sample is known to cause loss of myoglobin antigenicity. Our data on 50 muscle biopsy specimens indicate that myoglobin levels in the extracts were slightly underestimated (~10%). In fact, we found a myoglobin content of 3.7 ± 0.8 mg/g of muscle tissue from controls, as expected given previously published data [4]. In any case, we required the relative and not the absolute amount of myoglobin in the samples for our comparative approach.

We established that a myoglobin content of 2.5 µg for each sample was optimal for detecting comparable dystrophin bands with Western blot analysis. In fact, considering the myosin heavy-chain band on the post-blotted gel as the reference standard [1], we found fairly equal intensities of these bands from both pathological samples and controls. This is indirect approximate evidence that myoglobin nephelometric determinations were reliable. Consequently, dystrophin bands from suspected pathological samples may be compared with controls, leading to estimation of dystrophin abundance.

Serum myoglobin determination is routinely used for early diagnosis and monitoring of myocardial infarction. There is not widespread agreement regarding its application in detection of the carrier status of Duchenne and Becker muscular dystrophies [5]. We propose an automated, rapid, and commercially available procedure of myoglobin determination in muscle tissue extracts useful for Western blot study of dystrophin in the diagnosis of human dystrophinopathies. The procedure can also be employed in studies on high molecular weight muscle proteins for research purposes.

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