REFOLDING ENHANCING FACTORS OF *ENTAMOEBA HISTOLYTICA* CYSTEINE PROTEASE BY FRACTIONAL FACTORIAL SCREENING

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Introduction. EhCP-A1 is a cysteine protease highly related with human parasite *Entamoeba histolytica* virulence and a potential target for drug design (1,2). However, the biochemical characterization of EhCP-A1 and other parasite cysteine proteases have been poorly studied mainly by the intrinsic difficulties to obtain them in sufficient amount in native conformation. Recombinant EhCP-A1 is obtained with great yields in *E. coli* as inclusion bodies; some functional studies have been reported refolding of EhCP-A1 by dilution or dialysis but with very low yields. Most refolding buffers for CPs had been developed empirically and no information of which factor could be beneficial o detrimental for protein naturalization. CPs differ from others proteins as they are loosely-packed and refolding buffers must provide optimal conditions for refolding without leading to activation and processing (3). Refolding screenings had been shown to be a useful tool to determine the feasibility of refolding and to identify optimal refolding conditions for each individual protein or family proteins (4,5).
The aim of this work is to identify factors that improve EhCP-A1 refolding for its thermal and structural characterization applying a fractional factorial design.

Methods. A fractional factorial protein folding screen of 12 factors and 16 conditions was used with two levels for each factor. Factors evaluated were protein concentration, pH, redox conditions, ionic strength, presence of a chaotrope (urea), a polar additive (arginine), a divalent cation quelate (EDTA) and PEG (Table 1). Refolding was evaluated by fluorescence spectroscopy and the protein aggregation was determined by light-scattering. Also, the enzymatic activity of refolded EhCP-A1 was determined by using a fluorogenic substrate. The main effects were calculated by adding the response obtained when using the upper level and subtract the response obtained using the lower level, as previously reported (4).

Results. Eight factors at two levels were evaluated in the refolding of EhCP-A1 by three response parameters (Table 1). The main effects observed by light-scattering (pink) and fluorescence (blue) of all factors are shown in Figure 1. Positive light scattering values indicate that upper level leads to aggregation, while postive fluorescence values indicate that spectra maximum moves to higher wavelengths. These results indicate that lower protein concentrations, presence of arginine, slightly basic conditions, use of redox pair instead of reducing agent, absence of chaotropic agent, low salt concentration, absence of EDTA and PEG enhanced EhCP-A1 refolding.

Table 1. Evaluated factors in EhCP-A1 refolding.

Level	[Pt] ($\mu g\ mL^{-1}$)	Polar Add. (M)	pH	Red./Ox.*	Urea (M)	Ionic Str. (mM)	Dival. Cat. (M)	PEG (%)
upper	50	0.5	8.0	GSH/GSSG	0.5	250	0.1	0.05
lower	10	0.0	6.0	DTT	0.0	10	0.0	0.0

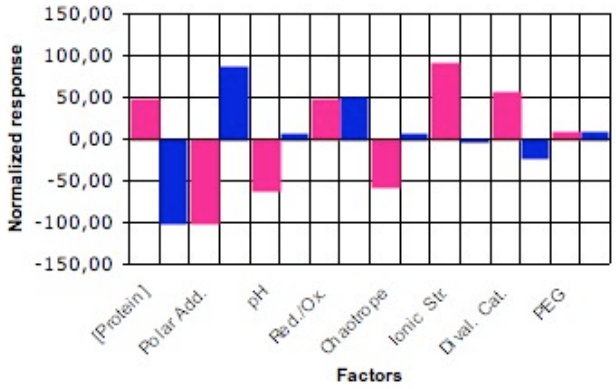

Fig.1 Plot of factor main effects by light scattering and fluorescence. Positive values in light scattering (pink) indicate that upper level leads to aggregation. Positive values in fluorescence (blue) indicate change to upper level moves maximum to higher wavelengths.

Conclusions. Factors that enhanced refolding of EhCP-A1, an *E. histolytica* CP were identified using fractional factorial screening.

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